METHODS OF TREATING HER2-POSITIVE CANCERS USING PD-1 AXIS BINDING ANTAGONISTS AND ANTI-HER2 ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application No. 61/917,264, filed December 17, 2013, which is hereby incorporated by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 146392022840SeqList.txt, date recorded: December 16, 2014, size: 37 KB).

FIELD OF THE INVENTION

[0003] This invention relates to methods of treating HER2-positive cancers by administering a PD-1 axis binding antagonist and an anti-HER2 antibody.

BACKGROUND OF THE INVENTION

[0004] The provision of two distinct signals to T-cells is a widely accepted model for lymphocyte activation of resting T lymphocytes by antigen-presenting cells (APCs). Lafferty et al, Aust. J. Exp. Biol. Med. Sci 53: 27-42 (1975). This model further provides for the discrimination of self from non-self and immune tolerance. Bretscher et al, Science 169: 1042-1049 (1970); Bretscher, P.A., Proc. Nat. Acad. Sci. USA 96: 185-190 (1999); Jenkins et al, J. Exp. Med. 165: 302-319 (1987). The primary signal, or antigen specific signal, is transduced through the T- cell receptor (TCR) following recognition of foreign antigen peptide presented in the context of the major histocompatibility-complex (MHC). The second or co-stimulatory signal is delivered to T-cells by co-stimulatory molecules expressed on antigen-presenting cells (APCs), inducing T-cells to promote clonal expansion, cytokine secretion and effector function. Lenschow et al., Ann. Rev. Immunol. 14:233 (1996). In the absence of co-stimulation, T-cells can become refractory to antigen stimulation, do not mount an effective immune response, and further may result in exhaustion or tolerance to foreign antigens.

[0005] In the two-signal model T-cells receive both positive and negative secondary co-stimulatory signals. The regulation of such positive and negative signals is critical to

maximize the host's protective immune responses, while maintaining immune tolerance and preventing autoimmunity. Negative secondary signals seem necessary for induction of T-cell tolerance, while positive signals promote T-cell activation. While the simple two-signal model still provides a valid explanation for naive lymphocytes, a host's immune response is a dynamic process, and co- stimulatory signals can also be provided to antigen-exposed T-cells. The mechanism of co-stimulation is of therapeutic interest because the manipulation of co-stimulatory signals has shown to provide a means to either enhance or terminate cell-based immune response. Recently, it has been discovered that T cell dysfunction or anergy occurs concurrently with an induced and sustained expression of the inhibitory receptor, programmed death 1 polypeptide (PD-1). As a result, therapeutic targeting of PD-1 and other molecules which signal through interactions with PD-1, such as programmed death ligand 1 (PD-Ll) and programmed death ligand 2 (PD-L2) are an area of intense interest.

[0006] PD-L1 is overexpressed in many cancers and is often associated with poor prognosis (Okazaki T et al., Intern. Immun. 2007 19(7):813) (Thompson RH et al., Cancer Res 2006, 66(7):3381). Interestingly, the majority of tumor infiltrating T lymphocytes predominantly express PD-1, in contrast to T lymphocytes in normal tissues and peripheral blood T lymphocytes indicating that up-regulation of PD-1 on tumor-reactive T cells can contribute to impaired antitumor immune responses (Blood 2009 114(8):1537). This may be due to exploitation of PD-L1 signaling mediated by PD-L1 expressing tumor cells interacting with PD-1 expressing T cells to result in attenuation of T cell activation and evasion of immune surveillance (Sharpe et al., Nat Rev 2002) (Keir ME et al., 2008 Annu. Rev. Immunol. 26:677). Therefore, inhibition of the PD-L1/PD-1 interaction may enhance CD8+ T cell-mediated killing of tumors.

[0007] Therapeutic targeting PD-1 and other molecules which signal through interactions with PD-1, such as programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2) are an area of intense interest. The inhibition of PD-L1 signaling has been proposed as a means to enhance T cell immunity for the treatment of cancer (e.g., tumor immunity) and infection, including both acute and chronic (e.g., persistent) infection. An optimal therapeutic treatment may combine blockade of PD-1 receptor/ligand interaction with an agent that directly inhibits tumor growth. There remains a need for an optimal therapy for treating, stabilizing, preventing, and/or delaying development of various cancers.

[0008] All references cited herein, including patent applications, patent publications, and UniProtKB/Swiss-Prot Accession numbers are herein incorporated by reference in their

entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY OF THE INVENTION

[0009] In one aspect, provided herein is a method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a human PD-1 axis binding antagonist and an anti-HER2 antibody.

[0010] In another aspect, provided herein is a method of enhancing immune function in an individual having cancer comprising administering an effective amount of a PD-1 axis binding antagonist and an anti-HER2 antibody.

[0011] In another aspect, provided herein is use of a human PD-1 axis binding antagonist in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising an anti-HER2 antibody and an optional pharmaceutically acceptable carrier.

[0012] In another aspect, provided herein is use of an anti-HER2 antibody in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the anti-HER2 antibody and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising a human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

[0013] In another aspect, provided herein is a composition comprising a human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in combination with a second composition, wherein the second composition comprises an anti-HER2 antibody and an optional pharmaceutically acceptable carrier.

[0014] In another aspect, provided herein is a composition comprising an anti-HER2 antibody and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in combination with a second composition, wherein the second composition comprises a human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

[0015] In another aspect, provided herein is a kit comprising a medicament comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising an anti-HER2 antibody and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual.

[0016] In another aspect, provided herein is a kit comprising a first medicament comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and a second medicament comprising an anti-HER2 antibody and an optional pharmaceutically acceptable carrier. In some embodiments, the kit further comprises a package insert comprising instructions for administration of the first medicament and the second medicament for treating or delaying progression of cancer in an individual.

[0017] In another aspect, provided herein is a kit comprising a medicament comprising an anti-HER2 antibody and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual.

[0018] In some embodiments of the methods, uses, compositions, and kits described above and herein, the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. In some embodiments, the PD-1 axis binding antagonist is an antibody. In some embodiments, the antibody is a humanized antibody, a chimeric antibody or a human antibody. In some embodiments, the antibody is an antigen binding fragment. In some embodiments, the antigen-binding fragment is selected from the group consisting of Fab, Fab', $F(ab')_2$, and Fv.

[0019] In some embodiments, the PD-1 axis binding antagonist is a PD-1 binding antagonist. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to its ligand binding partners. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2. In some embodiments, the PD-1 binding antagonist is an antibody. In some embodiments, the PD-1 binding antagonist is selected from the group consisting of MDX-1106 (nivolumab), MK-3475 (pembrolizumab, lambrolizumab), CT-011 (pidilizumab), and AMP-224.

[0020] In some embodiments, the PD-1 axis binding antagonist is a PD-L1 binding antagonist. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-

L1 to PD-1. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1. In some embodiments, the PD-L1 binding antagonist is an antibody. In some embodiments, the PD-L1 binding antagonist is selected from the group consisting of: YW243.55.S70, MPDL3280A, MDX-1105, and MEDI4736. In some embodiments, the anti-PD-L1 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21; and/or a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24. In some embodiment, the anti-PD-L1 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 or 26 and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:4. In some embodiments, the anti-PD-L1 antibody comprises the three heavy chain HVR sequences of antibody YW243.55.S70 and/or the three light chain HVR sequences of antibody YW24355.S70 described in WO 2010/077634 and U.S. Patent No. 8,217,149, which are incorporated herein by reference. In some embodiments, the anti-PD-L1 antibody comprises the heavy chain variable region sequence of antibody YW243.55.S70 and/or the light chain variable region sequence of antibody YW24355.S70.

[0021] In some embodiments, the PD-1 axis binding antagonist is a PD-L2 binding antagonist. In some embodiments, the PD-L2 binding antagonist is an antibody. In some embodiments, the PD-L2 binding antagonist is an immunoadhesin.

[0022] In some embodiments of the methods, uses, compositions, and kits described above and herein, the anti-HER2 antibody is trastuzumab (HERCEPTIN®, Genentech) or pertuzumab (PERJETA®, Genentech). In some embodiments, the anti-HER2 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:38, HVR-H2 sequence of SEQ ID NO:50, and HVR-H3 sequence of SEQ ID NO:40; and/or a light chain comprising HVR-L1 sequence of SEQ ID NO:41, HVR-L2 sequence of SEQ ID NO:42, and HVR-L3 sequence of SEQ ID NO:43. In some embodiments, the anti-HER2 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:34 and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:35. In some embodiments, the anti-HER2 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:36 and/or a light chain comprising the amino acid sequence of SEQ ID NO:37. In some embodiments that can be combined with any other embodiments, the anti-HER2 antibody is not trastuzumab or pertuzumab.

[0023] In some embodiments of the methods, uses, compositions, and kits described above and herein, the anti-HER2 antibody is a multispecific antibody. In some embodiments, the anti-HER2 antibody is a bispecific antibody. In some embodiments, the bispecific antibody comprises a first antigen binding domain that binds to HER2, and a second antigen binding domain that binds to CD3. In some embodiments, the second antigen binding domain binds to a human CD3 polypeptide. In some embodiments, the CD3 polypeptide is a human CD3ε polypeptide or a human CD3γ polypeptide. In some embodiments, the second antigen binding domain binds to a human CD3ε polypeptide or a human CD3 γ polypeptide in native T-cell receptor (TCR) complex in association with other TCR subunits. In some embodiments, the first antigen binding domain comprises a heavy chain variable region (V_HHER2) and a light chain variable region (V_LHER2), and the second antigen binding domain comprises a heavy chain variable region (V_HCD3) and a light chain variable region (V_LCD3). In some embodiments, the first antigen binding domain comprises a heavy chain variable region (V_HHER2) comprising HVR-H1 sequence of SEQ ID NO:38, HVR-H2 sequence of SEQ ID NO:50, and HVR-H3 sequence of SEQ ID NO:40; and/or a light chain variable region (V_LHER2) comprising HVR-L1 sequence of SEQ ID NO:41, HVR-L2 sequence of SEQ ID NO:42, and HVR-L3 sequence of SEQ ID NO:43. In some embodiment, the first antigen binding domain comprises a heavy chain variable region (V_HHER2) comprising the amino acid sequence of SEQ ID NO:34 and/or a light chain variable region (V_LHER2) comprising the amino acid sequence of SEQ ID NO:35. In some embodiments, the bispecific antibody is a single-chain bispecific antibody comprising the first antigen binding domain and the second antigen binding domain. In some embodiments, the single-chain bispecific antibody comprises variable regions, as arranged from N-terminus to C-terminus, selected from the group consisting of (1) V_HHER2-V_LHER2-V_HCD3-V_LCD3, (2) V_HCD3-V_LCD3-V_HHER2-V_LHER2, (3) V_HCD3-V_LCD3-V_LHER2-V_HHER2, (4) V_HHER2-V_LHER2-V_LCD3-V_HCD3, (5) V_LHER2-V_HHER2-V_HCD3-V_LCD3, or (6) V_LCD3-V_HCD3-V_HHER2-V_LHER2.

[0024] In some embodiments, the bispecific antibody comprises a first antigen binding domain that binds to HER2 and a second antigen binding domain that binds to CD3, wherein the first antigen binding domain comprises one or more heavy chain constant domains, wherein the one or more heavy chain constant domains are selected from a first CH1 ($CH1_1$) domain, a first CH2 ($CH2_1$) domain, a first CH3 ($CH3_1$) domain; and wherein the second antigen binding domain comprises one or more heavy chain constant domains, wherein the one or more heavy chain constant domains are selected from a second CH1 ($CH1_2$) domain,

second CH2 ($CH2_2$) domain, and a second CH3 ($CH3_2$) domain. In some embodiments, at least one of the one or more heavy chain constant domains of the first antigen binding domain is paired with another heavy chain constant domain of the second antigen binding domain. In some embodiments, the $CH3_1$ and $CH3_2$ domains each comprise a protuberance or cavity, and wherein the protuberance or cavity in the $CH3_1$ domain is positionable in the cavity or protuberance, respectively, in the $CH3_2$ domain. In some embodiments, the $CH3_1$ and $CH3_2$ domains meet at an interface between said protuberance and cavity. In some embodiments, the $CH2_1$ and $CH2_2$ domains each comprise a protuberance or cavity, and wherein the protuberance or cavity in the $CH2_1$ domain is positionable in the cavity or protuberance, respectively, in the $CH2_2$ domain. In some embodiments, the $CH2_1$ and $CH2_2$ domains meet at an interface between said protuberance and cavity.

[0025] In some embodiments, the antibody described herein (e.g., a PD-1 axis binding antagonist antibody, an anti-HER2 antibody, or a bispecific antibody that binds to HER2 and a CD3) comprises an aglycosylation site mutation. In some embodiments, the aglycosylation site mutation is a substitution mutation. In some embodiments, the substitution mutation is at amino acid residue N297, L234, L235, and/or D265 (EU numbering). In some embodiments, the substitution mutation is selected from the group consisting of N297G, N297A, L234A, L235A, and D265A. In some embodiments, the substitution mutation is a D265A mutation and an N297G mutation. In some embodiments, the aglycosylation site mutation reduces effector function of the antibody. In some embodiments, the PD-1 axis binding antagonist (e.g., an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-PD-L2 antibody) is a human IgG1 having Asn to Ala substitution at position 297 according to EU numbering.

[0026] In some embodiments of the methods, uses, compositions and kits described above and herein, the cancer is a HER2-positive cancer. In some embodiments, the cancer is breast cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, colon cancer, kidney cancer, esophageal cancer, prostate cancer, or other cancers described herein. In some embodiments, the individual has cancer or has been diagnosed with cancer. In some embodiments, the cancer cells in the individual express PD-L1. In some embodiments, the individual has cancer that is resistant to a HER2 targeted therapy. In some embodiments, the individual is refractory to a HER2 targeted therapy. In some embodiments, the HER2 targeted therapy is a treatment with an anti-HER2 antibody or an inhibitor of the HER2 pathway. In some embodiments, the HER2 targeted therapy is a treatment with trastuzumab (HERCEPTIN®, Genentech), pertuzumab (PERJETA®, Genentech), ado-trastuzumab emtansine (KADCYLA®, Genentech), or lapatinib.

[0027] In some embodiments of the methods, uses, compositions, and kits described above and herein, the treatment or administration of the human PD-1 axis binding antagonist and the anti-HER2 antibody results in a sustained response in the individual after cessation of the treatment. In some embodiments, the anti-HER2 antibody is administered before the PD-1 axis binding antagonist, simultaneous with the PD-1 axis binding antagonist, or after the PD-1 axis binding antagonist. In some embodiments, the PD-1 axis binding antagonist and the anti-HER2 antibody are in the same composition. In some embodiments, the PD-1 axis binding antagonist and the anti-HER2 antibody are in separate compositions.

[0028] In some embodiments of the methods, uses, compositions, and kits described above and herein, the PD-1 axis binding antagonist and/or the anti-HER2 antibody is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments of the methods, uses, compositions, and kits described above and herein, the treatment further comprises administering a chemotherapeutic agent for treating or delaying progression of cancer in an individual. In some embodiments, the individual has been treated with a chemotherapeutic agent before the combination treatment with the PD-1 axis binding antagonist and the anti-HER2 antibody. In some embodiments, the individual treated with the combination of the PD-1 axis binding antagonist and/or the anti-HER2 antibody is refractory to a chemotherapeutic agent treatment. Some embodiments of the methods, uses, compositions, and kits described throughout the application, further comprise administering a chemotherapeutic agent for treating or delaying progression of cancer.

[0029] In some embodiments of the methods, uses, compositions and kits described above and herein, CD8 T cells in the individual have enhanced priming, activation, proliferation and/or cytolytic activity relative to prior to the administration of the combination. In some embodiments, the number of CD8 T cells is elevated relative to prior to administration of the combination. In some embodiments, the CD8 T cell is an antigen-specific CD8 T cell. In some embodiments, Treg function is suppressed relative to prior to the administration of the combination. In some embodiments, T cell exhaustion is decreased relative to prior to the administration of the combination.

[0030] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in

the art. These and other embodiments of the invention are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] **FIG. 1** shows the generation of a full length HER2-CD3 bispecific antibody using knobs-into-holes technology and T cell independent properties of HER2-TDB. (**A**) Amino acid substitutions were generated to C_H3 domains of the 'knob' (α-HER2 4D5) and 'hole' (α-CD3 UCHT1.v9) heavy chains, which selectively promote heterodimerization to generate bispecific full length IgG1. (**B**) Overview of the TDB purification. ProA= Protein A affinity purification, HIC = hydrophobic interaction chromatography, QC = quality control, SEC = size exclusion chromatography. (**C**) Size exclusion chromatography demonstrated low levels of aggregate or single arms. (**D**) MS analysis indicated undetectable levels of homodimeric species. (**E**) Binding to SKBR-3 was determined by competition binding of ^{125}I-trastuzumab Fab with non-labeled trastuzumab (black), trastuzumab-fab (blue) or bispecific HER2-TDB (red). Data points shown represent the mean of 3 measurements. (**F**) Direct effect on proliferation of SKBR-3 cells was analyzed after 6 days of treatment with antibodies using CellTiter-Glo® Luminescent Cell Viability Assay. Data points shown represent the mean of 3 measurements. (**G**) The ability of trastuzumab, trastuzumab produced in *E. coli*, and HER2-TDB to mediate *in vitro* ADCC by NK cells was measured using assay detecting LDH released from lysed cells. Timepoint 4h. Error bars = S.D.

[0032] **FIG. 2** shows that target dependent T cell mediated cytotoxicity of HER2-TDB. (**A**) T cell activation was detected by staining cells for CD8/CD69/Granzyme B followed by FACS analysis. Effectors CD8+ T cells, target SKBR-3, E:T ratio 3:1, time point 48h. Data presented as mean of two repeats. (**B**) Soluble granzymes and perforin were detected from the media using ELISA and cytotoxicity using LDH release assay. Effectors PBMC, target SKBR-3, E:T ratio 30:1, time point 18h, ABs 10ng/ml. (**C**) Elevated caspase activity (Caspase 3/7 glo assay) and apoptosis (Cell Death Detection ELISA^{plus} assay) corresponded with LDH-release after treatment with 1ng/ml bispecific antibody. Effectors PBMC, targets SKBR-3, E:T ratio 10:1, time point 24h. Error bar = S.D. in panels C-F. (**D**) The ability of HER2-TDB to induce killing of HER2 (red) or vector-transfected (blue) 3T3 was measured using an LDH release assay. Effectors PBMC, E:T ratio 10:1, time point 19h. (**E**) Blocking HER2-arm binding using trastuzumab Fab (1 μg/ml, black) or soluble HER2 extracellular domain (1 μg/ml, blue) efficiently inhibited cytotoxic activity of HER2-TDB. Effectors CD8+ T cells, target BT474, E:T ratio 5:1, time point 24h. Cytotoxicity was detected using

an LDH release assay. (**F**) Killing activity of PBMC was compared before and after depletion of CD3 positive cells with CD3 MicroBeads. Target SKBR3, E:T ratio 20:1, time point 19h. Cytotoxicity was detected using FACS assay.

[0033] **FIG. 3** shows the characteristics of T cell activation and killing induced by HER2-TDB. (**A**) T cell activation was detected at various timepoints by staining cells for CD8, CD69, and CD107a followed by FACS analysis. T cell activation data presented as mean of two repeats. Effectors CD8+ T cells, target SKBR-3, E:T ratio 3:1. Cytotoxicity was detected using FACS assay. Effectors CD8+, target SKBR-3, E:T ratio 3:1, Error bar = S.D. in all panels. (**B**) Cytotoxicity was detected using LDH release assay. Effectors CD8+ T cells, target BT474, E:T ratio indicated in the figure, time point 19h. T cell activation was measured as in panel A.

[0034] **FIG. 4** demonstrates that activation of T cells by HER2-TDB induces T cell proliferation. (**A**) Proliferation of T cells was measured at day 3 as dilution of CFSE in CD8+/PI- cells with cell divisions. (**B-C**) HER2-TDB induced T cell expansion. Purified CD8+ T cells were labeled with CFSE according to manufacturer's protocol (Invitrogen, #C34554). CFSE-labeled CD8+ T cells were incubated with target cells in the presence or absence of TDB for 19 hours. T cells were collected, washed and cultured for 2-7 days (RPMI+10% FBS, +/- 20 ng/ml IL2). Live CD8+ cell number (CD8+/PI-) and the percentage of $CFSE_{dim}$ cells was detected by FACS.

[0035] **FIG. 5** demonstrates that HER2-TDB activity correlates with the target cell HER2 expression level. (**A**) HER2-levels in different cancer cells were detected by Western blot. (**B**) Cytotoxicity was detected using LDH release assay. Effectors PBMCs, E:T 25:1, time point 26h. (**C**) MCF-7 cells were labeled with CFSE and mixed with SKBR3 and PBMC (E:T 20:1) followed by 19h treatment with HER2-TDB. Cells were stained with anti-HER2 APC and PI. The number of living SKBR3 (HER2 high, PI-) and MCF7 (CFSE+. PI-) cells were analyzed by FACS and normalized to fluorescent beads. (**D**) BJAB cells were labeled with CFSE and mixed with SKBR3 and PBMCs (E:T 20:1) followed by 19h treatment with HER2-TDB. Cells were stained with anti-HER2 APC and PI. The number of living SKBR3 (HER2 high, PI-) and BJAB (CFSE+ PI-) cells was normalized to fluorescent beads. (**E**) HER2 copy number was previously reported (Aguilar et al., *Oncogene*, 18:6050-62, 1999). EC_{50} values were calculated from dose response data in **FIG. 6B**. Calculation of HER2 occupancy is described in text.

[0036] **FIG. 6** shows the efficient killing of HER2+ cancer cells refractory to anti-HER2 therapies and regardless of tissue type, PI3K pathway mutation status, or sensitivity to

trastuzumab or lapatinib. (**A**) Cytotoxicity against various cell lines was detected using LDH release assay. Effectors PBMC, E:T 10:1, time point 19h. (**B**) Parental and T-DM1 resistant BT474-M1 clones were treated with T-DM1 for 3 days. Cell viability was measured using Cell titer Glo. (**C**) Parental and T-DM1 resistant BT474-M1 clones were treated with HER2-TDB. Cytotoxicity was detected using FACS assay. Effectors CD8+ T cells, E:T ratio 3:1, time point 24h. Error bar = S.D.

[0037] **FIG. 7** shows the pharmacokinetic profile of HER2-TDB. Single intravenous doses of 10 mg/kg trastuzumab (open symbols) or HER2-TDB (black symbols) were injected into Sprague-Dawley Rats. Serum samples were assayed for test agent by ELISA. Mean +/- SD. HER2-TDB N=4, trastuzumab N=3.

[0038] **FIG. 8** demonstrates that HER2-TDB inhibits growth of HER2+ tumors. (**A**) *In vivo* efficacy of HER2-TDB was tested in NOD-SCID mice. $5x10^6$ MCF7-neo/HER2 cells were injected together with $1x10^7$ unstimulated human PBMC from two healthy donors (PBMC 1, 2). Mice (N=5-10) were treated with 0.5 mg/kg i.v. doses of HER2-TDB on days 0, 7 and 14. Tumor volumes from individual mice and fitted tumor volumes of treatment groups are presented; mice terminated prior to study end are shown as red traces whereas mice remaining on study to study end are shown as grey traces. Fitted tumor volume for each treatment group are shown as a solid black line with fitted tumor volume for comparator control group are shown as a dashed blue line. (**B**) MMTV-huHER2 transgenic animals with established mammary tumors were treated with 0.5 mpk 4D5/2C11-TDB (red; mCD3 reactive 2C11 surrogate arm; qwk x 5, IV, starting on day 0) or vehicle (black). (**C**) Progression of MMTV-huHER2 transgenic tumors and maximum percentage of tumor shrinkage by HER2-TDB treatment are shown. (**D**) 4D5/2C11-TDB (0.5 mpk, qwk x 5, IV, starting on day 0) is effective in treatment of large (>1000 mm^3) MMTV-huHER2 transgenic tumors. (**E**) Growth of MMTV-huHER2 transgenic tumors was not affected by control TDBs in which the CD3 arm was switched to human CD3 specific (4D5/SP34-TDB; blue), or in which the target arm was switched to irrelevant (CTRL/2C11-TDB; grey). (**F**) *In vivo* efficacy of HER2-TDB in huCD3 transgenic mice. Established CT26-HER2 tumors were treated with vehicle or with 0.5 mg/kg HER2-TDB (4D5/SP34-TDB) qwk x 3, IV, starting on day 0. (**G**) *In vivo* efficacy of HER2-TDB with mCD3 reactive 2C11 surrogate arm (4D5/2C11-TDB) was tested in Balb/c mice. Dosing was administered as described above. 15 mg/kg TDM-1 was dosed qwk x 3, IV. Control TDB is 2C11 paired with irrelevant target arm.

[0039] **FIG. 9** shows that CD3-TG T cells express both mouse and human CD3 at approximately 50% of the level of respective Balb/c mouse or human T cells. (**A-B**) T cells were extracted from spleens of CD3-TG (diamonds), BALB/c mice (squares) or from peripheral blood from healthy human donors (circles) and stained with mouse or human CD8 and either human CD3 (clone UCHT1; **A**) or mouse CD3 (clone 2C11; **B**). The figure is gated on CD8+ cells.

[0040] **FIG. 10** shows TDB mediated killing by CD3-TG splenic T cells. (**A-B**) T cells were extracted from spleens of CD3-TG (diamonds), BALB/c mice (squares) or from peripheral blood from healthy human donors (circles). *In vitro* killing activity of CT26-HER2 cells was tested using human CD3-specific (UCHT1.v9) HER2-TDB (**A**) or mouse CD3-specific (2C11) HER2-TDB (**B**). E:T = 20:1 Assay time: 40 hours. *In vitro* cytotoxicity was monitored by flow cytometry.

[0041] **FIG. 11** demonstrates that the anti-tumor activity of HER2-TDB is T cell dependent. 0.1 million CT26-HER2 antibodies were injected subcutaneously into BALB/c mice. Mice with established tumors were treated with vehicle or human CD3-specific HER2-TDB (0.5 mg/kg, qwx3, IV, n=10).

[0042] **FIG. 12** shows that T cells in CT26-HER2 tumors display the CD69 activation marker. 0.1 million CT26-HER2 were injected subcutaneously into BALB/c mice. Mice with established tumors were treated with vehicle, human CD3-specific HER2-TDB, or a CTRL-TDB with irrelevant target arm (0.5 mg/kg, qwx3, IV). Tumors were harvested 11-35 days after cell injection. Tissues were cut into small pieces and transferred into gentleMACS™ C-tubes (Miltenyi, # 130-093-237). Samples were digested with Collagenase D (1 mg/ml) and DNase I (0.2 mg/ml) in rotating incubator for 15 minutes then dissociated to achieve single cell suspension. After anti-CD16/CD32 FcR blocking, cells were stained with the cocktails of surface markers (N = 2/group,NT = non treated).

[0043] **FIG. 13** shows the expression of PD-1 and PD-L1 by CT-26-HER2 tumor-infiltrating T cells and CT-26-HER2 tumor cells, respectively. (**A**) FACS analysis demonstrated that CT-26-HER2 tumor-infiltrating T cells express PD-1. (**B**) FACS analysis demonstrated that CT-26-HER2 tumor cells express PD-L1.

[0044] **FIG. 14** shows that the PD1/PD-L1signaling limits the response to HER2-TDB. (**A**) Flow cytometric analysis revealed that stimulation of human T cells by TDB and target cells induced PD-1 expression. (**B**) Expression of PD-L1 in target cells (293) was sufficient to inhibit TDB-mediated T cell killing activity. Cytotoxicity of target cells was measured upon addition of primed T cells to 293 cells expressing PD-L1 (triangles), 293 cells expressing PD-

L1 treated in the presence of anti-PD-L1 antibody (circles), or vector-transfected, control 293 cells (squares). (**C**) The combination of HER2-TDB (4D5/SP34-TDB) and anti-PD-L1 antibody significantly inhibited growth of established CT26-HER2 tumors in huCD3 transgenic mice. TDB dosing was administered as in **FIG. 8F**, and anti-PD-L1 antibody (25A1) was dosed 10 mg/kg tiw x 3, IP. Control TDB binds to huCD3 but has an irrelevant target arm. TTP = time to tumor progression (2x day 0 volume). (**D**) The combination of HER2-TDB (4D5/SP34-TDB) and anti-PD-L1 antibody resulted in complete, long-term responses by treatment of CT26-HER2 tumors in huCD3 transgenic mice. Dosing was administered as described above. CR = no detectable tumor, PR = at least 50% tumor shrinkage from Day 0.

[0045] **FIG. 15** shows that the activity of HER2-TDB in NOD-SCID mice is dependent on human PBMCs. (**A**) $5x10^6$ MCF7-neo/HER2 cells were injected into mice without human PBMCs. Mice (n=7) were treated with 0.5 mg/kg i.v. doses of HER2-TDB on days 0, 7, and 14. Tumor volumes from individual mice and fitted tumor volumes of treatment groups are presented; mice terminated prior to study end are shown as red traces, whereas mice remaining to study end are shown as grey traces. Fitted tumor volumes for each treatment group are shown as a solid black line with fitted tumor volume for comparator control groups are shown as a dashed blue line. (**B**) Control TDB that shares the same CD3-arm as HER2-TDB but has an irrelevant non-binding target arm has no effect on tumor growth. $5x10^6$ MCF7-neo/HER2 cells were injected together with $1x10^7$ unstimulated human PBMCs. Mice were treated as in panel (**A**).

DETAILED DESCRIPTION

[0046] The inventors of this application demonstrated that PD-L1 expressed by cancer cells can inhibit the activity of T cell recruiting antibodies and this inhibition can be reversed by an anti-PD-L1 antibody. The data in the application show that the combination of a HER2 T cell dependent bispecific antibody (HER2-TDB) with anti-PD-L1 immune therapy resulted in enhanced inhibition of tumor growth, increased response rates and durable responses. The inventors demonstrated the benefit of combining two immune therapies: direct polyclonal recruitment of T cell activity together with inhibiting the T cell suppressive PD-1/PD-L1 signaling results in enhanced and durable long term responses.

[0047] In one aspect, provided herein are methods, compositions and uses for treating or delaying progression of cancer in an individual comprising administering an effective amount of a human PD-1 axis binding antagonist and an anti-HER2 antibody.

[0048] In another aspect, provided herein are methods, compositions and uses for enhancing immune function in an individual having cancer comprising administering an effective amount of a human PD-1 axis binding antagonist and an anti-HER2 antibody.

I. Definitions

[0049] Before describing the invention in detail, it is to be understood that this invention is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0050] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

[0051] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0052] It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

[0053] The term *"PD-1 axis binding antagonist"* refers to a molecule that inhibits the interaction of a PD-1 axis binding partner with either one or more of its binding partner, so as to remove T-cell dysfunction resulting from signaling on the PD-1 signaling axis – with a result being to restore or enhance T-cell function (*e.g.*, proliferation, cytokine production, target cell killing). As used herein, a PD-1 axis binding antagonist includes a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

[0054] The term *"PD-1 binding antagonist"* refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1, PD-L2. In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to one or more of its binding partners. In a specific aspect, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1 and/or PD-L2. For example, PD-1 binding antagonists include anti-PD-1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-1 with PD-L1 and/or PD-L2. In one

embodiment, a PD-1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-1 so as render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody. In a specific aspect, a PD-1 binding antagonist is MDX-1106 (nivolumab) described herein. In another specific aspect, a PD-1 binding antagonist is MK-3475 (lambrolizumab) described herein. In another specific aspect, a PD-1 binding antagonist is CT-011 (pidilizumab) described herein. In another specific aspect, a PD-1 binding antagonist is AMP-224 described herein.

[0055] The term "*PD-L1 binding antagonist*" refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L1 with either one or more of its binding partners, such as PD-1, B7-1. In some embodiments, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, the PD-L1 binding antagonist inhibits binding of PD-L1 to PD-1 and/or B7-1. In some embodiments, the PD-L1 binding antagonists include anti-PD-L1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L1 with one or more of its binding partners, such as PD-1, B7-1. In one embodiment, a PD-L1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L1 so as to render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, a PD-L1 binding antagonist is an anti-PD-L1 antibody. In a specific aspect, an anti-PD-L1 antibody is YW243.55.S70 described herein. In another specific aspect, an anti-PD-L1 antibody is MDX-1105 described herein. In still another specific aspect, an anti-PD-L1 antibody is MPDL3280A described herein. In still another specific aspect, an anti-PD-L1 antibody is MEDI4736 described herein.

[0056] The term "*PD-L2 binding antagonist*" refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In some embodiments, a PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to one or more of its binding partners. In a specific aspect, the PD-L2 binding antagonist inhibits binding of PD-L2 to PD-1. In some embodiments, the PD-L2 antagonists include anti-PD-L2 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins,

oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In one embodiment, a PD-L2 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L2 so as render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, a PD-L2 binding antagonist is an immunoadhesin.

[0057] The term "*dysfunction*" in the context of immune dysfunction, refers to a state of reduced immune responsiveness to antigenic stimulation. The term includes the common elements of both *exhaustion* and/or *anergy* in which antigen recognition may occur, but the ensuing immune response is ineffective to control infection or tumor growth.

[0058] The term "*dysfunctional*", as used herein, also includes refractory or unresponsive to antigen recognition, specifically, impaired capacity to translate antigen recognition into down-stream T-cell effector functions, such as proliferation, cytokine production (e.g., IL-2) and/or target cell killing.

[0059] The term "*anergy*" refers to the state of unresponsiveness to antigen stimulation resulting from incomplete or insufficient signals delivered through the T-cell receptor (*e.g.* increase in intracellular Ca^{+2} in the absence of ras-activation). T cell anergy can also result upon stimulation with antigen in the absence of co-stimulation, resulting in the cell becoming refractory to subsequent activation by the antigen even in the context of costimulation. The unresponsive state can often be overriden by the presence of Interleukin-2. Anergic T-cells do not undergo clonal expansion and/or acquire effector functions.

[0060] The term "*exhaustion*" refers to T cell exhaustion as a state of T cell dysfunction that arises from sustained TCR signaling that occurs during many chronic infections and cancer. It is distinguished from anergy in that it arises not through incomplete or deficient signaling, but from sustained signaling. It is defined by poor effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infection and tumors. Exhaustion can result from both extrinsic negative regulatory pathways (e.g., immunoregulatory cytokines) as well as cell intrinsic negative regulatory (costimulatory) pathways (PD-1, B7-H3, B7-H4, etc.).

[0061] "*Enhancing T-cell function*" means to induce, cause or stimulate a T-cell to have a sustained or amplified biological function, or renew or reactivate exhausted or inactive T-cells. Examples of enhancing T-cell function include: increased secretion of γ-interferon

from $CD8^+$ T-cells, increased proliferation, increased antigen responsiveness (*e.g.*, viral, pathogen, or tumor clearance) relative to such levels before the intervention. In one embodiment, the level of enhancement is as least 50%, alternatively 60%, 70%, 80%, 90%, 100%, 120%, 150%, 200%. The manner of measuring this enhancement is known to one of ordinary skill in the art.

[0062] A "*T cell dysfunctional disorder*" is a disorder or condition of T-cells characterized by decreased responsiveness to antigenic stimulation. In a particular embodiment, a T-cell dysfunctional disorder is a disorder that is specifically associated with inappropriate increased signaling through PD-1. In another embodiment, a T-cell dysfunctional disorder is one in which T-cells are anergic or have decreased ability to secrete cytokines, proliferate, or execute cytolytic activity. In a specific aspect, the decreased responsiveness results in ineffective control of a pathogen or tumor expressing an immunogen. Examples of T cell dysfunctional disorders characterized by T-cell dysfunction include unresolved acute infection, chronic infection and tumor immunity.

[0063] *"Tumor immunity"* refers to the process in which tumors evade immune recognition and clearance. Thus, as a therapeutic concept, tumor immunity is "treated" when such evasion is attenuated, and the tumors are recognized and attacked by the immune system. Examples of tumor recognition include tumor binding, tumor shrinkage and tumor clearance.

[0064] *"Immunogenecity"* refers to the ability of a particular substance to provoke an immune response. Tumors are immunogenic and enhancing tumor immunogenicity aids in the clearance of the tumor cells by the immune response. Examples of enhancing tumor immunogenicity include treatment with a PD-1 axis binding antagonist and an anti-HER2 antibody.

[0065] "*Sustained response*" refers to the sustained effect on reducing tumor growth after cessation of a treatment. For example, the tumor size may remain to be the same or smaller as compared to the size at the *beginning* of the administration phase. In some embodiments, the sustained response has a duration at least the same as the treatment duration, at least 1.5X, 2.0X, 2.5X, or 3.0X length of the treatment duration.

[0066] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile. "Pharmaceutically acceptable"

excipients (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

[0067] As used herein, the term "treatment" refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. For example, an individual is successfully "treated" if one or more symptoms associated with cancer are mitigated or eliminated, including, but are not limited to, reducing the proliferation of (or destroying) cancerous cells, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, and/or prolonging survival of individuals.

[0068] As used herein, "delaying progression of a disease" means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0069] An "effective amount" is at least the minimum amount required to effect a measurable improvement or prevention of a particular disorder. An effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the treatment are outweighed by the therapeutically beneficial effects. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In the case of cancer or tumor, an effective amount of the drug may have the effect in reducing the number of cancer cells; reducing the tumor size; inhibiting (*i.e.*, slow to some extent or desirably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and desirably stop)

tumor metastasis; inhibiting to some extent tumor growth; and/or relieving to some extent one or more of the symptoms associated with the disorder. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0070] As used herein, "in conjunction with" refers to administration of one treatment modality in addition to another treatment modality. As such, "in conjunction with" refers to administration of one treatment modality before, during, or after administration of the other treatment modality to the individual.

[0071] A "disorder" is any condition that would benefit from treatment including, but not limited to, chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question.

[0072] The terms "cell proliferative disorder" and "proliferative disorder" refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer. In one embodiment, the cell proliferative disorder is a tumor.

[0073] "Tumor," as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer", "cancerous", "cell proliferative disorder", "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

[0074] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include, but not limited to, squamous cell cancer (*e.g.*, epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic

cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, nodular melanomas, multiple myeloma and B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), Meigs' syndrome, brain, as well as head and neck cancer, and associated metastases. In certain embodiments, cancers that are amenable to treatment by the antibodies of the invention include breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, glioblastoma, non-Hodgkins lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, ovarian cancer, mesothelioma, and multiple myeloma. In some embodiments, the cancer is selected from: small cell lung cancer, gliblastoma, neuroblastomas, melanoma, breast carcinoma, gastric cancer, colorectal cancer (CRC), and hepatocellular carcinoma. Yet, in some embodiments, the cancer is selected from: non-small cell lung cancer, colorectal cancer, glioblastoma and breast carcinoma, including metastatic forms of those cancers.

[0075] The term "cytotoxic agent" as used herein refers to any agent that is detrimental to cells (e.g., causes cell death, inhibits proliferation, or otherwise hinders a cellular function). Cytotoxic agents include, but are not limited to, radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents; growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Exemplary cytotoxic agents can be selected from anti-microtubule agents, platinum coordination complexes, alkylating agents, antibiotic agents, topoisomerase II inhibitors,

antimetabolites, topoisomerase I inhibitors, hormones and hormonal analogues, signal transduction pathway inhibitors, non-receptor tyrosine kinase angiogenesis inhibitors, immunotherapeutic agents, proapoptotic agents, inhibitors of LDH-A, inhibitors of fatty acid biosynthesis, cell cycle signalling inhibitors, HDAC inhibitors, proteasome inhibitors, and inhibitors of cancer metabolism. In one embodiment the cytotoxic agent is a taxane. In one embodiment the taxane is paclitaxel or docetaxel. In one embodiment the cytotoxic agent is a platinum agent. In one embodiment the cytotoxic agent is an antagonist of EGFR. In one embodiment the antagonist of EGFR is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (*e.g.*, erlotinib). In one embodiment the cytotoxic agent is a RAF inhibitor. In one embodiment, the RAF inhibitor is a BRAF and/or CRAF inhibitor. In one embodiment the RAF inhibitor is vemurafenib. In one embodiment the cytotoxic agent is a PI3K inhibitor.

[0076] "Chemotherapeutic agent" includes compounds useful in the treatment of cancer. Examples of chemotherapeutic agents include erlotinib (TARCEVA®, Genentech/OSI Pharm.), bortezomib (VELCADE®, Millennium Pharm.), disulfiram, epigallocatechin gallate , salinosporamide A, carfilzomib, 17-AAG (geldanamycin), radicicol, lactate dehydrogenase A (LDH-A), fulvestrant (FASLODEX®, AstraZeneca), sunitib (SUTENT®, Pfizer/Sugen), letrozole (FEMARA®, Novartis), imatinib mesylate (GLEEVEC®, Novartis), finasunate (VATALANIB®, Novartis), oxaliplatin (ELOXATIN®, Sanofi), 5-FU (5-fluorouracil), leucovorin, Rapamycin (Sirolimus, RAPAMUNE®, Wyeth), Lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), Lonafamib (SCH 66336), sorafenib (NEXAVAR®, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), AG1478, alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including topotecan and irinotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); adrenocorticosteroids (including prednisone and prednisolone); cyproterone acetate; 5α-reductases including finasteride and dutasteride); vorinostat, romidepsin, panobinostat, valproic acid, mocetinostat dolastatin; aldesleukin, talc duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin;

nitrogen mustards such as chlorambucil, chlomaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin γ1I and calicheamicin ω1I (*Angew Chem. Intl. Ed. Engl.* **1994** 33:183-186); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® (doxorubicin), morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamnol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, TAXOL (paclitaxel; Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE® (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumberg,

Ill.), and TAXOTERE® (docetaxel, doxetaxel; Sanofi-Aventis); chloranmbucil; GEMZAR® (gemcitabine); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® (vinorelbine); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA®); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0077] Chemotherapeutic agent also includes (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX®; tamoxifen citrate), raloxifene, droloxifene, iodoxyfene , 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® (toremifine citrate); (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® (megestrol acetate), AROMASIN® (exemestane; Pfizer), formestanie, fadrozole, RIVISOR® (vorozole), FEMARA® (letrozole; Novartis), and ARIMIDEX® (anastrozole; AstraZeneca); (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide and goserelin; buserelin, tripterelin, medroxyprogesterone acetate, diethylstilbestrol, premarin, fluoxymesterone, all transretionic acid, fenretinide, as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) protein kinase inhibitors; (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; (vii) ribozymes such as VEGF expression inhibitors (*e.g.*, ANGIOZYME®) and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines, for example, ALLOVECTIN®, LEUVECTIN®, and VAXID®; PROLEUKIN®, rIL-2; a topoisomerase 1 inhibitor such as LURTOTECAN®; ABARELIX® rmRH; and (ix) pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0078] Chemotherapeutic agent also includes antibodies such as alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®, Genentech/Biogen Idec), pertuzumab (OMNITARG®, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar, Corixia), and the antibody drug conjugate, gemtuzumab ozogamicin (MYLOTARG®, Wyeth). Additional humanized monoclonal antibodies with therapeutic potential as agents in combination with the compounds of the invention include: apolizumab,

aselizumab, atlizumab, bapineuzumab, bivatuzumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidtuzumab, daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, siplizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, ustekinumab, visilizumab, and the anti–interleukin-12 (ABT-874/J695, Wyeth Research and Abbott Laboratories) which is a recombinant exclusively human-sequence, full-length IgG_1 λ antibody genetically modified to recognize interleukin-12 p40 protein.

[0079] Chemotherapeutic agent also includes "EGFR inhibitors," which refers to compounds that bind to or otherwise interact directly with EGFR and prevent or reduce its signaling activity, and is alternatively referred to as an "EGFR antagonist." Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, US Patent No. 4,943, 533, Mendelsohn *et al.*) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX®) and reshaped human 225 (H225) *(see*, WO 96/40210, Imclone Systems Inc.); IMC-11F8, a fully human, EGFR-targeted antibody (Imclone); antibodies that bind type II mutant EGFR (US Patent No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in US Patent No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF or Panitumumab (*see* WO98/50433, Abgenix/Amgen); EMD 55900 (Stragliotto *et al. Eur. J. Cancer* 32A:636-640 (1996)); EMD7200 (matuzumab) a humanized EGFR antibody directed against EGFR that competes with both EGF and TGF-alpha for EGFR binding (EMD/Merck); human EGFR antibody, HuMax-EGFR (GenMab); fully human antibodies known as E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6. 3 and E7.6. 3 and described in US 6,235,883; MDX-447 (Medarex Inc); and mAb 806 or humanized mAb 806 (Johns *et al., J. Biol. Chem.* 279(29):30375-30384 (2004)). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, *e.g.*, EP659439A2, Merck Patent GmbH). EGFR antagonists include small molecules such as

compounds described in US Patent Nos: 5,616,582, 5,457,105, 5,475,001, 5,654,307, 5,679,683, 6,084,095, 6,265,410, 6,455,534, 6,521,620, 6,596,726, 6,713,484, 5,770,599, 6,140,332, 5,866,572, 6,399,602, 6,344,459, 6,602,863, 6,391,874, 6,344,455, 5,760,041, 6,002,008, and 5,747,498, as well as the following PCT publications: WO98/14451, WO98/50038, WO99/09016, and WO99/24037. Particular small molecule EGFR antagonists include OSI-774 (CP-358774, erlotinib, TARCEVA® Genentech/OSI Pharmaceuticals); PD 183805 (CI 1033, 2-propenamide, N-[4-[(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-quinazolinyl]-, dihydrochloride, Pfizer Inc.); ZD1839, gefitinib (IRESSA®) 4-(3'-Chloro-4'-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline, AstraZeneca); ZM 105180 ((6-amino-4-(3-methylphenyl-amino)-quinazoline, Zeneca); BIBX-1382 (N8-(3-chloro-4-fluoro-phenyl)-N2-(1-methyl-piperidin-4-yl)-pyrimido[5,4-d]pyrimidine-2,8-diamine, Boehringer Ingelheim); PKI-166 ((R)-4-[4-[(1-phenylethyl)amino]-1H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol); (R)-6-(4-hydroxyphenyl)-4-[(1-phenylethyl)amino]-7H-pyrrolo[2,3-d]pyrimidine); CL-387785 (N-[4-[(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide); EKB-569 (N-[4-[(3-chloro-4-fluorophenyl)amino]-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide) (Wyeth); AG1478 (Pfizer); AG1571 (SU 5271; Pfizer); dual EGFR/HER2 tyrosine kinase inhibitors such as lapatinib (TYKERB®, GSK572016 or N-[3-chloro-4-[(3 fluorophenyl)methoxy]phenyl]-6[5[[[2methylsulfonyl)ethyl]amino]methyl]-2-furanyl]-4-quinazolinamine).

[0080] Chemotherapeutic agents also include "tyrosine kinase inhibitors" including the EGFR-targeted drugs noted in the preceding paragraph; small molecule HER2 tyrosine kinase inhibitor such as TAK165 available from Takeda; CP-724,714, an oral selective inhibitor of the ErbB2 receptor tyrosine kinase (Pfizer and OSI); dual-HER inhibitors such as EKB-569 (available from Wyeth) which preferentially binds EGFR but inhibits both HER2 and EGFR-overexpressing cells; lapatinib (GSK572016; available from Glaxo-SmithKline), an oral HER2 and EGFR tyrosine kinase inhibitor; PKI-166 (available from Novartis); pan-HER inhibitors such as canertinib (CI-1033; Pharmacia); Raf-1 inhibitors such as antisense agent ISIS-5132 available from ISIS Pharmaceuticals which inhibit Raf-1 signaling; non-HER targeted TK inhibitors such as imatinib mesylate (GLEEVEC®, available from Glaxo SmithKline); multi-targeted tyrosine kinase inhibitors such as sunitinib (SUTENT®, available from Pfizer); VEGF receptor tyrosine kinase inhibitors such as vatalanib (PTK787/ZK222584, available from Novartis/Schering AG); MAPK extracellular regulated

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

[0081] Chemotherapeutic agents also include dexamethasone, interferons, colchicine, metoprine, cyclosporine, amphotericin, metronidazole, alemtuzumab, alitretinoin, allopurinol, amifostine, arsenic trioxide, asparaginase, BCG live, bevacuzimab, bexarotene, cladribine, clofarabine, darbepoetin alfa, denileukin, dexrazoxane, epoetin alfa, elotinib, filgrastim, histrelin acetate, ibritumomab, interferon alfa-2a, interferon alfa-2b, lenalidomide, levamisole, mesna, methoxsalen, nandrolone, nelarabine, nofetumomab, oprelvekin, palifermin, pamidronate, pegademase, pegaspargase, pegfilgrastim, pemetrexed disodium, plicamycin, porfimer sodium, quinacrine, rasburicase, sargramostim, temozolomide, VM-26, 6-TG, toremifene, tretinoin, ATRA, valrubicin, zoledronate, and zoledronic acid, and pharmaceutically acceptable salts thereof.

[0082] Chemotherapeutic agents also include hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, triamcinolone acetonide, triamcinolone alcohol, mometasone, amcinonide, budesonide, desonide, fluocinonide, fluocinolone acetonide, betamethasone, betamethasone sodium phosphate, dexamethasone, dexamethasone sodium phosphate, fluocortolone, hydrocortisone-17-butyrate, hydrocortisone-17-valerate, aclometasone dipropionate, betamethasone valerate, betamethasone dipropionate, prednicarbate, clobetasone-17-butyrate, clobetasol-17-propionate, fluocortolone caproate, fluocortolone pivalate and fluprednidene acetate; immune selective anti-inflammatory

peptides (ImSAIDs) such as phenylalanine-glutamine-glycine (FEG) and its D-isomeric form (feG) (IMULAN BioTherapeutics, LLC); anti-rheumatic drugs such as azathioprine, ciclosporin (cyclosporine A), D-penicillamine, gold salts, hydroxychloroquine, leflunomideminocycline, sulfasalazine, tumor necrosis factor alpha (TNFα) blockers such as etanercept (Enbrel), infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia), golimumab (Simponi), Interleukin 1 (IL-1) blockers such as anakinra (Kineret), T cell costimulation blockers such as abatacept (Orencia), Interleukin 6 (IL-6) blockers such as tocilizumab (ACTEMERA®); Interleukin 13 (IL-13) blockers such as lebrikizumab; Interferon alpha (IFN) blockers such as Rontalizumab; Beta 7 integrin blockers such as rhuMAb Beta7; IgE pathway blockers such as Anti-M1 prime; Secreted homotrimeric LTa3 and membrane bound heterotrimer LTa1/β2 blockers such as Anti-lymphotoxin alpha (LTa); radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); miscellaneous investigational agents such as thioplatin, PS-341, phenylbutyrate, ET-18- OCH_3, or farnesyl transferase inhibitors (L-739749, L-744832); polyphenols such as quercetin, resveratrol, piceatannol, epigallocatechine gallate, theaflavins, flavanols, procyanidins, betulinic acid and derivatives thereof; autophagy inhibitors such as chloroquine; delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; acetylcamptothecin, scopolectin, and 9-aminocamptothecin); podophyllotoxin; tegafur (UFTORAL®); bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine; perifosine, COX-2 inhibitor (*e.g.* celecoxib or etoricoxib), proteosome inhibitor (*e.g.* PS341); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASAR™); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

[0083] Chemotherapeutic agents also include non-steroidal anti-inflammatory drugswith analgesic, antipyretic and anti-inflammatory effects. NSAIDs include non-selective inhibitors

of the enzyme cyclooxygenase. Specific examples of NSAIDs include aspirin, propionic acid derivatives such as ibuprofen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin and naproxen, acetic acid derivatives such as indomethacin, sulindac, etodolac, diclofenac, enolic acid derivatives such as piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam and isoxicam, fenamic acid derivatives such as mefenamic acid, meclofenamic acid, flufenamic acid, tolfenamic acid, and COX-2 inhibitors such as celecoxib, etoricoxib, lumiracoxib, parecoxib, rofecoxib, rofecoxib, and valdecoxib. NSAIDs can be indicated for the symptomatic relief of conditions such as rheumatoid arthritis, osteoarthritis, inflammatory arthropathies, ankylosing spondylitis, psoriatic arthritis, Reiter's syndrome, acute gout, dysmenorrhoea, metastatic bone pain, headache and migraine, postoperative pain, mild-to-moderate pain due to inflammation and tissue injury, pyrexia, ileus, and renal colic.

[0084] A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell either in vitro or in vivo. In one embodiment, growth inhibitory agent is growth inhibitory antibody that prevents or reduces proliferation of a cell expressing an antigen to which the antibody binds. In another embodiment, the growth inhibitory agent may be one which significantly reduces the percentage of cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., The Molecular Basis of Cancer, Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (W.B. Saunders, Philadelphia, 1995), e.g., p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0085] By "radiation therapy" is meant the use of directed gamma rays or beta rays to induce sufficient damage to a cell so as to limit its ability to function normally or to destroy the cell altogether. It will be appreciated that there will be many ways known in the art to

determine the dosage and duration of treatment. Typical treatments are given as a one-time administration and typical dosages range from 10 to 200 units (Grays) per day.

[0086] A "subject" or an "individual" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

[0087] The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

[0088] An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, an antibody is purified (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of, for example, a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using, for example, Coomassie blue or silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0089] "Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0090] The term "constant domain" refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The constant domain contains the C_H1, C_H2 and C_H3 domains (collectively, CH) of the heavy chain and the CHL (or CL) domain of the light chain.

[0091] The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain may be referred to as "V_H." The variable domain of the light chain may be referred to as "V_L." These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

[0092] The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[0093] The "light chains" of antibodies (immunoglobulins) from any mammalian species can be assigned to one of two clearly distinct types, called kappa ("κ") and lambda ("λ"), based on the amino acid sequences of their constant domains.

[0094] The term IgG "isotype" or "subclass" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions.

[0095] Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be

further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, γ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al. *Cellular and Mol. Immunology*, 4th ed. (W.B. Saunders, Co., 2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

[0096] The terms "full length antibody," "intact antibody" and "whole antibody" are used herein interchangeably to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain an Fc region.

[0097] A "naked antibody" for the purposes herein is an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

[0098] "Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. In some embodiments, the antibody fragment described herein is an antigen-binding fragment. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0099] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0100] "Fv" is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three HVRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0101] The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0102] "Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see, *e.g.*, Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York, 1994), pp. 269-315.

[0103] The term "diabodies" refers to antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

[0104] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, e.g., the individual antibodies comprising the population are identical except for possible mutations, e.g., naturally occurring mutations, that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected

target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

[0105] The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the invention may be made by a variety of techniques, including, for example, the hybridoma method (e.g., Kohler and Milstein, *Nature*, 256:495-97 (1975); Hongo *et al.*, *Hybridoma*, 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567), phage-display technologies (see, e.g., Clackson *et al.*, *Nature*, 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggemann *et al.*, *Year in Immunol.* 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg et al., *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

[0106] The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to

corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see, *e.g.*, U.S. Pat. No. 4,816,567; and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)). Chimeric antibodies include PRIMATTZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with the antigen of interest.

[0107] "Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a HVR of the recipient are replaced by residues from a HVR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, *e.g.*, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409.

[0108] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991);

Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.*, 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0109] A "species-dependent antibody" is one which has a stronger binding affinity for an antigen from a first mammalian species than it has for a homologue of that antigen from a second mammalian species. Normally, the species-dependent antibody "binds specifically" to a human antigen (e.g., has a binding affinity (Kd) value of no more than about $1x10^{-7}$ M, preferably no more than about $1x10^{-8}$ M and preferably no more than about $1x10^{-9}$ M) but has a binding affinity for a homologue of the antigen from a second nonhuman mammalian species which is at least about 50 fold, or at least about 500 fold, or at least about 1000 fold, weaker than its binding affinity for the human antigen. The species-dependent antibody can be any of the various types of antibodies as defined above, but preferably is a humanized or human antibody.

[0110] The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, *e.g.*, Xu et al., *Immunity* 13:37-45 (2000); Johnson and Wu, in *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, N.J., 2003). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, *e.g.*, Hamers-Casterman et al., *Nature* 363:446-448 (1993); Sheriff et al., *Nature Struct. Biol.* 3:733-736 (1996).

[0111] A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers

instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

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

[0112] HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat et al., *supra*, for each of these definitions.

[0113] "Framework" or "FR" residues are those variable domain residues other than the HVR residues as herein defined.

[0114] The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., *supra*. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0115] The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g., Kabat *et al.*, *Sequences of Immunological Interest*. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat *et al.*, *supra*). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

[0116] The expression "linear antibodies" refers to the antibodies described in Zapata et al. (1995 *Protein Eng*, 8(10):1057-1062). Briefly, these antibodies comprise a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[0117] As use herein, the term "binds", "specifically binds to" or is "specific for" refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody that binds to or specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, *e.g.,* by a radioimmunoassay (RIA). In certain embodiments, an antibody that specifically binds to a target has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, or ≤ 0.1 nM. In certain embodiments, an antibody specifically binds to an epitope on a protein that is conserved among the protein from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

II. PD-1 Axis Binding Antagonists

[0118] Provided herein is a method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an anti-HER2 antibody. Also provided herein is a method of enhancing immune function in an individual having cancer comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an anti-HER2 antibody. For example, a PD-1 axis binding antagonist includes a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. PD-1 (programmed death 1) is also referred to in the art as "programmed cell death 1", PDCD1, CD279 and SLEB2. PD-L1 (programmed death ligand 1) is also referred to in the art as "programmed cell death 1 ligand 1", PDCD1LG1, CD274, B7-H, and PDL1. PD-L2 (programmed death ligand 2) is also referred to in the art as "programmed cell death 1 ligand 2", PDCD1LG2, CD273, B7-DC, Btdc, and PDL2. In some embodiments, PD-1, PD-L1, and PD-L2 are human PD-1, PD-L1 and PD-L2.

[0119] In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect the PD-1 ligand binding

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

[0120] In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody (e.g., a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of MDX-1106 (nivolumab), MK-3475 (lambrolizumab), and CT-011 (pidilizumab). In some embodiments, the PD-1 binding antagonist is an immunoadhesin (e.g., an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (e.g., an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 binding antagonist is AMP-224. In some embodiments, the PD-L1 binding antagonist is anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 binding antagonist is selected from the group consisting of YW243.55.S70, MPDL3280A, MDX-1105, and MEDI4736. Antibody YW243.55.S70 is an anti-PD-L1 described in WO 2010/077634. MDX-1105, also known as BMS-936559, is an anti-PD-L1 antibody described in WO2007/005874. MEDI4736, is an anti-PD-L1 monoclonal antibody described in WO2011/066389 and US2013/034559. Nivolumab, also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO®, is an anti-PD-1 antibody described in WO2006/121168. Pembrolizumab, also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA®, and SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. CT-011, also known as hBAT, hBAT-1 or pidilizumab, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PD-L2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

[0121] In some embodiments, the PD-1 axis binding antagonist is an anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 antibody is capable of inhibiting binding between PD-L1 and PD-1 and/or between PD-L1 and B7-1. In some embodiments, the anti-PD-L1 antibody is a monoclonal antibody. In some embodiments, the anti-PD-L1 antibody is an antibody fragment selected from the group consisting of Fab, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments. In some embodiments, the anti-PD-L1 antibody is a humanized antibody. In some embodiments, the anti-PD-L1 antibody is a human antibody.

[0122] Examples of anti-PD-L1 antibodies useful for the methods of this invention, and methods for making thereof are described in PCT patent application WO 2010/077634,

WO2007/005874, WO2011/066389, and US2013/034559, which are incorporated herein by reference. The anti-PD-L1 antibodies useful in this invention, including compositions containing such antibodies, may be used in combination with an anti-HER2 antibody to treat cancer.

Anti-PD1 antibodies

[0123] In some embodiments, the anti-PD-1 antibody is MDX-1106. Alternative names for "MDX-1106" include MDX-1106-04, ONO-4538, BMS-936558 or Nivolumab. In some embodiments, the anti-PD-1 antibody is Nivolumab (CAS Registry Number: 946414-94-4). In a still further embodiment, provided is an isolated anti-PD-1 antibody comprising a heavy chain variable region comprising the heavy chain variable region amino acid sequence from SEQ ID NO:1 and/or a light chain variable region comprising the light chain variable region amino acid sequence from SEQ ID NO:2. In a still further embodiment, provided is an isolated anti-PD-1 antibody comprising a heavy chain and/or a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the heavy chain sequence:
QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDG
SKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVT
VSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPE
FLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT
KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP
QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:1), and

(b) the light chain sequences has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the light chain sequence:
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGI
PARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:2).

Anti-PDL1 antibodies

[0124] In some embodiments, the antibody in the formulation comprises at least one tryptophan (e.g., at least two, at least three, or at least four) in the heavy and/or light chain sequence. In some embodiments, amino acid tryptophan is in the CDR regions, framework regions and/or constant regions of the antibody. In some embodiments, the antibody comprises two or three tryptophan residues in the CDR regions. In some embodiments, the antibody in the formulation is an anti-PDL1 antibody. PD-L1 (programmed death ligand 1), also known as PDL1, B7-H1, B7-4, CD274, and B7-H, is a transmembrane protein, and its interaction with PD-1 inhibits T-cell activation and cytokine production. In some embodiments, the anti-PDL1 antibody described herein binds to human PD-L1. Examples of anti-PDL1 antibodies that can be used in the methods described herein are described in PCT patent application WO 2010/077634 A1 and US 8,217,149, which are incorporated herein by reference.

[0125] In some embodiments, the anti-PDL1 antibody is capable of inhibiting binding between PD-L1 and PD-1 and/or between PD-L1 and B7-1. In some embodiments, the anti-PDL1 antibody is a monoclonal antibody. In some embodiments, the anti-PDL1 antibody is an antibody fragment selected from the group consisting of Fab, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments. In some embodiments, the anti-PDL1 antibody is a humanized antibody. In some embodiments, the anti-PDL1 antibody is a human antibody.

[0126] Anti-PDL1 antibodies described in WO 2010/077634 A1 and US 8,217,149 may be used in the methods described herein. In some embodiments, the anti-PDL1 antibody comprises a heavy chain variable region sequence of SEQ ID NO:3 and/or a light chain variable region sequence of SEQ ID NO:4. In a still further embodiment, provided is an isolated anti-PDL1 antibody comprising a heavy chain and/or a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the heavy chain sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG TLVTVSA (SEQ ID NO:3), and

(b) the light chain sequences has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO:4).

[0127] In one embodiment, the anti-PDL1 antibody comprises a heavy chain variable region polypeptide comprising an HVR-H1, HVR-H2 and HVR-H3 sequence, wherein:

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

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

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

further wherein: X_1 is D or G; X_2 is S or L; X_3 is T or S. In one specific aspect, X_1 is D; X_2 is S and X_3 is T.

[0128] In another aspect, the polypeptide further comprises variable region heavy chain framework sequences juxtaposed between the HVRs according to the formula: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a further aspect, the framework sequences are VH subgroup III consensus framework. In a still further aspect, at least one of the framework sequences is the following:

HC-FR1 is EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO:8)

HC-FR2 is WVRQAPGKGLEWV (SEQ ID NO:9)

HC-FR3 is RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR (SEQ ID NO:10)

HC-FR4 is WGQGTLVTVSA (SEQ ID NO:11).

[0129] In a still further aspect, the heavy chain polypeptide is further combined with a variable region light chain comprising an HVR-L1, HVR-L2 and HVR-L3, wherein:

(a) the HVR-L1 sequence is RASQ$X_4X_5X_6TX_7X_8$A (SEQ ID NO:12);

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

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

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

[0130] In a still further aspect, the light chain further comprises variable region light chain framework sequences juxtaposed between the HVRs according to the formula: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still

further aspect, the framework sequences are VL kappa I consensus framework. In a still further aspect, at least one of the framework sequence is the following:

LC-FR1 is DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO:15)

LC-FR2 is WYQQKPGKAPKLLIY (SEQ ID NO:16)

LC-FR3 is GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO:17)

LC-FR4 is FGQGTKVEIKR (SEQ ID NO:18).

[0131] In another embodiment, provided is an isolated anti-PDL1 antibody or antigen binding fragment comprising a heavy chain and a light chain variable region sequence, wherein:

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

(i) the HVR-H1 sequence is GFTFSX$_1$SWIH; (SEQ ID NO:5)

(ii) the HVR-H2 sequence is AWIX$_2$PYGGSX$_3$YYADSVKG (SEQ ID NO:6)

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

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

(i) the HVR-L1 sequence is RASQX$_4$X$_5$X$_6$TX$_7$X$_8$A (SEQ ID NO:12)

(ii) the HVR-L2 sequence is SASX$_9$LX$_{10}$S; and (SEQ ID NO:13)

(iii) the HVR-L3 sequence is QQX$_{11}$X$_{12}$X$_{13}$X$_{14}$PX$_{15}$T; (SEQ ID NO:14)

wherein: X$_1$ is D or G; X$_2$ is S or L; X$_3$ is T or S; X$_4$ is D or V; X$_5$ is V or I; X$_6$ is S or N; X$_7$ is A or F; X$_8$ is V or L; X$_9$ is F or T; X$_{10}$ is Y or A; X$_{11}$ is Y, G, F, or S; X$_{12}$ is L, Y, F or W; X$_{13}$ is Y, N, A, T, G, F or I; X$_{14}$ is H, V, P, T or I; X$_{15}$ is A, W, R, P or T. In a specific aspect, X$_1$ is D; X$_2$ is S and X$_3$ is T. In another aspect, X$_4$ is D; X$_5$ is V; X$_6$ is S; X$_7$ is A; X$_8$ is V; X$_9$ is F; X$_{10}$ is Y; X$_{11}$ is Y; X$_{12}$ is L; X$_{13}$ is Y; X$_{14}$ is H; X$_{15}$ is A. In yet another aspect, X$_1$ is D; X$_2$ is S and X$_3$ is T, X$_4$ is D; X$_5$ is V; X$_6$ is S; X$_7$ is A; X$_8$ is V; X$_9$ is F; X$_{10}$ is Y; X$_{11}$ is Y; X$_{12}$ is L; X$_{13}$ is Y; X$_{14}$ is H and X$_{15}$ is A.

[0132] In a further aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-

(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and 11. In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

[0133] In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region if IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0134] In yet another embodiment, provided is an anti-PDL1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

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

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

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

[0135] In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-

(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and 11. In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

[0136] In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region if IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0137] In another further embodiment, provided is an isolated anti-PDL1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

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

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG TLVTVSS (SEQ ID NO:25), and/or

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

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO:4).

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%. In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and WGQGTLVTVSS (SEQ ID NO:27).

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

[0138] In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region if IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect, the minimal effector function results from production in prokaryotic cells. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0139] In a further aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II,

or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences is the following:

HC-FR1	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	(SEQ ID NO:29)
HC-FR2	WVRQAPGKGLEWVA	(SEQ ID NO:30)
HC-FR3	RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR	(SEQ ID NO:10)
HC-FR4	WGQGTLVTVSS	(SEQ ID NO:27).

[0140] In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences is the following:

LC-FR1	DIQMTQSPSSLSASVGDRVTITC	(SEQ ID NO:15)
LC-FR2	WYQQKPGKAPKLLIY	(SEQ ID NO:16)
LC-FR3	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	(SEQ ID NO:17)
LC-FR4	FGQGTKVEIK	(SEQ ID NO:28).

[0141] In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region if IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0142] In yet another embodiment, provided is an anti-PDL1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

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

(d) the light chain further comprises an HVR-L1, HVR-L2 and an HVR-L3 sequence having at least 85% sequence identity to RASQDVSTAVA (SEQ ID

NO:22), SASFLYS (SEQ ID NO:23) and QQYLYHPAT (SEQ ID NO:24), respectively.

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

[0143] In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and WGQGTLVTVSSASTK (SEQ ID NO:31).

[0144] In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18. In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region if IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0145] In a still further embodiment, provided is an isolated anti-PDL1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

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

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG

STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG TLVTVSSASTK (SEQ ID NO:26), or

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

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO:4).

[0146] In some embodiments, provided is an isolated anti-PDL1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:4. In some embodiments, provided is an isolated anti-PDL1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:26. In some embodiments, provided is an isolated anti-PDL1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:4 and the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:26. In some embodiments, one, two, three, four or five amino acid residues at the N-terminal of the heavy and/or light chain may be deleted, substituted or modified.

[0147] In a still further embodiment, provided is an isolated anti-PDL1 antibody comprising a heavy chain and a light chain sequence, wherein:

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

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG

TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID
NO:32), and/or

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

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:33)

In a still further embodiment, provided is an isolated anti-PDL1 antibody comprising a heavy chain and a light chain sequence, wherein:

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

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG
STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ
ID NO:54), and/or

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

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:33).

[0148] In some embodiments, provided is an isolated anti-PDL1 antibody comprising a heavy chain and a light chain sequence, wherein the light chain sequence has at least 85%, at

least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:33. In some embodiments, provided is an isolated anti-PDL1 antibody comprising a heavy chain and a light chain sequence, wherein the heavy chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:32 or 54. In some embodiments, provided is an isolated anti-PDL1 antibody comprising a heavy chain and a light chain sequence, wherein the light chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:33 and the heavy chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:32 or 54.

[0149] In some embodiments, the isolated anti-PDL1 antibody is aglycosylated. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. Removal of glycosylation sites form an antibody is conveniently accomplished by altering the amino acid sequence such that one of the above-described tripeptide sequences (for N-linked glycosylation sites) is removed. The alteration may be made by substitution of an asparagine, serine or threonine residue within the glycosylation site another amino acid residue (e.g., glycine, alanine or a conservative substitution).

[0150] In any of the embodiments herein, the isolated anti-PDL1 antibody can bind to a human PD-L1, for example a human PD-L1 as shown in UniProtKB/Swiss-Prot Accession No.Q9NZQ7.1, or a variant thereof.

[0151] In a still further embodiment, provided is an isolated nucleic acid encoding any of the antibodies described herein. In some embodiments, the nucleic acid further comprises a vector suitable for expression of the nucleic acid encoding any of the previously described anti-PDL1 antibodies. In a still further specific aspect, the vector is in a host cell suitable for expression of the nucleic acid. In a still further specific aspect, the host cell is a eukaryotic cell or a prokaryotic cell. In a still further specific aspect, the eukaryotic cell is a mammalian cell, such as Chinese hamster ovary (CHO) cell.

[0152] The antibody or antigen binding fragment thereof, may be made using methods known in the art, for example, by a process comprising culturing a host cell containing nucleic acid encoding any of the previously described anti-PDL1 antibodies or antigen-binding fragment in a form suitable for expression, under conditions suitable to produce such antibody or fragment, and recovering the antibody or fragment.

III. Anti-HER2 Antibodies

[0153] Provided herein is a method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an anti-HER2 antibody. Also provided herein is a method of enhancing immune function in an individual having cancer comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an anti-HER2 antibody.

[0154] Provided herein are antibodies that bind to a human epidermal growth factor receptor 2 (HER2). Alternative names for "HER2" include ERBB2, Neu, CD340, and p185. The term "HER2" as used herein, refers to any native HER2 from any human source. The term encompasses "full-length" and unprocessed HER2 as well as any form of HER2 that results from processing in the cell (e.g., mature protein). The term also encompasses naturally occurring variants and isoforms of HER2, e.g., splice variants or allelic variants. For example, descriptions of HER2 and sequences are provided at www.uniprot.org/uniprot/P04626.

[0155] In some embodiments, the anti-HER antibody binds to HER2 and inhibits cell proliferation or growth of HER2+ cancer cells. In some embodiments, the anti-HER2 antibody binds to HER2 and inhibits dimerization of HER2 with other HER receptors. In some embodiments, the anti-HER2 antibody is trastuzumab or pertuzumab.

In some embodiments, the antigen binding domain of an antibody that binds to a HER2 comprises a heavy chain variable region (V_HHER2) comprising the amino acid sequence: EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG

YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW GQGTLVTVSS (SEQ ID NO:34), and /or

a light chain variable region (V_LHER2) comprising the amino acid sequence: DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK (SEQ ID NO:35).

In some embodiments, the heavy chain of the anti-HER2 antibody comprises the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:36), and/or the light chain of the anti-HER2 antibody comprises the amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:37).

In some embodiments, the anti-HER2 antibody comprises a heavy chain variable region comprising an HVR-H1 sequence of DTYIH (SEQ ID NO:38), an HVR-H2 sequence of RIYPTNGYTRYADSVKG (SEQ ID NO:50), and an HVR-H3 sequence of WGGDGFYAMDY (SEQ ID NO:40) and a light chain variable region comprising an HVR-L1 sequence of RASQDVNTAVA (SEQ ID NO:41), an HVR-L2 sequence of SASFLYS (SEQ ID NO:42), and an HVR-L3 sequence of QQHYTTPPT (SEQ ID NO:43).

Bispecific Antibodies

[0156] In some embodiments, the anti-HER2 is a multispecific antibody or a bispecific antibody. In some embodiments, the bispecific antibody comprises a first antigen binding domain that binds a HER2, and a second antigen binding domain that binds to a human CD3 polypeptide.

[0157] CD3 (cluster of differentiation 3) T-cell co-receptor is a protein complex and is composed of four distinct chains. In mammals, the complex contains a CD3γ chain, a CD3δ chain, and two CD3ε chains. These chains associate with the T-cell receptor (TCR) and the ζ-chain to generate an activation signal in T lymphocytes. The TCR, ζ-chain, and CD3 molecules together form the TCR complex. The term "CD3" as used herein, refers to any native CD3 from any human source. The term encompasses "full-length" and unprocessed protein as well as any form of the protein or one or more of the CD3 chains (polypeptides) that result from processing in the cell (e.g., mature polypeptides). The term also encompasses naturally occurring variants and isoforms of CD3, e.g., splice variants or allelic variants. For example, descriptions of CD3γ chain, CD3δ chain, and CD3ε chains and sequences are provided at www.uniprot.org/uniprot/P04234, www.uniprot.org/uniprot/P07766, and www.uniprot.org/uniprot/P09693.

[0158] In some embodiments, the bispecific antibody binds to a human CD3 epsilon (CD3ε) polypeptide. In some embodiments, the bispecific antibody binds to a human CD3 epsilon polypeptide in native T-cell receptor (TCR) complex in association with other TCR subunits. In some embodiments, the bispecific antibody binds to a human CD3 gamma (CD3γ) polypeptide. In some embodiments, the bispecific antibody binds a human CD3 gamma polypeptide in native T-cell receptor (TCR) complex in association with other TCR subunits.

[0159] In one aspect, assays are provided for identifying anti-CD3 antibodies thereof having biological activity. Biological activity may include, for example, binding to a CD3 polypeptide (e.g., CD3 on the surface of a T cell), or a peptide fragment thereof, either *in vivo*, *in vitro*, or *ex vivo*. In the case of a multispecific (*e.g.*, bispecific) anti-CD3 antibody of the invention (*e.g.*, a TDB antibody having one anti-HER2 arm and another arm that recognizes a CD3 polypeptide), biological activity may also include, for example, effector cell activation (e.g., T cell (e.g., CD8+ and/or CD4+ T cell) activation), effector cell population expansion (i.e., an increase in T cell count), target cell population reduction (i.e., a decrease in the population of cells expressing HER2 on their cell surfaces), and/or target cell killing. Antibodies having such biological activity *in vivo* and/or *in vitro* are provided. In certain embodiments, an antibody of the invention is tested for such biological activity.

[0160] In some embodiments, the antigen binding domain of a bispecific antibody that binds to a HER2 comprises a heavy chain variable region (V_HHER2) comprising the amino acid sequence of SEQ ID NO:34, and a light chain variable region (V_LHER2) comprising the amino acid sequence of SEQ ID NO:35. In some embodiments, the antigen binding domain

that binds to a HER2 comprises a heavy chain variable region comprising an HVR-H1 sequence of DTYIH (SEQ ID NO:38), an HVR-H2 sequence of RIYPTNGYTRYASDVKG (SEQ ID NO:39), and an HVR-H3 sequence of WGGDGFYAMDY (SEQ ID NO:40) and comprises a light chain comprising an HVR-L1 sequence of RASQDVNTAVA (SEQ ID NO:41), an HVR-L2 sequence of SASFLYS (SEQ ID NO:42), and an HVR-L3 sequence of QQHYTTPPT (SEQ ID NO:43). In some embodiments, the antigen binding domain that binds to a HER2 comprises a heavy chain variable region comprising an HVR-H1 sequence of DTYIH (SEQ ID NO:38), an HVR-H2 sequence of RIYPTNGYTRYADSVKG (SEQ ID NO:50), and an HVR-H3 sequence of WGGDGFYAMDY (SEQ ID NO:40) and a light chain variable region comprising an HVR-L1 sequence of RASQDVNTAVA (SEQ ID NO:41), an HVR-L2 sequence of SASFLYS (SEQ ID NO:42), and an HVR-L3 sequence of QQHYTTPPT (SEQ ID NO:43). In some embodiments, the antigen binding domain that binds to HER2 comprises a heavy chain variable region comprising an HVR-H1 sequence of DTYIH (SEQ ID NO:38), an HVR-H2 sequence of RIYPTNGYTRYDPKFQD (SEQ ID NO:51), and an HVR-H3 sequence of WGGDGFYAMDY (SEQ ID NO:40) and comprises a light chain variable region comprising an HVR-L1 sequence of KASQDVNTAVA (SEQ ID NO:52), an HVR-L2 sequence of SASFRYT (SEQ ID NO:53), and an HVR-L3 sequence of QQHYTTPPT (SEQ ID NO:43).

[0161] In some embodiments, the antigen binding domain of a bispecific antibody that binds to a CD3 comprises a heavy chain variable region (V_HCD3) amino acid sequences and a light chain variable region (V_LCD3) amino acid sequences as described in Zhu et al., *Int J Cancer* 62:319-24, 1995 and Rodrigues et al., *Int J Cancer Suppl* 7:45-50, 1992. In some embodiments, the antigen binding domain that binds to a CD3 comprises a heavy chain variable region comprising an HVR-H1 sequence of GYTMN (SEQ ID NO:44), an HVR-H2 sequence of LINPYKGVSTYNQKFKD (SEQ ID NO:45), and an HVR-H3 sequence of SGYYGDSDWYFDV (SEQ ID NO:46) and comprises a light chain comprising an HVR-L1 sequence of RASQDIRNYLN (SEQ ID NO:47), an HVR-L2 sequence of YTSRLES (SEQ ID NO:48), and an HVR-L3 sequence of QQGNTLPWT (SEQ ID NO:49). See CDR sequences of antibody huxCD3v9 in Rodrigues et al., *Int. J. Cancer:* Supplement 7, 45-50, 1992. In some embodiments, the antigen binding domain that binds to a human CD3 polypeptide comprises the VH and VL sequences described in WO2004/106381, WO2005/061547, WO2007/042261, WO2008/119567, and Rodrigues et al., *Int J Cancer Suppl* 7:45-50, 1992.

[0162] In some embodiments, the first antigen binding domain of the bispecific antibody comprises one or more heavy chain constant domains, wherein the one or more heavy chain constant domains are selected from a first CH1 ($CH1_1$) domain, a first CH2 ($CH2_1$) domain, a first CH3 ($CH3_1$) domain; and the second antigen binding domain of the bispecific antibody comprises one or more heavy chain constant domains, wherein the one or more heavy chain constant domains are selected from a second CH1 ($CH1_2$) domain, second CH2 ($CH2_2$) domain, and a second CH3 ($CH3_2$) domain. In some embodiments, at least one of the one or more heavy chain constant domains of the first antigen binding domain is paired with another heavy chain constant domain of the second antigen binding domain. In some embodiments, the $CH3_1$ and $CH3_2$ domains each comprise a protuberance or cavity, and wherein the protuberance or cavity in the $CH3_1$ domain is positionable in the cavity or protuberance, respectively, in the $CH3_2$ domain. In some embodiments, the $CH3_1$ and $CH3_2$ domains meet at an interface between said protuberance and cavity. Examplary sets of amino acid substitutions in $CH3_1$ and $CH3_2$ domains are shown in Table 2 herein. In some embodiments, the $CH2_1$ and $CH2_2$ domains each comprise a protuberance or cavity, and wherein the protuberance or cavity in the $CH2_1$ domain is positionable in the cavity or protuberance, respectively, in the $CH2_2$ domain. In some embodiments, the $CH2_1$ and $CH2_2$ domains meet at an interface between said protuberance and cavity. In some embodiments, the $CH3_1$ and/or $CH3_2$ domain of an IgG contain one or more amino acid substitutions at residues selected from the group consisting of 347, 349, 350, 351, 366, 368, 370, 392, 394, 395, 398, 399, 405, 407, and 409 according to the amino acid numbering as shown in FIG. 5 of the U.S. Pat. No. 8,216,805. In some embodiments, the protuberance comprises one or more introduced residues selected from the group consisting of arginine (R) residue, phenylalanine (F) residue, tyrosine (Y) residue, and tryptophan (W) residue. In some embodiments, the cavity comprises one or more introduced residues selected from the group consisting of alanine (A) residue, serine (S) residue, threonine (T) residue, and valine (V) residue. In some embodiments, the CH3 and/or CH2 domains are from an IgG (e.g., IgG1 subtype, IgG2 subtype, IgG2A subtype, IgG2B subtype, IgG3, subtype, or IgG4 subtype). In some embodiments, one CH3 domain of the bispecific antibody comprises amino acid substitution T366Y, and the other CH3 domain comprises amino acid substitution Y407T. In some embodiments, one CH3 domain comprises amino acid substitution T366W, and the other CH3 domain comprises amino acid substitution Y407A. In some embodiments, one CH3 domain comprises amino acid substitution F405A, and the other CH3 domain comprises amino acid substitution T394W. In some embodiments, one CH3 domain comprises amino

acid substitutions T366Y and F405A, and the other CH3 domain comprises amino acid substitutions T394W and Y407T. In some embodiments, one CH3 domain comprises amino acid substitutions T366W and F405W, and the other CH3 domain comprises amino acid substitutions T394S and Y407A. In some embodiments, one CH3 domain comprises amino acid substitutions F405W and Y407A, and the other CH3 domain comprises amino acid substitutions T366W and T394S. In some embodiments, one CH3 domain comprises amino acid substitution F405W, and the other CH3 domain comprises amino acid substitution T394S. The mutations are denoted by the original residue, followed by the position using the Kabat numbering system, and then the import residues. See also numbering in FIG. 5 of U.S. Pat. No. 8,216,805.

[0163] In some embodiments, the bispecific antibody having a heavy chain Fc region comprises an aglycosylation site mutation. In some embodiments, the aglycosylation site mutation is a substitution mutation. In some embodiments, the substitution mutation is at amino acid residue N297, L234, L235, and/or D265 (EU numbering). In some embodiments, the substitution mutation is selected from the group consisting of N297G, N297A, L234A, L235A, and D265A. In some embodiments, the substitution mutation is a D265A mutation and an N297G mutation.. In some embodiments, the aglycosylation site mutation reduces effector function of the anti-HER2 antibody.

[0164] In some embodiments, the bispecific antibody is a single-chain bispecific antibody comprising the first antigen binding domain and the second antigen binding domain. In some embodiments, the single-chain bispecific antibody comprises variable regions, as arranged from N-terminus to C-terminus, selected from the group consisting of (1) V_HHER2-V_LHER2-V_HCD3-V_LCD3, (2) V_HCD3-V_LCD3-V_HHER2-V_LHER2, (3) V_HCD3-V_LCD3-V_LHER2-V_HHER2, (4) V_HHER2-V_LHER2-V_LCD3-V_HCD3, (5) V_LHER2-V_HHER2-V_HCD3-V_LCD3, and (6) V_LCD3-V_HCD3-V_HHER2-V_LHER2.

IV. Antibody Preparation

[0165] The antibody described herein is prepared using techniques available in the art for generating antibodies, exemplary methods of which are described in more detail in the following sections.

[0166] The antibody is directed against an antigen of interest (*i.e.*, PD-L1 (such as a human PD-L1), HER2, or CD3 (such as a human CD3)). Preferably, the antigen is a biologically important polypeptide and administration of the antibody to a mammal suffering from a disorder can result in a therapeutic benefit in that mammal.

[0167] In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of ≤ 1μM, ≤ 150 nM, ≤ 100 nM, ≤ 50 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, *e.g.,* from 10^{-9} M to 10^{-13} M).

[0168] In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, *e.g.,* Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest. The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.,* about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.,* for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20 ™; Packard) is added, and the plates are counted on a TOPCOUNT ™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0169] According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE ®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N*'- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of

approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, *e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

(i) Antigen Preparation

[0170] Soluble antigens or fragments thereof, optionally conjugated to other molecules, can be used as immunogens for generating antibodies. For transmembrane molecules, such as receptors, fragments of these (e.g. the extracellular domain of a receptor) can be used as the immunogen. Alternatively, cells expressing the transmembrane molecule can be used as the immunogen. Such cells can be derived from a natural source (e.g. cancer cell lines) or may be cells which have been transformed by recombinant techniques to express the transmembrane molecule. Other antigens and forms thereof useful for preparing antibodies will be apparent to those in the art.

(ii) Certain Antibody-Based Methods

[0171] Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N=C=NR$, where R and R^1 are different alkyl groups.

[0172] Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 µg or 5 µg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution

intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

[0173] Monoclonal antibodies of the invention can be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), and further described, e.g., in Hongo *et al.*, *Hybridoma*, 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981), and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) regarding human-human hybridomas. Additional methods include those described, for example, in U.S. Pat. No. 7,189,826 regarding production of monoclonal human natural IgM antibodies from hybridoma cell lines. Human hybridoma technology (Trioma technology) is described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0174] For various other hybridoma techniques, see, *e.g.*, US 2006/258841; US 2006/183887 (fully human antibodies), US 2006/059575; US 2005/287149; US 2005/100546; US 2005/026229; and U.S. Pat. Nos. 7,078,492 and 7,153,507. An exemplary protocol for producing monoclonal antibodies using the hybridoma method is described as follows. In one embodiment, a mouse or other appropriate host animal, such as a hamster, is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Antibodies are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of a polypeptide of the invention or a fragment thereof, and an adjuvant, such as monophosphoryl lipid A (MPL)/trehalose dicrynomycolate (TDM) (Ribi Immunochem. Research, Inc., Hamilton, Mont.). A polypeptide of the invention (e.g., antigen) or a fragment thereof may be prepared using methods well known in the art, such as recombinant methods, some of which are further described herein. Serum from immunized animals is assayed for anti-antigen antibodies, and booster immunizations are optionally administered. Lymphocytes from animals producing anti-antigen antibodies are isolated. Alternatively, lymphocytes may be immunized *in vitro*.

[0175] Lymphocytes are then fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. See, *e.g.*, Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986). Myeloma cells may be used that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Exemplary myeloma cells include, but are not limited to, murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0176] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium, e.g., a medium that contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells. Preferably, serum-free hybridoma cell culture methods are used to reduce use of animal-derived serum such as fetal bovine serum, as described, for example, in Even *et al.*, *Trends in Biotechnology*, 24(3), 105-108 (2006).

[0177] Oligopeptides as tools for improving productivity of hybridoma cell cultures are described in Franek, *Trends in Monoclonal Antibody Research*, 111-122 (2005). Specifically, standard culture media are enriched with certain amino acids (alanine, serine, asparagine, proline), or with protein hydrolyzate fractions, and apoptosis may be significantly suppressed by synthetic oligopeptides, constituted of three to six amino acid residues. The peptides are present at millimolar or higher concentrations.

[0178] Culture medium in which hybridoma cells are growing may be assayed for production of monoclonal antibodies that bind to an antibody of the invention. The binding specificity of monoclonal antibodies produced by hybridoma cells may be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoadsorbent assay (ELISA). The binding affinity of the monoclonal antibody can be determined, for example, by Scatchard analysis. See, *e.g.*, Munson *et al.*, *Anal. Biochem.*, 107:220 (1980).

[0179] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods. See, *e.g.*, Goding, *supra.* Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, hybridoma cells may be grown *in vivo* as ascites tumors in an animal. Monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography. One procedure for isolation of proteins from hybridoma cells is described in US 2005/176122 and U.S. Pat. No. 6,919,436. The method includes using minimal salts, such as lyotropic salts, in the binding process and preferably also using small amounts of organic solvents in the elution process.

(iii) Library-Derived Antibodies

[0180] Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics such as the methods described in Example 3. Additional methods are reviewed, *e.g.,* in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.,* in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0181] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.,* from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive

libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0182] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

(iv) Chimeric, Humanized and Human Antibodies

[0183] In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, *e.g.,* in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (*e.g.,* a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0184] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, *e.g.,* CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.,* the antibody from which the HVR residues are derived), *e.g.,* to restore or improve antibody specificity or affinity.

[0185] Humanized antibodies and methods of making them are reviewed, *e.g.,* in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, *e.g.,* in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60

(2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[0186] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, *e.g.,* Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, *e.g.,* Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, *e.g.,* Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, *e.g.,* Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

[0187] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

[0188] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, *e.g.,* U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, *e.g.,* by combining with a different human constant region.

[0189] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, *e.g.,* Kozbor *J. Immunol.*, 133: 3001

(1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0190] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

(v) Antibody Fragments

[0191] Antibody fragments may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved access to solid tumors. For a review of certain antibody fragments, see Hudson et al. (2003) *Nat. Med.* 9:129-134.

[0192] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); and Brennan et al., *Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from E. coli, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form $F(ab')_2$ fragments (Carter et al., *Bio/Technology* 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Fab and $F(ab')_2$ fragment with increased *in vivo* half-life comprising salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv).

See WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. Fv and scFv are the only species with intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during in vivo use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv. See *Antibody Engineering*, ed. Borrebaeck, *supra*. The antibody fragment may also be a "linear antibody", e.g., as described in U.S. Pat. No. 5,641,870, for example. Such linear antibodies may be monospecific or bispecific.

(vi) Multispecific Antibodies

[0193] Multispecific antibodies have binding specificities for at least two different epitopes, where the epitopes are usually from different antigens. While such molecules normally will only bind two different epitopes (i.e. bispecific antibodies, BsAbs), antibodies with additional specificities such as trispecific antibodies are encompassed by this expression when used herein. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. $F(ab')_2$ bispecific antibodies).

[0194] Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein et al., *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker et al., *EMBO J.*, 10:3655-3659 (1991).

[0195] One approach known in the art for making bispecific antibodies is the "knobs-into-holes" or "protuberance-into-cavity" approach (see, *e.g.,* US Pat. No. 5,731,168). In this approach, two immunoglobulin polypeptides (*e.g.,* heavy chain polypeptides) each comprise an interface. An interface of one immunoglobulin polypeptide interacts with a corresponding interface on the other immunoglobulin polypeptide, thereby allowing the two immunoglobulin polypeptides to associate. These interfaces may be engineered such that a "knob" or "protuberance" (these terms may be used interchangeably herein) located in the interface of one immunoglobulin polypeptide corresponds with a "hole" or "cavity" (these terms may be used interchangeably herein) located in the interface of the other immunoglobulin polypeptide. In some embodiments, the hole is of identical or similar size to

the knob and suitably positioned such that when the two interfaces interact, the knob of one interface is positionable in the corresponding hole of the other interface. Without wishing to be bound to theory, this is thought to stabilize the heteromultimer and favor formation of the heteromultimer over other species, for example homomultimers. In some embodiments, this approach may be used to promote the heteromultimerization of two different immunoglobulin polypeptides, creating a bispecific antibody comprising two immunoglobulin polypeptides with binding specificities for different epitopes.

[0196] In some embodiments, a knob may be constructed by replacing a small amino acid side chain with a larger side chain. In some embodiments, a hole may be constructed by replacing a large amino acid side chain with a smaller side chain. Knobs or holes may exist in the original interface, or they may be introduced synthetically. For example, knobs or holes may be introduced synthetically by altering the nucleic acid sequence encoding the interface to replace at least one "original" amino acid residue with at least one "import" amino acid residue. Methods for altering nucleic acid sequences may include standard molecular biology techniques well known in the art. The side chain volumes of various amino acid residues are shown in the following table. In some embodiments, original residues have a small side chain volume (*e.g.,* alanine, asparagine, aspartic acid, glycine, serine, threonine, or valine), and import residues for forming a knob are naturally occurring amino acids and may include arginine, phenylalanine, tyrosine, and tryptophan. In some embodiments, original residues have a large side chain volume (*e.g.,* arginine, phenylalanine, tyrosine, and tryptophan), and import residues for forming a hole are naturally occurring amino acids and may include alanine, serine, threonine, and valine.

Table 1. Properties of amino acid residues

Amino acid	One-letter abbreviation	Mass[a] (daltons)	Volume[b] (Å^3)	Accessible surface area[c] (Å^2)
Alanine (Ala)	A	71.08	88.6	115
Arginine (Arg)	R	156.20	173.4	225
Asparagine (Asn)	N	114.11	117.7	160
Aspartic Acid (Asp)	D	115.09	111.1	150
Cysteine (Cys)	C	103.14	108.5	135
Glutamine (Gln)	Q	128.14	143.9	180
Glutamic Acid (Glu)	E	129.12	138.4	190
Glycine (Gly)	G	57.06	60.1	75
Histidine (His)	H	137.15	153.2	195
Isoleucine (Ile)	I	113.17	166.7	175
Leucine (Leu)	L	113.17	166.7	170
Lysine (Lys)	K	128.18	168.6	200
Methionine (Met)	M	131.21	162.9	185
Phenylalanine (Phe)	F	147.18	189.9	210
Proline (Pro)	P	97.12	122.7	145
Serine (Ser)	S	87.08	89.0	115
Threonine (Thr)	T	101.11	116.1	140
Tryptophan (Trp)	W	186.21	227.8	255
Tyrosine (Tyr)	Y	163.18	193.6	230
Valine (Val)	V	99.14	140.0	155

[a]Molecular weight of amino acid minus that of water. Values from Handbook of Chemistry and Physics, 43rd ed. Cleveland, Chemical Rubber Publishing Co., 1961.
[b]Values from A.A. Zamyatnin, Prog. Biophys. Mol. Biol. 24:107-123, 1972.
[c]Values from C. Chothia, J. Mol. Biol. 105:1-14, 1975. The accessible surface area is defined in Figures 6-20 of this reference.

[0197] In some embodiments, original residues for forming a knob or hole are identified based on the three-dimensional structure of the heteromultimer. Techniques known in the art for obtaining a three-dimensional structure may include X-ray crystallography and NMR. In some embodiments, the interface is the CH3 domain of an immunoglobulin constant domain. In these embodiments, the CH3/CH3 interface of human IgG_1 involves sixteen residues on

each domain located on four anti-parallel β-strands. Without wishing to be bound to theory, mutated residues are preferably located on the two central anti-parallel β-strands to minimize the risk that knobs can be accommodated by the surrounding solvent, rather than the compensatory holes in the partner CH3 domain. In some embodiments, the mutations forming corresponding knobs and holes in two immunoglobulin polypeptides correspond to one or more pairs provided in the following table.

Table 2. Exemplary sets of corresponding knob-and hole-forming mutations

CH3 of first immunoglobulin	CH3 of second immunoglobulin
T366Y	Y407T
T366W	Y407A
F405A	T394W
Y407T	T366Y
T366Y:F405A	T394W:Y407T
T366W:F405W	T394S:Y407A
F405W:Y407A	T366W:T394S
F405W	T394S

Mutations are denoted by the original residue, followed by the position using the Kabat numbering system, and then the import residue (all residues are given in single-letter amino acid code). Multiple mutations are separated by a colon.

[0198] In some embodiments, an immunoglobulin polypeptide comprises a CH3 domain comprising one or more amino acid substitutions listed in Table 2 above. In some embodiments, a bispecific antibody comprises a first immunoglobulin polypeptide comprising a CH3 domain comprising one or more amino acid substitutions listed in the left column of Table 2, and a second immunoglobulin polypeptide comprising a CH3 domain comprising one or more corresponding amino acid substitutions listed in the right column of Table 2.

[0199] Following mutation of the DNA as discussed above, polynucleotides encoding modified immunoglobulin polypeptides with one or more corresponding knob- or hole-forming mutations may be expressed and purified using standard recombinant techniques and cell systems known in the art. See, e.g., U.S. Pat. Nos. 5,731,168; 5,807,706; 5,821,333; 7,642,228; 7,695,936; 8,216,805; U.S. Pub. No. 2013/0089553; and Spiess et al., Nature Biotechnology 31: 753-758, 2013. Modified immunoglobulin polypeptides may be produced using prokaryotic host cells, such as *E. coli*, or eukaryotic host cells, such as CHO cells.

Corresponding knob- and hole-bearing immunoglobulin polypeptides may be expressed in host cells in co-culture and purified together as a heteromultimer, or they may be expressed in single cultures, separately purified, and assembled *in vitro*. In some embodiments, two strains of bacterial host cells (one expressing an immunoglobulin polypeptide with a knob, and the other expressing an immunoglobulin polypeptide with a hole) are co-cultured using standard bacterial culturing techniques known in the art. In some embodiments, the two strains may be mixed in a specific ratio, *e.g.,* so as to achieve equal expression levels in culture. In some embodiments, the two strains may be mixed in a 50:50, 60:40, or 70:30 ratio. After polypeptide expression, the cells may be lysed together, and protein may be extracted. Standard techniques known in the art that allow for measuring the abundance of homo-multimeric vs. hetero-multimeric species may include size exclusion chromatography. In some embodiments, each modified immunoglobulin polypeptide is expressed separately using standard recombinant techniques, and they may be assembled together *in vitro*. Assembly may be achieved, for example, by purifying each modified immunoglobulin polypeptide, mixing and incubating them together in equal mass, reducing disulfides (*e.g.,* by treating with dithiothreitol), concentrating, and reoxidizing the polypeptides. Formed bispecific antibodies may be purified using standard techniques including cation-exchange chromatography and measured using standard techniques including size exclusion chromatography. For a more detailed description of these methods, see Speiss et al., *Nat Biotechnol* 31:753-8, 2013. In some embodiments, modified immunoglobulin polypeptides may be expressed separately in CHO cells and assembled *in vitro* using the methods described above.

[0200] According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is typical to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two

polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0201] In one embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 121:210 (1986).

[0202] According to another approach described in WO96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. One interface comprises at least a part of the C_H 3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[0203] Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

[0204] Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., *Science*, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal

dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[0205] Recent progress has facilitated the direct recovery of Fab'-SH fragments from E. coli, which can be chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.*, 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody.

[0206] Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. *See* Gruber et al, *J. Immunol*, 152:5368 (1994).

[0207] Another technique for making bispecific antibody fragments is the "bispecific T cell engager" or BiTE® approach (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 includes two single chain Fv (scFv) fragments, each having a variable heavy chain (V_H) and a variable light chain (V_L) 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 scFv fragments. Each scFv recognizes a different epitope, and these epitopes may be specific for different cell types, such that cells of two different cell types are brought into close proximity or tethered when each scFv is engaged with its cognate epitope. One particular embodiment of this approach includes a scFv recognizing a cell-surface antigen expressed by an immune cell, *e.g.,* a CD3 polypeptide on a T cell, linked to another scFv that recognizes a cell-surface antigen expressed by a target cell, such as a malignant or tumor cell.

[0208] As it is a single polypeptide, the bispecific T cell engager may be expressed using any prokaryotic or eukaryotic cell expression system known in the art, *e.g.,* a CHO cell line. However, specific purification techniques (see, *e.g.,* EP1691833) may be necessary to separate monomeric bispecific T cell engagers from other multimeric species, which may have biological activities other than the intended activity of the monomer. In one exemplary purification scheme, a solution containing secreted polypeptides is first subjected to a metal affinity chromatography, and polypeptides are eluted with a gradient of imidazole concentrations. This eluate is further purified using anion exchange chromatography, and polypeptides are eluted using with a gradient of sodium chloride concentrations. Finally, this eluate is subjected to size exclusion chromatography to separate monomers from multimeric species.

[0209] Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. *Tuft et al. J. Immunol.* 147: 60 (1991).

(vii) Single-Domain Antibodies

[0210] In some embodiments, an antibody of the invention is a single-domain antibody. A single-domain antibody is a single polypeptide chain comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, Mass.; *see*, *e.g.*, U.S. Pat. No. 6,248,516 B1). In one embodiment, a single-domain antibody consists of all or a portion of the heavy chain variable domain of an antibody.

(viii) Antibody Variants

[0211] In some embodiments, amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody may be prepared by introducing appropriate changes into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for

example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

(ix) Substitution, Insertion, and Deletion Variants

[0212] In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "conservative substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.,* retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

Table 3. Exemplary Substitutions.

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

[0213] Amino acids may be grouped according to common side-chain properties:

- **a.** hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- **b.** neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- **c.** acidic: Asp, Glu;
- **d.** basic: His, Lys, Arg;
- **e.** residues that influence chain orientation: Gly, Pro;
- **f.** aromatic: Trp, Tyr, Phe.

[0214] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0215] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.,* improvements) in certain biological properties (*e.g.,* increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.,* using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0216] Alterations (*e.g.,* substitutions) may be made in HVRs, *e.g.,* to improve antibody affinity. Such alterations may be made in HVR "hotspots," *i.e.,* residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, *e.g.,* Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.,* in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.,* error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.,* 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.,* using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0217] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.,* conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0218] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by

Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0219] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.*, for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

(x) Glycosylation variants

[0220] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0221] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, *e.g.*, Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0222] In one embodiment, antibody variants are provided comprising an Fc region wherein a carbohydrate structure attached to the Fc region has reduced fucose or lacks fucose, which may improve ADCC function. Specifically, antibodies are contemplated herein

that have reduced fusose relative to the amount of fucose on the same antibody produced in a wild-type CHO cell. That is, they are characterized by having a lower amount of fucose than they would otherwise have if produced by native CHO cells (*e.g.,* a CHO cell that produce a native glycosylation pattern, such as, a CHO cell containing a native FUT8 gene). In certain embodiments, the antibody is one wherein less than about 50%, 40%, 30%, 20%, 10%, or 5% of the N-linked glycans thereon comprise fucose. For example, the amount of fucose in such an antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. In certain embodiments, the antibody is one wherein none of the N-linked glycans thereon comprise fucose, *i.e.,* wherein the antibody is completely without fucose, or has no fucose or is afucosylated. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.,* between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, *e.g.,* US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (see, *e.g.,* Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[0223] Antibody variants are further provided with bisected oligosaccharides, *e.g.,* in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC

function. Examples of such antibody variants are described, *e.g.,* in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); US 2005/0123546 (Umana *et al.*), and Ferrara et al., Biotechnology and Bioengineering, 93(5): 851-861 (2006). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.,* in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

[0224] In certain embodiments, the antibody variants comprising an Fc region described herein are capable of binding to an FcγRIII. In certain embodiments, the antibody variants comprising an Fc region described herein have ADCC activity in the presence of human effector cells or have increased ADCC activity in the presence of human effector cells compared to the otherwise same antibody comprising a human wild-type IgG1Fc region.

(xi) Fc region variants

[0225] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.,* a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

[0226] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc(RIII only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed

(see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.,* in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, *e.g.,* C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, 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)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, *e.g.,* Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0227] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent 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 (US Patent No. 7,332,581).

[0228] Certain antibody variants with improved or diminished binding to FcRs are described. (See, *e.g.,* U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[0229] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.,* substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues). In an exemplary embodiment, the antibody comprising the following amino acid substitutions in its Fc region: S298A, E333A, and K334A.

[0230] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.,* either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.,* as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

[0231] Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in

US2005/0014934A1 (Hinton et al.)). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.,* substitution of Fc region residue 434 (US Patent No. 7,371,826). See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

(xii) Antibody Derivatives

[0232] The antibodies of the invention can be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. In certain embodiments, the moieties suitable for derivatization of the antibody are water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

(xiii) Vectors, Host Cells, and Recombinant Methods

[0233] Antibodies may also be produced using recombinant methods. For recombinant production of an anti-antigen antibody, nucleic acid encoding the antibody is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the

following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(a) Signal Sequence Component

[0234] An antibody of the invention may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (*e.g.*, cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process a native antibody signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, a factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders), or acid phosphatase leader, the *C. albicans* glucoamylase leader, or the signal described in WO 90/13646. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

(b) Origin of Replication

[0235] Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ, plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter.

(c) Selection Gene Component

[0236] Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

[0237] One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

[0238] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up antibody-encoding nucleic acid, such as DHFR, glutamine synthetase (GS), thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, *etc.*

[0239] For example, cells transformed with the DHFR gene are identified by culturing the transformants in a culture medium containing methotrexate (Mtx), a competitive antagonist of DHFR. Under these conditions, the DHFR gene is amplified along with any other co-transformed nucleic acid. A Chinese hamster ovary (CHO) cell line deficient in endogenous DHFR activity (e.g., ATCC CRL-9096) may be used.

[0240] Alternatively, cells transformed with the GS gene are identified by culturing the transformants in a culture medium containing L-methionine sulfoximine (Msx), an inhibitor of GS. Under these conditions, the GS gene is amplified along with any other co-transformed nucleic acid. The GS selection/amplification system may be used in combination with the DHFR selection/amplification system described above.

[0241] Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding an antibody of interest, wild-type DHFR gene, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, *e.g.*, kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.

[0242] A suitable selection gene for use in yeast is the *trp*1 gene present in the yeast plasmid YRp7 (Stinchcomb *et al.*, *Nature*, 282:39 (1979)). The *trp*1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, *Genetics*, 85:12 (1977). The presence of the *trp*1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu*2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu*2 gene.

[0243] In addition, vectors derived from the 1.6 μm circular plasmid pKD1 can be used for transformation of *Kluyveromyces* yeasts. Alternatively, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis*. Van den Berg,

Bio/Technology, 8:135 (1990). Stable multi-copy expression vectors for secretion of mature recombinant human serum albumin by industrial strains of *Kluyveromyces* have also been disclosed. Fleer *et al.*, *Bio/Technology*, 9:968-975 (1991).

(d) Promoter Component

[0244] Expression and cloning vectors generally contain a promoter that is recognized by the host organism and is operably linked to nucleic acid encoding an antibody. Promoters suitable for use with prokaryotic hosts include the *pho*A promoter, β-lactamase and lactose promoter systems, alkaline phosphatase promoter, a tryptophan (trp) promoter system, and hybrid promoters such as the tac promoter. However, other known bacterial promoters are suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding an antibody.

[0245] Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

[0246] Examples of suitable promoter sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

[0247] Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657. Yeast enhancers also are advantageously used with yeast promoters.

[0248] Antibody transcription from vectors in mammalian host cells can be controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus, Simian Virus 40 (SV40), or from

heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[0249] The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Reyes *et al.*, *Nature* 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

(e) Enhancer Element Component

[0250] Transcription of a DNA encoding an antibody of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature* 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the antibody-encoding sequence, but is preferably located at a site 5' from the promoter.

(f) Transcription Termination Component

[0251] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

(g) Selection and Transformation of Host Cells

[0252] Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, *e.g.*, *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (*e.g.*, *B. licheniformis* 41P disclosed in DD 266,710 published 12 Apr. 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting.

[0253] Full length antibody, antibody fusion proteins, and antibody fragments can be produced in bacteria, in particular when glycosylation and Fc effector function are not needed, such as when the therapeutic antibody is conjugated to a cytotoxic agent (e.g., a toxin) that by itself shows effectiveness in tumor cell destruction. Full length antibodies have greater half-life in circulation. Production in E. coli is faster and more cost efficient. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Pat. No. 5,648,237 (Carter et. al.), U.S. Pat. No. 5,789,199 (Joly et al.), U.S. Pat. No. 5,840,523 (Simmons et al.), which describes translation initiation region (TIR) and signal sequences for optimizing expression and secretion. *See also* Charlton, *Methods in Molecular Biology*, *Vol.* 248 (B. K. C. Lo, ed., Humana Press, Totowa, N.J., 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*. After expression, the antibody may be isolated from the E. coli cell paste in a soluble fraction and can be purified through, e.g., a protein A or G column depending on the isotype. Final purification can be carried out similar to the process for purifying antibody expressed e.g., in CHO cells.

[0254] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces* hosts such as, *e.g.*, *K. lactis*, *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilarum* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, *e.g.*, *Neurospora*, *Penicillium*,

Tolypocladium, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*. For a review discussing the use of yeasts and filamentous fungi for the production of therapeutic proteins, see, e.g., Gerngross, *Nat. Biotech.* 22:1409-1414 (2004).

[0255] Certain fungi and yeast strains may be selected in which glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. *See*, *e.g.*, Li et al., *Nat. Biotech.* 24:210-215 (2006) (describing humanization of the glycosylation pathway in *Pichia pastoris*); and Gerngross et al., *supra*.

[0256] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, *e.g.*, the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the invention, particularly for transfection of *Spodoptera frugiperda* cells.

[0257] Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, duckweed (*Leninaceae*), alfalfa (*M. truncatula*), and tobacco can also be utilized as hosts. *See*, *e.g.*, U.S. Pat. Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

[0258] Vertebrate cells may be used as hosts, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2). Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including $DHFR^-$ CHO cells (Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as NS0 and Sp2/0.

For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology,* Vol. 248 (B. K. C. Lo, ed., Humana Press, Totowa, N.J., 2003), pp. 255-268.

[0259] Host cells are transformed with the above-described expression or cloning vectors for antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

(h) Culturing the Host Cells

[0260] The host cells used to produce an antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, *Meth. Enz.* 58:44 (1979), Barnes *et al.*, *Anal. Biochem.* 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

(xiv) Purification of Antibody

[0261] When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a

commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[0262] The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, hydrophobic interaction chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being among one of the typically preferred purification steps. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark *et al.*, *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss *et al.*, *EMBO J.* 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C_H3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[0263] In general, various methodologies for preparing antibodies for use in research, testing, and clinical are well-established in the art, consistent with the above-described methodologies and/or as deemed appropriate by one skilled in the art for a particular antibody of interest.

C. Selecting Biologically Active Antibodies

[0264] Antibodies produced as described above may be subjected to one or more "biological activity" assays to select an antibody with beneficial properties from a therapeutic perspective or selecting formulations and conditions that retain biological activity of the antibody. The antibody may be tested for its ability to bind the antigen against which it was raised. For example, methods known in the art (such as ELISA, Western Blot, etc.) may be used.

[0265] For example, for an anti-PDL1 antibody, the antigen binding properties of the antibody can be evaluated in an assay that detects the ability to bind to PDL1. In some embodiments, the binding of the antibody may be determined by saturation binding; ELISA; and/or competition assays (e.g. RIA's), for example. Also, the antibody may be subjected to other biological activity assays, e.g., in order to evaluate its effectiveness as a therapeutic. Such assays are known in the art and depend on the target antigen and intended use for the antibody. For example, the biological effects of PD-L1 blockade by the antibody can be assessed in CD8+T cells, a lymphocytic choriomeningitis virus (LCMV) mouse model and/or a syngeneic tumor model e.g., as described in US Patent 8,217,149.

[0266] To screen for antibodies which bind to a particular epitope on the antigen of interest (e.g., those which block binding of the anti-PDL1 antibody of the example to PD-L1), a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping, e.g. as described in Champe et al., *J. Biol. Chem.* 270:1388-1394 (1995), can be performed to determine whether the antibody binds an epitope of interest.

D. Pharmaceutical Compositions and Formulations

[0267] Also provided herein are pharmaceutical compositions and formulations comprising a PD-1 axis binding antagonist and/or an antibody described herein (such as an anti-PD-L1 antibody, an anti-HER2 antibody, or a bispecific antibody that binds HER2 and CD3) and a pharmaceutically acceptable carrier.

[0268] Pharmaceutical compositions and formulations as described herein can be prepared by mixing the active ingredients (such as an antibody or a polypeptide) and/or an anti-HER2 antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino

acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0269] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

In some embodiments, the anti-PDL1 antibody described herein is in a formulation comprising the antibody in a concentration of about 60 mg/mL, histidine acetate in a concentration of about 20 mM, sucrose in a concentration of about 120 mM, and polysorbate (e.g., polysorbate 20) in a concentration of 0.04% (w/v), and the formulation has a pH of about 5.8. In some embodiments, the anti-PDL1 antibody described herein is in a formulation comprising the antibody in a concentration of about 125 mg/mL, histidine acetate in a concentration of about 20 mM, sucrose is in a concentration of about 240 mM, and polysorbate (e.g., polysorbate 20) in a concentration of 0.02% (w/v), and the formulation has a pH of about 5.5. In some embodiments, the anti-HER2 antibody described herein is in a formulation comprising the antibody, α,α-trehalose dihydrate, L-histidine HCL buffer, L-histidine and a polysorbate. In some embodiments, the anti-HER2 antibody described herein is in a formulation comprising the antibody in a concentration of about 22±2 mg/mL, histidine in a concentration of about 4.4 mM, trehalose in a concentration of about 54 mM, and polysorbate 20 in a concentration of about 0.009%, and the formulation has a pH of about 6.0.

[0270] The composition and formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0271] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[0272] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

IV. Methods of Treatment

[0273] Provided herein are methods for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an anti-HER2 antibody. In some embodiments, the treatment results in a sustained response in the individual after cessation of the treatment. The methods described herein may find use in treating conditions where enhanced immunogenicity is desired such as increasing tumor immunogenicity for the treatment of cancer. Also provided herein are methods of enhancing immune function in an individual having cancer comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an anti-HER2 antibody. Any of the PD-1 axis binding antagonists and the anti-HER2 antibodies known in the art or described herein may be used in the methods.

[0274] In some embodiments, the individual is a human. In some embodiments, the individual has HER-2 positive cancer. In some embodiments, HER-2 positive cancer is breast cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, colon cancer, kidney cancer, esophageal cancer, or prostate cancer. In some embodiments, the breast cancer is a breast carcinoma or a breast adenocarcinoma. In some embodiments, the breast carcinoma is an invasive ductal carcinoma. In some embodiments, the lung cancer is a lung adenocarcinoma. In some embodiments, the colon cancer is a colorectal adenocarcinoma. In some embodiments, the cancer cells in the individual express PD-L1. In some embodiments, the cancer cells in the individual express HER-2 protein at a level that is detectable (e.g., detectable using methods known in the art).

[0275] In some embodiments, the individual has been treated with a HER2 targeted therapy before the combination treatment with a PD-1 axis binding antagonist and an anti-HER2 antibody. In some embodiments, the HER2 targeted therapy includes treatment with one or more antibodies, *e.g.,* trastuzumab or pertuzumab. In some embodiments, the HER2 targeted therapy includes treatment with one or more antibody-drug conjugates, *e.g.,* ado-trastuzumab emtansine (KADCYLA®, Genentech). In some embodiments, the HER2 targeted therapy includes treatment with one or more small molecules, *e.g.,* lapatinib. In some embodiments, the individual has cancer that is resistant to one or more HER2 targeted therapies. In some embodiments, resistance to HER2 targeted therapy includes recurrence of cancer or refractory cancer. Recurrence may refer to the reappearance of cancer, in the original site or a new site, after treatment. In some embodiments, resistance to HER2 targeted therapy includes progression of the cancer during treatment with the HER2 targeted therapy. In some embodiments, resistance to HER2 targeted therapy includes cancer that does not response to treatment. The cancer may be resistant at the beginning of treatment or it may become resistant during treatment. In some embodiments, the cancer is at early stage or at late stage.

[0276] In some embodiments, the combination therapy of the invention comprises administration of a PD-1 axis binding antagonist and an anti-HER2 antibody. The PD-1 axis binding antagonist and the anti-HER2 antibody may be administered in any suitable manner known in the art. For example, The PD-1 axis binding antagonist and the anti-HER2 antibody may be administered sequentially (at different times) or concurrently (at the same time). In some embodiments, the PD-1 axis binding antagonist is in a separate composition as the anti-HER2 antibody. In some embodiments, the PD-1 axis binding antagonist is in the same composition as the anti-HER2 antibody.

[0277] The PD-1 axis binding antagonist and the anti-HER2 antibody may be administered by the same route of administration or by different routes of administration. In some embodiments, the PD-1 axis binding antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments, the anti-HER2 antibody is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. An effective amount of the PD-1 axis binding antagonist and the anti-HER2 antibody may be administered for prevention or treatment of disease. The appropriate dosage of the PD-1 axis binding antagonist and/or the anti-HER2 antibody may be determined based on the type of

disease to be treated, the type of the PD-1 axis binding antagonist and the anti-HER2 antibody, the severity and course of the disease, the clinical condition of the individual, the individual's clinical history and response to the treatment, and the discretion of the attending physician.

[0278] As a general proposition, the therapeutically effective amount of the antibody administered to human will be in the range of about 0.01 to about 50 mg/kg of patient body weight whether by one or more administrations. In some embodiments, the antibody used is about 0.01 to about 45 mg/kg, about 0.01 to about 40 mg/kg, about 0.01 to about 35 mg/kg, about 0.01 to about 30 mg/kg, about 0.01 to about 25 mg/kg, about 0.01 to about 20 mg/kg, about 0.01 to about 15 mg/kg, about 0.01 to about 10 mg/kg, about 0.01 to about 5 mg/kg, or about 0.01 to about 1 mg/kg administered daily, for example. In some embodiments, the antibody is administered at 15 mg/kg. However, other dosage regimens may be useful. In one embodiment, an anti-PDL1 antibody described herein is administered to a human at a dose of about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg or about 1400 mg on day 1 of 21-day cycles. The dose may be administered as a single dose or as multiple doses (e.g., 2 or 3 doses), such as infusions. The dose of the antibody administered in a combination treatment may be reduced as compared to a single treatment. The progress of this therapy is easily monitored by conventional techniques.

[0279] In some embodiments, the methods may further comprise an additional therapy. The additional therapy may be radiation therapy, surgery (*e.g.*, lumpectomy and a mastectomy), chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, immunotherapy, bone marrow transplantation, nanotherapy, monoclonal antibody therapy, or a combination of the foregoing. The additional therapy may be in the form of adjuvant or neoadjuvant therapy. In some embodiments, the additional therapy is the administration of small molecule enzymatic inhibitor or anti-metastatic agent. In some embodiments, the additional therapy is the administration of side-effect limiting agents (*e.g.*, agents intended to lessen the occurrence and/or severity of side effects of treatment, such as anti-nausea agents, etc.). In some embodiments, the additional therapy is radiation therapy. In some embodiments, the additional therapy is surgery. In some embodiments, the additional therapy is a combination of radiation therapy and surgery. In some embodiments, the additional therapy is gamma irradiation. In some embodiments, the additional therapy is therapy targeting PI3K/AKT/mTOR pathway, HSP90 inhibitor, tubulin inhibitor, apoptosis inhibitor,

and/or chemopreventative agent. The additional therapy may be one or more of the chemotherapeutic agents described herein.

Other Combination Therapies

[0280] Also provided herein are methods for treating or delaying progression of cancer in an individual comprising administering to the individual a human PD-1 axis binding antagonist in conjunction with another anti-cancer agent or cancer therapy. In the embodiments described herein, the method may further comprise administering an anti-HER2 antibody described herein for treating a HER2 positive cancer.

[0281] In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a chemotherapy or chemotherapeutic agent. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a radiation therapy or radiotherapeutic agent. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a targeted therapy or targeted therapeutic agent. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an immunotherapy or immunotherapeutic agent, for example a monoclonal antibody.

[0282] Without wishing to be bound to theory, it is thought that enhancing T cell stimulation, by promoting an activating co-stimulatory molecule or by inhibiting a negative co-stimulatory molecule, may promote tumor cell death thereby treating or delaying progression of cancer. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an agonist directed against an activating co-stimulatory molecule. In some embodiments, an activating co-stimulatory molecule may include CD40, CD226, CD28, OX40, GITR, CD137, CD27, HVEM, or CD127. In some embodiments, the agonist directed against an activating co-stimulatory molecule is an agonist antibody that binds to CD40, CD226, CD28, OX40, GITR, CD137, CD27, HVEM, or CD127. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antagonist directed against an inhibitory co-stimulatory molecule. In some embodiments, an inhibitory co-stimulatory molecule may include CTLA-4 (also known as CD152), PD-1, TIM-3, BTLA, VISTA, LAG-3, B7-H3, B7-H4, IDO, TIGIT, MICA/B, or arginase. In some embodiments, the antagonist directed against an inhibitory co-stimulatory molecule is an antagonist antibody that binds to CTLA-4, PD-1, TIM-3, BTLA, VISTA, LAG-3, B7-H3, B7-H4, IDO, TIGIT, MICA/B, or arginase.

[0283] In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antagonist directed against CTLA-4 (also known as CD152), *e.g.,* a blocking antibody. In some embodiments, a PD-1 axis binding antagonist may be

administered in conjunction with ipilimumab (also known as MDX-010, MDX-101, or Yervoy®). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with tremelimumab (also known as ticilimumab or CP-675,206). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antagonist directed against B7-H3 (also known as CD276), *e.g.,* a blocking antibody. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with MGA271. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antagonist directed against a TGF beta, *e.g.,* metelimumab (also known as CAT-192), fresolimumab (also known as GC1008), or LY2157299.

[0284] In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a treatment comprising adoptive transfer of a T cell (*e.g.,* a cytotoxic T cell or CTL) expressing a chimeric antigen receptor (CAR). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a treatment comprising adoptive transfer of a T cell comprising a dominant-negative TGF beta receptor, *e.g,* a dominant-negative TGF beta type II receptor. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a treatment comprising a HERCREEM protocol (see, e.g., ClinicalTrials.gov Identifier NCT00889954).

[0285] In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an agonist directed against CD137 (also known as TNFRSF9, 4-1BB, or ILA), *e.g.,* an activating antibody. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with urelumab (also known as BMS-663513). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an agonist directed against CD40, *e.g.,* an activating antibody. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with CP-870893. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an agonist directed against OX40 (also known as CD134), *e.g.,* an activating antibody. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an anti-OX40 antibody (e.g., AgonOX). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an agonist directed against CD27, *e.g.,* an activating antibody. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with CDX-1127. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antagonist directed against indoleamine-2,3-dioxygenase (IDO). In some embodiments, with the IDO antagonist is 1-methyl-D-tryptophan (also known as 1-D-MT).

[0286] In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antibody-drug conjugate. In some embodiments, the antibody-drug conjugate comprises mertansine or monomethyl auristatin E (MMAE). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with and anti-NaPi2b antibody-MMAE conjugate (also known as DNIB0600A or RG7599). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with trastuzumab emtansine (also known as T-DM1, ado-trastuzumab emtansine, or KADCYLA®, Genentech). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with DMUC5754A. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antibody-drug conjugate targeting the endothelin B receptor (EDNBR), *e.g.,* an antibody directed against EDNBR conjugated with MMAE.

[0287] In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an angiogenesis inhibitor. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antibody directed against a VEGF, *e.g.,* VEGF-A. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with bevacizumab (also known as AVASTIN®, Genentech). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antibody directed against angiopoietin 2 (also known as Ang2). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with MEDI3617.

[0288] In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antineoplastic agent. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an agent targeting CSF-1R (also known as M-CSFR or CD115). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with anti-CSF-1R (also known as IMC-CS4). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an interferon, for example interferon alpha or interferon gamma. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with Roferon-A (also known as recombinant Interferon alpha-2a). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with GM-CSF (also known as recombinant human granulocyte macrophage colony stimulating factor, rhu GM-CSF, sargramostim, or Leukine®). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with IL-2 (also known as aldesleukin or Proleukin®). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with IL-12. In some

embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antibody targeting CD20. In some embodiments, the antibody targeting CD20 is obinutuzumab (also known as GA101 or Gazyva®) or rituximab. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antibody targeting GITR. In some embodiments, the antibody targeting GITR is TRX518.

[0289] In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a cancer vaccine. In some embodiments, the cancer vaccine is a peptide cancer vaccine, which in some embodiments is a personalized peptide vaccine. In some embodiments the peptide cancer vaccine is a multivalent long peptide, a multi-peptide, a peptide cocktail, a hybrid peptide, or a peptide-pulsed dendritic cell vaccine (see, e.g., Yamada et al., Cancer Sci, 104:14-21, 2013). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an adjuvant. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a treatment comprising a TLR agonist, *e.g.,* Poly-ICLC (also known as Hiltonol®), LPS, MPL, or CpG ODN. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with tumor necrosis factor (TNF) alpha. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with IL-1. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with HMGB1. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an IL-10 antagonist. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an IL-4 antagonist. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an IL-13 antagonist. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an HVEM antagonist. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an ICOS agonist, *e.g.,* by administration of ICOS-L, or an agonistic antibody directed against ICOS. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a treatment targeting CX3CL1. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a treatment targeting CXCL9. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a treatment targeting CXCL10. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a treatment targeting CCL5. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an LFA-1 or ICAM1 agonist. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a Selectin agonist.

[0290] In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a targeted therapy. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an inhibitor of B-Raf. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with vemurafenib (also known as Zelboraf®). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with dabrafenib (also known as Tafinlar®). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with erlotinib (also known as Tarceva®). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an inhibitor of a MEK, such as MEK1 (also known as MAP2K1) or MEK2 (also known as MAP2K2). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with cobimetinib (also known as GDC-0973 or XL-518). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with trametinib (also known as Mekinist®). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an inhibitor of K-Ras. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an inhibitor of c-Met. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with onartuzumab (also known as MetMAb). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an inhibitor of Alk. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with AF802 (also known as CH5424802 or alectinib). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an inhibitor of a phosphatidylinositol 3-kinase (PI3K). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with BKM120. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with idelalisib (also known as GS-1101 or CAL-101). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with perifosine (also known as KRX-0401). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an inhibitor of an Akt. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with MK2206. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with GSK690693. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with GDC-0941. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an inhibitor of mTOR. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with sirolimus (also known as rapamycin). In some

embodiments, a PD-1 axis binding antagonist may be administered in conjunction with temsirolimus (also known as CCI-779 or Torisel®). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with everolimus (also known as RAD001). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with ridaforolimus (also known as AP-23573, MK-8669, or deforolimus). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with OSI-027. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with AZD8055. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with INK128. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with a dual PI3K/mTOR inhibitor. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with XL765. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with GDC-0980. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with BEZ235 (also known as NVP-BEZ235). In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with BGT226. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with GSK2126458. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with PF-04691502. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with PF-05212384 (also known as PKI-587).

V. Articles of Manufacture or Kits

[0291] In another embodiment of the invention, an article of manufacture or a kit is provided comprising a PD-1 axis binding antagonist and/or an anti-HER2 antibody. In some embodiments, the article of manufacture or kit further comprises package insert comprising instructions for suing the PD-1 axis binding antagonist in conjunction with an anti-HER2 antibody to treat or delay progression of cancer in an individual or to enhance immune function of an individual having cancer. Any of the PD-1 axis binding antagonist and/or anti-HER antibodies described herein may be included in the article of manufacture or kits.

[0292] In some embodiments, the PD-1 axis binding antagonist and the anti-HER2 antibody are in the same container or separate containers. Suitable containers include, for example, bottles, vials, bags and syringes. The container may be formed from a variety of materials such as glass, plastic (such as polyvinyl chloride or polyolefin), or metal alloy (such as stainless steel or hastelloy). In some embodiments, the container holds the formulation

and the label on, or associated with, the container may indicate directions for use. The article of manufacture or kit may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. In some embodiments, the article of manufacture further includes one or more of another agent (e.g., a chemotherapeutic agent, and anti-neoplastic agent). Suitable containers for the one or more agent include, for example, bottles, vials, bags and syringes.

[0293] The specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

EXAMPLES

[0294] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: HER2 T cell dependent bispecific antibody (HER2-TDB) for treatment of HER2 positive cancers

[0295] Based on recent clinical success of tumor immunotherapies that block immune suppressive mechanisms to restore T cell function, there is a profound interest in the clinical development of T cell targeted therapies. To meet this demand, described herein is a trastuzumab-based HER2 T cell dependent bispecific antibody (HER2-TDB). This full-length human IgG format bispecific antibody conditionally activated T cells resulting in lysis of HER2 expressing cancer cells at low picomolar concentrations. Importantly, HER2-TDB was able to eliminate cells refractory to currently approved HER2 therapies. The potent anti-tumor activity of HER2-TDB was demonstrated using four model systems including MMTV-

huHER2 and huCD3 transgenic mice. These results demonstrated inhibitory effect of PD-L1 expression on the activity of bispecific T cell recruiting antibodies. This resistance mechanism was reversed by anti-PD-L1 antibody treatment and combination of HER2-TDB and anti-PD-L1 immune therapy resulted in enhanced inhibition of tumor growth, increased response rates and durable responses.

Materials and Methods

Antibody expression and purification

[0296] The 'knob' arm of HER2 huIgG1 TDB was humanized anti-HER2 4D5 (trastuzumab) (Carter et al., *Proc Natl Acad Sci USA*, 89:4285-9, 1992) and 'hole' arm was humanized anti-CD3 UCHT1.v9 (Zhu et al., *Int J Cancer*, 62:319-24, 1995). The huIgG1 bispecific antibodies were produced by two different approaches as described earlier (Spiess et al., *Nat Biotechnol.*, 2013): co-culture of bacteria expressing each of the two antibody arms, or by expressing each arm separately and then annealing them *in vitro*.

[0297] To avoid immune response towards the TDB, a murine IgG2a isotype HER2-TDB was used in experiments with immune competent mice. For expression as muIgG2a, equivalent knob-into-hole mutations (Atwell et al., *J Mol Biol.*, 270:26-35, 1997) were introduced into the Fc region, as well as D265A and N297G (EU numbering) to abolish effector function. In muIgG2a HER2-TDBs the "knob" arm is murine anti-HER2 4D5 and the "hole" is either chimeric anti-murine CD3 2C11 (Leo et al., *Proc Natl Acad Sci USA*, 84:1374-8, 1987) (4D5/2C11-TDB) or mouse anti-hu CD3 SP34 (Pessano et al., *The EMBO journal*, 4:337-44, 1985) (4D5/SP34-TDB). The muIgG2a bispecific antibodies were expressed in CHO cells and assembled by *in vitro* assembly. Bispecific antibodies were purified from contaminants by hydrophobic interaction chromatography (HIC) as described elsewhere (Speiss et al., *Nat Biotechnol* 31:753-8, 2013). The resulting material was analyzed for endotoxin levels using an Endosafe® portable test system (Charles River, USA) and when needed, the endotoxin content was reduced by washing the protein with 0.1% Triton X-114.

Antibody characterization

[0298] The molecular weight of the bispecific antibody was analyzed by mass spectrometry (LC-ESI/TOF) as described before (Jackman et al., *The Journal of biological chemistry*, 285:20850-9, 2010). The antibodies were also analyzed by analytical size exclusion chromatography in a Zenix™ SEC-300 column (Sepax Technologies, USA) using an Agilent 1,100 HPLC system (Agilent Technologies, USA). The presence of residual antibody fragments was quantified by electrohoresis using a 2100 Bioanalyzer and a Protein 230 Chip (Agilent Technologies).

HER2-TDB affinity

[0299] The competitive Scatchard assay was described in detail elsewhere (Ramirez-Carrozzi et al., *Nature immunology*, 12:1159-66, 2011).

Breast cancer cell proliferation

[0300] Breast cancer cell proliferation/viability was detected using CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI). For the assay, 5×10^3 cells/well were plated in 96-well plates and incubated overnight for cell attachment before treatments.

Blood cell fractionation

[0301] PBMCs were separated from the blood of healthy volunteers using lymphocyte separation medium (MP biomedicals, Solon, OH). CD8+ cells were extracted from PBMC using human CD8+ Isolation Kit from Miltenyi (#130-094-156) by negative selection. CD3+-depletion was done using CD3+ MicroBeads from Miltenyi (#130-050-101).

***In vitro* cytotoxicity assays (*In vitro* ADCC, T cell killing)**

[0302] *In vitro* cytotoxicity assays (Cytotoxicity Detection Kit; LDH; Roche, Mannheim, Germany) were performed as previously described (Junttila et al., *Cancer Res.*, 70:4481-9, 2010). Alternatively, in vitro cytotoxicity was monitored by flow cytometry. Target cells were labeled with CFSE (Invitrogen, #C34554). The labeled target cells and CD8+ cells were mixed with or without TDB for 4-26 hours. At the end of the incubation, the cells were lifted by trypsin and collected from the plate. The cells were resuspended in equal volume of PBS + 2% FBS + 1 mM EDTA + propidium iodine (PI). Flow cytometry analysis was done on a FACSCalibur in automation format. The number of live target cells was counted by gating on CFSE+/PI negative cells. The percentage of cytotoxicity was calculated as follows: % cytotoxicity (live target cell number w/o TDB – live target cell number w/TDB) / (live target cell number w/o TDB) x 100.

Analysis of T cell activation

[0303] Cells were stained with CD8-FITC (BD Bioscience, 555634) CD69-PE (BD Bioscience, 555531) and CD107a-Alexa-Fluor647 (eBioscience, 51-1079). Alternatively, cells were fixed and permeabilized with Cytofix/CytoPerm™ solution (BD Bioscience, 554722) and stained with anti-granzyme B-Alexa-Fluor647 (BD Bioscience, 560212).

Detection of soluble granzymes and perforin

[0304] Soluble perforin (Cell Sciences), granzyme A and granzyme B (eBioscience) were detected from growth media by ELISA according to manufacturer's protocols.

PD-1 induction and effect of PD-L1 expression on TDB activity

[0305] Purified CD8+ T cells from human peripheral blood were primed with 100 ug/ml of HER2-TDB and SKBR3 cell at 3:1 ratio for 24h. After 24 hours incubation the cell pellet was digested with Non-Enzyme Cell Dissociation Solution (Sigma, #C5789) at 37C for 10 min and CD8+ T cells recovered using Human CD8+ Microbeads (Miltenyi, #130-045-201). The primed-CD8+ T cells were used for in vitro cytotoxicity assay. In flat-bottom 96 well plate, CFSE-labeled 293 cells or 293-PDL1 cells were mixed with primed effector cells in 3:1 ratio in the presence or absence of HER2-TDB and anti-PD-L1 antibody (clone 6E11, mIgG2A, D265A and N297A). After 24 hours, cytotoxicity was measured by counting live CFSE+ target cells by flow cytometry.

Pharmacokinetic (PK) study in rats

[0306] Eight rats (n = 4/group) were randomized into two dosing groups that received a single intravenous (IV) bolus of either HER2-TDB or trastuzumab at 10 mg/kg. Samples were taken from 4 rats per group at time points through 35 days post dose. Approximately 0.2 mL of whole blood was collected via the jugular vein (under CO_2/O_2 anesthesia). The samples were allowed to clot and centrifuged under refrigeration (5 °C for 10 minutes at 2000 x g) to obtain serum. Serum samples were assayed for human IgG by ELISA, where Donkey anti-huFc coated to microtiter plate is used to capture the humanized anti HER2 antibodies in circulation and goat anti-huFc-HRP (mouse adsorbed) for detection. PK parameters were determined with a 2-compartment method (Model 7) using WinNonlin®, version 5.2.1 (Pharsight Corp., Mountain View, CA).

***In vivo* efficacy**

[0307] NOD/SCID mice (NOD.CB17-*Prkdcscid*/J, Jackson Labs West) were implanted with 0.36mg, 60 day sustained release estrogen pellets (Innovative Research of America) 1 to 3 days prior to cell inoculation, subcutaneously over the opposite flank of tumor inoculation. On Day 0, 5 million MCF7 neo/HER2 and 10 million non-activated human PBMCs in HBSS-matrigel were inoculated in right 2/3 mammary fat-pad. The first treatments were administered 2 hours post-inoculation. All treatments were administered 1x/week by i. v. tail vein injection for a total of 3 doses.

[0308] MMTV-huHER2 transgenic mice have been previously described (Finkle et al., *Clinical Cancer Research* 10:2499-511, 2004). For experiments with syngeneic tumors, 0.1 million CT26-HER2 cells were injected subcutaneously to Balb/c or human CD3ε transgenic mice (de la Hera et al., *J Exp Med.*, 173:7-17, 1991). Treatment of mice with established tumors is indicated in the figure legends. To avoid immune response towards the TDB, a murine IgG2A version of the HER2-TDB was used in experiments with immune competent

mice. Anti-PD-L1 antibody clone 25A1 (mIgG2A, D265A and N297A) was used for therapeutic blockade of PD-L1.

Results

Generation and purification of full length HER2-CD3 bispecific antibody (HER2-TDB) using knobs-into-holes technology

[0309] HER2-TDB was generated using a knobs-into-holes strategy (Jefferis, *Trends in pharmacological sciences*, 30:356-62, 2009) (**FIG. 1A**). The anti-CD3 arm (UCHT1.v9; hole) and the anti-HER2 arm (4D5; trastuzumab; knob) were expressed in separate *E. coli* cultures or, alternatively, co-cultured (**FIG. 1B**). The fully assembled antibody was isolated on Protein A and then purified from antibody fragments by hydrophobic interaction chromatography. Size exclusion chromatography showed a very low level of aggregation (**FIG. 1C**, <0.2% to 0.9%) and mass spectrometry analysis showed a main mass deconvolution peak corresponding to the heterodimer and the absence of significant amounts of either homodimer (**FIG. 1D**). These results demonstrate that high quality HER2-TDB can be efficiently produced using standard expression and purification methods.

T cell independent properties of HER2-TDB

[0310] Unlike trastuzumab, HER2-TDB is monovalent and is produced in *E. coli*. The T cell independent properties of HER2-TDB were compared to trastuzumab and trastuzumab-Fab fragments (**FIG. 1E-F**). Target arm binding affinity of HER2-TDB by Scatchard analysis (K_D=5.4nM, **FIG. 1E**) was similar to monovalent trastuzumab Fab (K_D=3.9nM) and lower than the affinity of bivalent trastuzumab to HER2 (K_D=0.7nM). The K_D for CD3-arm binding affinity to Jurkat cells was 4.7 nM (not shown). The ability of HER2-TDB to directly inhibit SKBR3 proliferation was reduced as compared to bivalent trastuzumab (**FIG. 1F**). Antibodies produced in *E. coli* are not glycosylated, which results in impaired FcγR binding, which is required to mediate antibody-dependent cell-mediated cytotoxicity (ADCC) (Jefferis, *Trends in pharmacological sciences*, 30:356-62, 2009; Simmons et al., *Journal of immunological methods*, 263:133-47, 2002) . *E. coli* produced trastuzumab and HER2 TDB were unable to induce NK cell mediated ADCC (**FIG. 1G**).

Target dependent T cell activation and cytotoxicity

[0311] T cell activation was not detected when CD8+ cells were incubated with HER2-TDB or target cells that do not express human HER2 (BJAB cells, **FIG. 2A**). A robust T cell activation was seen when HER2+ SKBR3 cells were used as targets accompanied by release of cytotoxic granules. Soluble perforin, granzyme A and B were detected in the growth media by ELISA (**FIG. 2B**), but only when all the key components (HER2-TDB, T cells, HER2

expressing cells) were included in the reaction. Granule exocytosis coincided with significant HER2-TDB induced elevation of caspase 3/7 activity, apoptosis and cytotoxicity (lactate dehydrogenase (LDH) release; **FIG. 2C**).

[0312] No killing of vector-transfected 3T3-cells was detected (**FIG. 2D**); in contrast, the HER2 transfected 3T3-cells were very efficiently killed. Addition of HER2-ECD or trastuzumab Fab to the killing assay efficiently inhibited the killing activity (**FIG. 2E**). To confirm T cell dependence of killing, CD3+ cells were depleted from the PBMC (**FIG. 2F**). The depletion resulted in loss of target cell killing activity.

Kinetics of T cell activation and killing induced by HER2-TDB

[0313] Early signs of T cell activation (CD69) appeared 4h after HER2-TDB treatment was initiated (**FIG. 3A**). However, late activation markers (extracellular CD107a) were detected later at the 24h time point. Activation of T cells was reflected in killing of HER2+ breast cancer cells (**FIG. 3A**). No significant killing activity was detected at 4-12h. Robust killing was detected at 24h and killing activity increased over time.

HER2-TDB induces T cell proliferation

[0314] Cytotoxicity was significantly reduced by effector cell titration (**FIG. 3B**). However even with an E:T ratio of ≤1:1 a weak LDH signal and robust activation of T cells was detected. To investigate whether HER2-TDB induces T cell proliferation, CD8+ T cells, target cells (SKBR3) and 0.1 ug/ml HER2-TDB were co-cultured, followed by T cell culture in absence of target cells and HER2-TDB. After 3 days 75% of the T cells pulsed with TDB and target cells had undergone a cell division (**FIG. 4**), however the cell number did not increase. Supplementing the growth media with IL-2 (20 ng/ml) provided a survival signal to CD8+ cells, and a robust T cell proliferation was detected in the T cells, but only if they were exposed to both HER2-TDB and target cells (**FIG. 4**).

HER2-TDB activity correlates with the target cell HER2 expression level

[0315] To investigate the relationship between target copy number and TDB activity, a panel of cancer cell lines with pre-determined number of HER2-receptors on the cell membrane was selected (**FIG. 5A & E**, (Aguilar et al., *Oncogene*, 18:6050-62, 1999)). HER2 amplified/overexpressing cell lines were significantly more sensitive to the TDB mediated killing (p=0.015, t-test) and were efficiently lysed at femtomolar to low picomolar concentrations (EC_{50}=0.8-3 pM; **FIG. 5B**). Cell lines expressing low levels of HER2 were significantly less sensitive to HER2-TDB antibody (EC_{50}=33-51 pM). As low as <1000 copies of target antigen was sufficient to support T cell killing.

[0316] Next, MCF7 (low HER2 expression) or BJAB cells (no HER2 expression) were co-targeted with HER2 amplified SKBR3 cells in the same killing assay. No killing of MCF7 cells was detectable at the EC_{50} for SKBR3 killing (**FIG. 5C**). No significant killing of BJAB cells was detectable at any HER2-TDB concentration (**FIG. 5D**).

Very low target occupancy is sufficient for TDB activity

[0317] HER2 occupancy at EC_{50} for HER2-TDB was calculated using formula $[D]/[D]+K_D$ (where the D = drug and K_D for HER2-TDB was 5.4 nM). In all tested cell lines, less than 1% target occupancy was sufficient for efficient killing (**FIG. 5E**), and in the case of the high HER2 expressing cell lines, the required occupancy was even lower (0.01-0.05%). The calculated absolute number of TDB bound to HER2 at the EC_{50} was as low as 10-150 in the low expressing cell lines. These results showcase the extreme potency of HER2-TDB and are consistent with studies of TCR triggering which suggest as few as 1-25 TCRs need to be engaged to trigger T cell responses (Irvine et al., *Nature*, 419:845-9, 2002; Purbhoo et al., *Nature immunology*, 5:524-30, 2004; Sykulev et al., *Immunity*, 4:565-71, 1996).

HER2-TDB is efficient in killing of HER2+ cancer cells refractory to anti-HER2 therapies

[0318] Next, cell lines that have previously been shown to express high levels of HER2 but are insensitive to the direct cellular effects of trastuzumab and lapatinib *in vitro* were examined (Junttila et al., *Cancer Cell*, 15:429-40, 2009; Junttila et al., *Breast Cancer Res Treat*, 2010). For some cell lines, activation of the PI3K pathway due to acquired activating mutations in the PI3K catalytic subunit (KPL4, HCC202) or by PTEN loss (HCC1596) may cause resistance. Sensitivity of the cell lines to T-DM1 has been previously reported (Junttila et al., *Breast Cancer Res Treat*, 2010; Lewis Phillips et al., *Cancer Res.*, 68:9280-90, 2008). EC_{50} for HER2-TDB mediated killing was in the femtomolar or low picomolar range (**FIG. 6A**). In addition, HER2-TDB was effective in killing HER2+ lung cancer cells. Using two independent cell line models (BT474, **FIG. 6B-C**; KPL-4, not shown), acquired resistance to T-DM1 did not affect the sensitivity to HER2-TDB.

Pharmacokinetics of HER2-TDB in rat

[0319] To assess the pharmacokinetic (PK) profile of HER2-TDB, Sprague-Dawley rats were administered a single intravenous (IV) dose of 10 mg/kg of either HER2-TDB or trastuzumab. HER2-TDB does not cross react with rat CD3 or rat HER2 and displayed a biphasic disposition typical of an IgG1 with of a short distribution phase and slow elimination phase (**FIG. 7**). Both the clearance and half-life of HER2-TDB were similar to trastuzumab, and within expected range of a typical IgG1 in rats.

HER2-TDB inhibits tumor growth in vivo in immuno-compromised mice

[0320] *In vivo* efficacy of HER2-TDB was tested in NOD-SCID mice, which lack endogenous functional T and B cells and have reduced levels of NK, DC and macrophage cell types. MCF7-neo/HER2 cells were grafted together with non-activated human PBMCs from healthy donors to mammary fat pads of mice. Mice were dosed intravenously on a weekly schedule with 0.5 mg/kg of HER2-TDB or control-TDB, starting on the day of tumor cell inoculation. HER2-TDB prevented growth of HER2 expressing tumors (**FIG. 8A**). As expected no efficacy was detected in mice when huPBMC were omitted (**FIG. 15A**). A control TDB that shares the same CD3-arm as HER2-TDB (but has an irrelevant target arm that does not bind to MCF7-neo/HER2, human PBMC or mouse cells) had no effect on the tumor growth (**FIG. 15B**).

HER2-TDB causes regression of large mammary tumors in huHER2 transgenic mice

[0321] To model the activity of HER2-TDB in immuno-competent mice, human MMTV-huHER2 transgenic mice were used (Finkle et al.; Clinical Cancer Research; 10:2499-511; 2004), and a surrogate TDB using a mouse CD3 reactive antibody clone 2C11 (Leo et al., *Proc Natl Acad Sci USA*, 84:1374-8, 1987) was generated. The *in vitro* activity of 4D5/2C11-TDB was similar to human CD3 reactive HER2-TDB (**FIG. 10**). With the exception of one tumor, 4D5/2C11-TDB resulted in regression (**FIG. 8B-C**). >50% tumor regression was detected in 57% mice and 43% mice had no detectable tumor. Responders included tumors that were >1000 mm^3 at the start of the treatment (**FIG. 8D**). Tumor growth was not affected by control TDBs, in which the CD3 arm was switched to human CD3 specific, or the target arm was switched to irrelevant (**FIG. 8E**).

HER2-TDB inhibits growth of established tumors in immuno-competent mice

[0322] Human CD3ε transgenic mice (CD3-TG, (de la Hera et al., *J Exp Med.*, 173:7-17, 1991)) were used to model the activity of HER2-TDB in immuno-competent mice. CD3-TG T cells express both mouse and human CD3 on approximately 50% of respective Balb/c mouse or human T cells (**FIG. 9**). CD3-TG T cells killed human HER2 expressing target cells *in vitro* (**FIG. 10**), although killing activity of mouse splenic T cells was consistently lower compared to human peripheral T cells. Human HER2 transfected CT26 tumor cells were grown in the CD3-TG mice subcutaneously and established tumors were treated with weekly 0.5 mg/kg IV doses of HER2-TDB. HER2-TDB clearly inhibited the growth of established tumors, but the effect was transient and no complete responses were seen (**FIG. 8F**). The activity of HER2-TDB was dependent on T cells, since HER2-TDB had no effect in non-CD3 transgenic mice (**FIG. 11**). The *in vivo* responses detected in Balb/c mice using

4D5/2C11 TDB were similar to the responses seen in CD3-TG mice with human specific CD3-arm based TDB (**FIG. 8F-G**). Despite incomplete responses, HER2-TDB significantly prolonged the time to tumor progression (Log-Rank p-value < 0.0001). Control-TDB with irrelevant tumor arm had no effect on tumor growth. In addition, the tumors were insensitive to T-DM1 (**FIG. 8G**).

PD-L1 expression in target cells inhibits HER2-TDB activity

[0323] The cellular composition of the CT26-HER2 tumors was further analyzed to characterize the incomplete tumor response. 10-30% of CD45+ cells in CT26-HER2 tumors were CD8+ T cells (**FIG. 12-13**). Almost all T cells displayed markers of activation and were positive for PD-1 (80-95% CD69+, 95% PD-1+). All CD45- cells were positive for PD-L1. To test whether the PD-1/PD-L1 signaling interferes with HER2-TDB activity, human T cells were used. Upregulation of PD-1 in T cells was detected upon overnight co-culture with SKBR3 cells and HER2-TDB (**FIG. 14A**). T cells were then transferred on PD-L1 or vector transfected 293 cells. 293 cells express low levels of HER2, and the primed T cells efficiently killed the 293 cells, but only when the HER2-TDB was added (**FIG. 14B**). Expression of PD-L1 in 293 cells significantly inhibited the killing activity, but this inhibition was completely reversed by PD-L1 blocking antibody. Together these results demonstrate the therapeutic benefit of HER2-TDB and antiPD-L1 combination treatment.

HER2-TDB + anti-PDL1 combination is effective in treatment of established CT26-HER2 tumors

[0324] In the next experiment using CD3-TG mice, a similar transient but significant response was seen with the HER2-TDB. In contrast to previous study, 2 complete responses were observed (**FIG. 14C**). Tumor growth was significantly slower in both of the single agent cohorts compared to the control mice, and the combination of HER2-TDB and PD-L1 blockade further improved the response (**FIG. 14C**). Combination resulted in durable responses; 60% of the mice lived tumor free until the study was terminated at 80 days after the first dose (not shown). In a repeat study (**FIG. 14D**), all mice responded to the combination, with 82% showing complete responses, and tumor growth was controlled by the treatment in all but one mouse in the combination cohort. In summary, combination of HER2-TDB with anti-PD-L1 immune therapy resulted in enhanced inhibition of tumor growth, increased response rates and durable responses.

[0325] The activity of HER2-TDB was characterized in this study and no evidence of T cell activation without HER2 binding was found. When target-expressing cells were present, HER2-TDB treatment resulted in a robust activation of T cells, release of cytotoxic granules,

and death of the HER2 expressing cells. Importantly, no bystander effect on non-target expressing cells was detected in conditions where most HER2+ cells in the same culture were killed. HER2-TDB induced proliferation and polyclonal expansion of T cells which may be critical for amplification of tumor-infiltrating lymphocytes.

[0326] The potency of HER2-TDB was consistently in the low picomolar to femtomolar range. Furthermore, as few as 10-500 HER2-bound TDBs were sufficient to induce significant *in vitro* cytotoxicity. As few as ~1000 copies of HER2 on the plasma membrane were sufficient to induce killing. These studies also demonstrated a correlation between target expression levels and *in vitro* sensitivity to HER2-TDB.

[0327] Finally, recruitment of T cell killing activity with HER2-TDB is dependent on HER2 expression, but independent of HER2 signaling pathway, which suggests that HER2-TDB may be efficient in treatment of tumors that are refractory to current anti-HER2 therapies. In accordance, data demonstrated equal activity in treatment of multiple trastuzumab/lapatinib resistant cell lines compared to sensitive cells. Resistance in these cells is generated by various mechanisms affecting HER2 pathway. Data presented here suggest that switching to alternative mechanism of action by using HER2-TDB may broadly enable overcoming resistance to antibody-drug conjugates (e.g., T-DM1), targeted small molecule inhibitors (e.g., lapatinib) and therapeutic monoclonal antibodies that block the pathway signaling (e.g. trastuzumab). The study demonstrated the potent *in vivo* activity of HER2-TDB using four independent model systems, including dramatic responses in MMTV-huHER2 transgenic mice. HuCD3 transgenic mice can be used as a novel efficacy model for the huCD3 targeting molecules. Importantly, this study discovered that PD-L1 expressed by the tumor cells can inhibit the activity of T cell recruiting antibodies and that this inhibition can be reversed by antiPD-L1 antibody. The finding suggests a potential general resistance mechanism for T cell recruiting molecules with vast diagnostic impact. The finding also provides a mechanistic rationale for combination of HER2-TDB with the PD-L1 blockade, which resulted in significant enhancement of responses and durable long term cures.

[0328] Taken together, this study presents a new immune-therapy for HER2+ breast cancer with an alternative, extremely potent mechanism of action that is broadly effective in cells resistant to current HER2 targeted therapies. Several significant advances are provided to bispecific T cell recruiting antibodies: i) charactering a critical resistance mechanism, ii) discovering a potential diagnosic, iii) introducing a novel huCD3 transgenic efficacy model and iv) significantly improving the drug-like properties by using technology based on full length antibodies with natural architecture. The benefit of combining two immune therapies:

direct polyclonal recruitment of T cell activity together with inhibiting the T cell suppressive PD-1/PD-L1 signaling) results enhanced and durable long term responses, was demonstrated.

Sequences of the antibody used in the Examples

α-PDL1 Light Chain Variable Region:
DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO:4)

α-PDL1 Heavy Chain Variable Region:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG
STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG
TLVTVSSASTK (SEQ ID NO:26)

α-PDL1 Full Length Light Chain:
DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:33)

α-PDL1 Full Length Heavy Chain:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG
STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:32)

CLAIMS

WHAT IS CLAIMED IS:

1. A method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a human PD-1 axis binding antagonist and an anti-HER2 antibody.

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

3. The method of claim 2, wherein the PD-1 axis binding antagonist is a PD-1 binding antagonist.

4. The method of claim 3, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to its ligand binding partners.

5. The method of claim 4, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1.

6. The method of claim 4, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2.

7. The method of claim 4, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2.

8. The method of claim 4, wherein the PD-1 binding antagonist is an antibody.

9. The method of claim 4, wherein the PD-1 binding antagonist is selected from the group consisting of MDX-1106 (nivolumab), MK-3475 (lambrolizumab), CT-011 (pidilizumab), and AMP-224.

10. The method of claim 2, wherein the PD-1 axis binding antagonist is a PD-L1 binding antagonist.

11. The method of claim 10, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1.

12. The method of claim 10, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1.

13. The method of claim 10, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1.

14. The method of any one of claims 11-13, wherein the PD-L1 binding antagonist is an antibody.

15. The method of claim 10, wherein the PD-L1 binding antagonist is selected from the group consisting of: YW243.55.S70, MPDL3280A, MDX-1105, and MEDI4736.

16. The method of claim 14, wherein the antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21; and a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24.

17. The method of claim 14, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 or 26 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4.

18. The method of claim 2, wherein the PD-1 axis binding antagonist is a PD-L2 binding antagonist.

19. The method of claim 18, wherein the PD-L2 binding antagonist is an antibody.

20. The method of claim 18, wherein the PD-L2 binding antagonist is an immunoadhesin.

21. The method of any one of claims 1-20, wherein the anti-HER2 antibody is trastuzumab or pertuzumab.

22. The method of any one of claims 1-20, wherein the anti-HER2 antibody comprises a heavy chain variable region comprising HVR-H1 sequence of SEQ ID NO:38, HVR-H2 sequence of SEQ ID NO:50, and HVR-H3 sequence of SEQ ID NO:40; and/or a light chain variable region comprising HVR-L1 sequence of SEQ ID NO:41, HVR-L2 sequence of SEQ ID NO:42, and HVR-L3 sequence of SEQ ID NO:43.

23. The method of any one of claims 1-20, wherein the anti-HER2 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:34 and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:35.

24. The method of any one of claims 1-20, wherein the anti-HER2 antibody is a multispecific antibody.

25. The method of any one of claims 1-20, wherein the anti-HER2 antibody is a bispecific antibody.

26. The method of claim 25, wherein the bispecific antibody comprises a first antigen binding domain that binds to HER2, and a second antigen binding domain that binds to CD3.

27. The method of claim 26, wherein the first antigen binding domain comprises a heavy chain variable region (V_HHER2) and a light chain variable region (V_LHER2), and the second antigen binding domain comprises a heavy chain variable region (V_HCD3) and a light chain variable region (V_LCD3).

28. The method of claim 27, wherein the first antigen binding domain comprises a heavy

chain variable region (V_HHER2) comprising HVR-H1 sequence of SEQ ID NO:38, HVR-H2 sequence of SEQ ID NO:50, and HVR-H3 sequence of SEQ ID NO:40; and/or a light chain variable region (V_LHER2) comprising HVR-L1 sequence of SEQ ID NO:41, HVR-L2 sequence of SEQ ID NO:42, and HVR-L3 sequence of SEQ ID NO:43.

29. The method of claim 28, wherein the heavy chain variable region (V_HHER2) comprises the amino acid sequence of SEQ ID NO:34 and/or a light chain variable region (V_LHER2) comprises the amino acid sequence of SEQ ID NO:35.

30. The method of any one of claims 26-29, wherein the second antigen binding domain binds to a human CD3 polypeptide.

31. The method of claim 30, wherein the CD3 polypeptide is a human CD3ε polypeptide or a human CD3γ polypeptide.

32. The method of claim 31, wherein the second antigen binding domain binds to a human CD3ε polypeptide or a human CD3 γ polypeptide in a native T-cell receptor (TCR) complex in association with other TCR subunits.

33. The method of any one of claims 25-32, wherein the bispecific antibody is a single-chain bispecific antibody comprising the first antigen binding domain and the second antigen binding domain.

34. The method of claim 33, wherein the single-chain bispecific antibody comprises variable regions, as arranged from N-terminus to C-terminus, selected from the group consisting of (1) V_HHER2-V_LHER2-V_HCD3-V_LCD3, (2) V_HCD3-V_LCD3-V_HHER2-V_LHER2, (3) V_HCD3-V_LCD3-V_LHER2-V_HHER2, (4) V_HHER2-V_LHER2-V_LCD3-V_HCD3, (5) V_LHER2-V_HHER2-V_HCD3-V_LCD3, or (6) V_LCD3-V_HCD3-V_HHER2-V_LHER2.

35. The method of any one of claims 25-32, wherein (a) the first antigen binding domain comprises one or more heavy chain constant domains, wherein the one or more heavy chain constant domains are selected from a first CH1 ($CH1_1$) domain, a first CH2 ($CH2_1$) domain, a first CH3 ($CH3_1$) domain; and (b) the second antigen binding domain comprises one or more heavy chain constant domains, wherein the one or more heavy chain constant domains are selected from a second CH1 ($CH1_2$) domain, second CH2 ($CH2_2$) domain, and a second CH3 ($CH3_2$) domain.

36. The method of claim 35, wherein at least one of the one or more heavy chain constant domains of the first antigen binding domain is paired with another heavy chain constant domain of the second antigen binding domain.

37. The method of claim 36, wherein the $CH3_1$ and $CH3_2$ domains each comprise a protuberance or cavity, and wherein the protuberance or cavity in the $CH3_1$ domain is

positionable in the cavity or protuberance, respectively, in the $CH3_2$ domain.

38. The method of claim 37, wherein the $CH3_1$ and $CH3_2$ domains meet at an interface between said protuberance and cavity.

39. The method of any one of claims 35-38, wherein the $CH2_1$ and $CH2_2$ domains each comprise a protuberance or cavity, and wherein the protuberance or cavity in the $CH2_1$ domain is positionable in the cavity or protuberance, respectively, in the $CH2_2$ domain.

40. The method of claim 39, wherein the $CH2_1$ and $CH2_2$ domains meet at an interface between said protuberance and cavity.

41. The method of any one of claims 1-40, wherein the anti-HER2 antibody comprises an aglycosylation site mutation.

42. The method of claim 41, wherein the aglycosylation site mutation is a substitution mutation.

43. The method of claim 42 wherein the substitution mutation is at amino acid residue N297, L234, L235, and/or D265 (EU numbering).

44. The method of claim 43, wherein the substitution mutation is selected from the group consisting of N297G, N297A, L234A, L235A, and D265A.

45. The method of claim 43, wherein the substitution mutation is a D265A mutation and an N297G mutation.

46. The method of any one of claims 41-45, wherein the aglycosylation site mutation reduces effector function of the anti-HER2 antibody.

47. The method of any one of claims 1-46, wherein the cancer is a HER2-positive cancer.

48. The method of claim 47, wherein the cancer is breast cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, colon cancer, kidney cancer, esophageal cancer, or prostate cancer.

49. The method of any one of claims 1-48, wherein the individual has cancer or has been diagnosed with cancer.

50. The method of claim 49, wherein cancer cells in the individual express PD-L1.

51. The method of claim 49 or 50, wherein the individual has cancer that is resistant to a HER2 targeted therapy.

52. The method of claim 49 or 50, wherein the individual is refractory to a HER2 targeted therapy.

53. The method of claim 51 or 52, wherein the HER2 targeted therapy is a treatment with an anti-HER2 antibody or an inhibitor of the HER2 pathway.

54. The method of claim 53, wherein the HER2 targeted therapy is a treatment with

trastuzumab, pertuzumab, ado-trastuzumab emtansine, or lapatinib.

55. The method of any one of claims 1-54, wherein the treatment results in a sustained response in the individual after cessation of the treatment.

56. The method of any one of claims 1-55, wherein the anti-HER2 antibody is administered before the PD-1 axis binding antagonist, simultaneous with the PD-1 axis binding antagonist, or after the PD-1 axis binding antagonist.

57. A method of enhancing immune function in an individual having cancer comprising administering an effective amount of a PD-1 axis binding antagonist and an anti-HER2 antibody.

58. The method of claim 57, wherein CD8 T cells in the individual have enhanced priming, activation, proliferation and/or cytolytic activity relative to prior to the administration of the PD-1 axis binding antagonist and the anti-HER2 antibody.

59. The method of claim 57, wherein the number of CD8 T cells is elevated relative to prior to administration of the combination.

60. The method of claim 59, wherein the CD8 T cell is an antigen-specific CD8 T cell.

61. The method of claim 57, wherein Treg function is suppressed relative to prior to the administration of the combination.

62. The method of claim 57, wherein T cell exhaustion is decreased relative to prior to the administration of the combination.

63. The method of any one of claims 57-62, wherein the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

64. The method of claim 63, wherein the PD-1 axis binding antagonist is a PD-1 binding antagonist.

65. The method of claim 64, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to its ligand binding partners.

66. The method of claim 65, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1.

67. The method of claim 65, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2.

68. The method of claim 65, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2.

69. The method of claim 65, wherein the PD-1 binding antagonist is an antibody.

70. The method of claim 65, wherein the PD-1 binding antagonist is selected from the

group consisting of MDX-1106 (nivolumab), MK-3475 (lambrolizumab), CT-011 (pidilizumab), and AMP-224.

71. The method of claim 63, wherein the PD-1 axis binding antagonist is a PD-L1 binding antagonist.

72. The method of claim 71, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1.

73. The method of claim 71, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1.

74. The method of claim 71, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1.

75. The method of any one of claims 71-74, wherein the PD-L1 binding antagonist is an antibody.

76. The method of claim 71, wherein the PD-L1 binding antagonist is selected from the group consisting of: YW243.55.S70, MPDL3280A, MDX-1105, and MEDI4736.

77. The method of claim 75, wherein the antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21; and a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24.

78. The method of claim 75, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 or 26 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4.

79. The method of claim 63, wherein the PD-1 axis binding antagonist is a PD-L2 binding antagonist.

80. The method of claim 79, wherein the PD-L2 binding antagonist is an antibody.

81. The method of claim 79, wherein the PD-L2 binding antagonist is an immunoadhesin.

82. The method of any one of claims 57-81, wherein the anti-HER2 antibody is trastuzumab or pertuzumab.

83. The method of any one of claims 57-81, wherein the anti-HER2 antibody comprises a heavy chain variable region comprising HVR-H1 sequence of SEQ ID NO:38, HVR-H2 sequence of SEQ ID NO:50, and HVR-H3 sequence of SEQ ID NO:40; and/or a light chain variable region comprising HVR-L1 sequence of SEQ ID NO:41, HVR-L2 sequence of SEQ ID NO:42, and HVR-L3 sequence of SEQ ID NO:43.

84. The method of any one of claims 57-81, wherein the anti-HER2 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:34 and/or a

light chain variable region comprising the amino acid sequence of SEQ ID NO:35.

85. The method of any one of claims 57-81, wherein the anti-HER2 antibody is a multispecific antibody.

86. The method of any one of claims 57-81, wherein the anti-HER2 antibody is a bispecific antibody.

87. The method of claim 86, wherein the bispecific antibody comprises a first antigen binding domain that binds to HER2, and a second antigen binding domain that binds to CD3.

88. The method of claim 87, wherein the first antigen binding domain comprises a heavy chain variable region (V_HHER2) and a light chain variable region (V_LHER2), and the second antigen binding domain comprises a heavy chain variable region (V_HCD3) and a light chain variable region (V_LCD3).

89. The method of claim 88, wherein the wherein the first antigen binding domain comprises a heavy chain variable region (V_HHER2) comprising HVR-H1 sequence of SEQ ID NO:38, HVR-H2 sequence of SEQ ID NO:50, and HVR-H3 sequence of SEQ ID NO:40; and/or a light chain variable region (V_LHER2) comprising HVR-L1 sequence of SEQ ID NO:41, HVR-L2 sequence of SEQ ID NO:42, and HVR-L3 sequence of SEQ ID NO:43.

90. The method of claim 88, wherein the heavy chain variable region (V_HHER2) comprises the amino acid sequence of SEQ ID NO:34 and/or the light chain variable region (V_LHER2) comprises the amino acid sequence of SEQ ID NO:35.

91. The method of any one of claims 87-90, wherein the second antigen binding domain binds to a human CD3 polypeptide.

92. The method of claim 91, wherein the CD3 polypeptide is a human CD3ε polypeptide or a human CD3γ polypeptide.

93. The method of claim 91, wherein the second antigen binding domain binds to a human CD3ε polypeptide or a human CD3 γ polypeptide in a native T-cell receptor (TCR) complex in association with other TCR subunits.

94. The method of any one of claims 87-90, wherein the bispecific antibody is a single-chain bispecific antibody comprising the first antigen binding domain and the second antigen binding domain.

95. The method of claim 94, wherein the single-chain bispecific antibody comprises variable regions, as arranged from N-terminus to C-terminus, selected from the group consisting of (1) V_HHER2-V_LHER2-V_HCD3-V_LCD3, (2) V_HCD3-V_LCD3-V_HHER2-V_LHER2, (3) V_HCD3-V_LCD3-V_LHER2-V_HHER2, (4) V_HHER2-V_LHER2-V_LCD3-V_HCD3, (5) V_LHER2-V_HHER2-V_HCD3-V_LCD3, or (6) V_LCD3-V_HCD3-V_HHER2-V_LHER2.

96. The method of any one of claims 87-93, wherein (a) the first antigen binding domain comprises one or more heavy chain constant domains, wherein the one or more heavy chain constant domains are selected from a first CH1 ($CH1_1$) domain, a first CH2 ($CH2_1$) domain, a first CH3 ($CH3_1$) domain; and (b) the second antigen binding domain comprises one or more heavy chain constant domains, wherein the one or more heavy chain constant domains are selected from a second CH1 ($CH1_2$) domain, second CH2 ($CH2_2$) domain, and a second CH3 ($CH3_2$) domain.

97. The method of claim 96, wherein at least one of the one or more heavy chain constant domains of the first antigen binding domain is paired with another heavy chain constant domain of the second antigen binding domain.

98. The method of claim 97, wherein the $CH3_1$ and $CH3_2$ domains each comprise a protuberance or cavity, and wherein the protuberance or cavity in the $CH3_1$ domain is positionable in the cavity or protuberance, respectively, in the $CH3_2$ domain.

99. The method of claim 98, wherein the $CH3_1$ and $CH3_2$ domains meet at an interface between said protuberance and cavity.

100. The method of any one of claims 96-99, wherein the $CH2_1$ and $CH2_2$ domains each comprise a protuberance or cavity, and wherein the protuberance or cavity in the $CH2_1$ domain is positionable in the cavity or protuberance, respectively, in the $CH2_2$ domain.

101. The method of claim 100, wherein the $CH2_1$ and $CH2_2$ domains meet at an interface between said protuberance and cavity.

102. The method of any one of claims 57-101, wherein the anti-HER2 antibody comprises an aglycosylation site mutation.

103. The method of claim 102, wherein the aglycosylation site mutation is a substitution mutation.

104. The method of claim 103, wherein the substitution mutation is at amino acid residue N297, L234, L235, and/or D265 (EU numbering).

105. The method of claim 104, wherein the substitution mutation is selected from the group consisting of N297G, N297A, L234A, L235A, and D265A.

106. The method of claim 104, wherein the substitution mutation is a D265A mutation and an N297G mutation.

107. The method of any one of claims 102-106, wherein the aglycosylation site mutation reduces effector function of the anti-HER2 antibody.

108. The method of any one of claims 57-107, wherein the cancer is a HER2-positive cancer.

109. The method of claim 108, wherein the cancer is breast cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, colon cancer, kidney cancer, esophageal cancer, or prostate cancer.

110. The method of any one of claims 57-109, wherein cancer cells in the individual express PD-L1.

111. The method of any one of claims 57-110, wherein the individual has cancer that is resistant to a HER2 targeted therapy.

112. The method of any one of claims 57-110, wherein the individual is refractory to a HER2 targeted therapy.

113. The method of claim 111 or 112, wherein the HER2 targeted therapy is a treatment with an anti-HER2 antibody or an inhibitor of the HER2 pathway.

114. The method of claim 113, wherein the HER2 targeted therapy is a treatment with trastuzumab, pertuzumab, ado-trastuzumab emtansine, or lapatinib.

115. The method of any one of claims 1-114, wherein the PD-1 axis binding antagonist and/or the anti-HER2 antibody are administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally.

116. The method of any one of claims 1-115, further comprising administering a chemotherapeutic agent for treating or delaying progression of cancer.

117. Use of a human PD-1 axis binding antagonist in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising an anti-HER2 antibody and an optional pharmaceutically acceptable carrier.

118. Use of an anti-HER2 antibody in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the anti-HER2 antibody and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising a human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

119. A composition comprising a human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in

combination with a second composition, wherein the second composition comprises an anti-HER2 antibody and an optional pharmaceutically acceptable carrier.

120. A composition comprising an anti-HER2 antibody and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in combination with a second composition, wherein the second composition comprises a human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

121. A kit comprising a medicament comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising an anti-HER2 antibody and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual.

122. A kit comprising a first medicament comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and a second medicament comprising an anti-HER2 antibody and an optional pharmaceutically acceptable carrier.

123. The kit of claim 122, wherein the kit further comprises a package insert comprising instructions for administration of the first medicament and the second medicament for treating or delaying progression of cancer in an individual.

124. A kit comprising a medicament comprising an anti-HER2 antibody and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual.