INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/012754

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

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

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

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

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

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

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

see additional sheet

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-125

Invention 1: Anti-PD1 antibody comprising a heavy chain comprising the amino acid sequence of SeqIdNo.91 and a light chain comprising the amino acid sequence of SeqIdNo.94; composition, nucleic acid, expression vector, host cell, production method and uses relating thereto

1.1. claims: 1-125

Inventions 2-21: As for invention 1, but relating respectively to the heavy and light chain sequences as recited in claims 65-84.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/012754

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006121168 A1	16-11-2006	AU 2006244885 A1	16-11-2006
		BR PI0610235 A2	08-06-2010
		CA 2607147 A1	16-11-2006
		CN 103059138 A	24-04-2013
		DK 2161336 T3	28-10-2013
		EP 1896582 A1	12-03-2008
		EP 2161336 A1	10-03-2010
		EP 2418278 A2	15-02-2012
		EP 2439272 A2	11-04-2012
		EP 2439273 A2	11-04-2012
		ES 2427646 T3	31-10-2013
		HK 1140793 A1	27-06-2014
		IL 187108 A	30-06-2011
		IL 208642 A	30-08-2012
		JP 4361545 B2	11-11-2009
		JP 5028700 B2	19-09-2012
		JP 2006340714 A	21-12-2006
		JP 2009155338 A	16-07-2009
		JP 2012158605 A	23-08-2012
		JP 2014077015 A	01-05-2014
		KR 20080011428 A	04-02-2008
		KR 20130032908 A	02-04-2013
		KR 20130114226 A	16-10-2013
		NZ 563193 A	28-05-2010
		PT 2161336 E	03-10-2013
		RU 2010135087 A	27-02-2012
		TW I379898 B	21-12-2012
		US 2009217401 A1	27-08-2009
		US 2013133091 A1	23-05-2013
		US 2014212422 A1	31-07-2014
		US 2014294852 A1	02-10-2014
		US 2014328833 A1	06-11-2014
		US 2014348743 A1	27-11-2014
		WO 2006121168 A1	16-11-2006
WO 2009114335 A2	17-09-2009	EP 2262837 A2	22-12-2010
		US 2011008369 A1	13-01-2011
		WO 2009114335 A2	17-09-2009
WO 2009101611 A1	20-08-2009	AU 2009213738 A1	20-08-2009
		CA 2715166 A1	20-08-2009
		CN 101970499 A	09-02-2011
		EP 2242773 A1	27-10-2010
		JP 2011512332 A	21-04-2011
		JP 2015038080 A	26-02-2015
		US 2011117085 A1	19-05-2011
		US 2014302032 A1	09-10-2014
		WO 2009101611 A1	20-08-2009
WO 2004056875 A1	08-07-2004	AT 514713 T	15-07-2011
		AU 2003288675 A1	14-07-2004
		AU 2010235966 A1	11-11-2010
		BR 0316880 A	25-10-2005
		CA 2508660 A1	08-07-2004
		CN 1753912 A	29-03-2006
		CN 101899114 A	01-12-2010
		EP 1576014 A1	21-09-2005
		ES 2367430 T3	03-11-2011

Form PCT/ISA/210 (patent family annex) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/012754

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		HK 1083510 A1	16-12-2011
		IL 169152 A	30-11-2010
		JP 4511943 B2	28-07-2010
		JP 2006521783 A	28-09-2006
		JP 2010189395 A	02-09-2010
		MX PA05006828 A	08-09-2005
		US 2004213795 A1	28-10-2004
		US 2006210567 A1	21-09-2006
		US 2008311117 A1	18-12-2008
		US 2010028330 A1	04-02-2010
		WO 2004056875 A1	08-07-2004
WO 2008083174 A2	10-07-2008	AU 2007339897 A1	10-07-2008
		BR PI0720724 A2	01-04-2014
		CA 2673870 A1	10-07-2008
		CN 101663323 A	03-03-2010
		CN 103536915 A	29-01-2014
		EP 2079760 A2	22-07-2009
		EP 2133365 A2	16-12-2009
		JP 5623747 B2	12-11-2014
		JP 2010514791 A	06-05-2010
		JP 2014210793 A	13-11-2014
		KR 20090094380 A	04-09-2009
		NZ 578650 A	22-12-2011
		NZ 594510 A	29-06-2012
		NZ 596237 A	27-07-2012
		NZ 600281 A	28-03-2013
		RU 2009128686 A	10-02-2011
		RU 2012153968 A	20-06-2014
		SG 183663 A1	27-09-2012
		US 2010040614 A1	18-02-2010
		US 2012251537 A1	04-10-2012
		WO 2008083174 A2	10-07-2008
WO 2010036959 A2	01-04-2010	AU 2009296392 A1	01-04-2010
		CA 2738252 A1	01-04-2010
		CN 102264762 A	30-11-2011
		EP 2342226 A2	13-07-2011
		JP 2012503984 A	16-02-2012
		KR 20110079673 A	07-07-2011
		US 2011271358 A1	03-11-2011
		US 2013291136 A1	31-10-2013
		WO 2010036959 A2	01-04-2010

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(54) Title: CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY

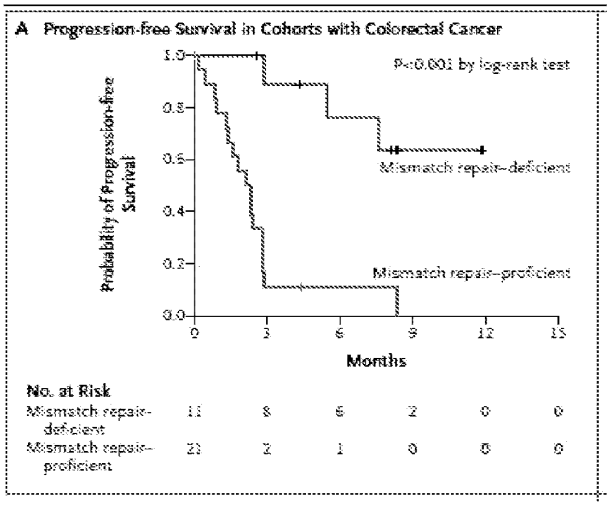

Fig. 2A

(57) Abstract: Blockade of immune checkpoints such as cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1) shows promise in patients with cancer. Inhibitory antibodies directed at these receptors have been shown to break immune tolerance and promote anti-tumor immunity. These agents work particularly well in patients with a certain category of tumor. Such tumors may be particularly susceptible to treatment because of the multitude of neoantigens which they produce.

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CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY

[01] This invention was made with government support under CA43460 and CA62924 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD OF THE INVENTION

[02] This invention is related to the area of cancer. In particular, it relates to cancer therapy.

BACKGROUND OF THE INVENTION

[03] Microsatellite instability (MSI) is the accumulation of sequencing errors in microsatellites. This occurs in tumors with deficiency in DNA mismatch repair. MSI is present in Lynch Syndrome which is an inherited cancer syndrome that predisposes patients to colon, endometrial, gastric cancer, ovarian, small intestine, liver, hepatobiliary, upper urinary tract, brain, and prostate cancer. MSI is also present in 10-20% of sporadic colorectal, gastric, prostate, lung, ampullary, and endometrial cancers. Between 0.3% and 13% of pancreatic cancers are reported to be MSI as well.

[04] The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in solid malignancies such as ovarian, colorectal and pancreatic cancer, hepatocellular carcinoma, malignant MEL and RCC. TILs can be expanded *ex vivo* and re-infused, inducing durable objective tumor responses in cancers such as melanoma.

[05] The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface

of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. The ligands for PD- 1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in various tumors. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. High expression of PD-L1 on tumor cells (and to a lesser extent of PD-L2) has been found to correlate with poor prognosis and survival in various cancer types, including renal cell carcinoma (RCC), pancreatic carcinoma, hepatocellular carcinoma, ovarian carcinoma and non-small cell lung cancer (NSCLC). Furthermore, PD-1 has been suggested to regulate tumor-specific T cell expansion in patients with malignant MEL. The observed correlation of clinical prognosis with PD-L1 expression in multiple cancers suggests that the PD- 1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

[06] Blockade of immune checkpoints such as cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1) is showing promise in patients with cancer. CTLA-4 and PD-1 are upregulated on activated T cells and provide inhibitory signals to T cells undergoing activation. Inhibitory antibodies directed at these receptors have been shown to break immune tolerance and promote anti-tumor immunity. MK-3475 is a humanized monoclonal IgG4 antibody against PD-1 and is showing activity in multiple tumor types including melanoma and non-small cell lung cancer (NSCLC). Previously, activity of a different PD-1 blocking antibody, BMS-936558, a fully humanized monoclonal IgG4 antibody, also showed activity in melanoma, NSCLC, and a complete response in a single patient with colorectal cancer.

[07] MK-3475 (previously known as SCH 900475) is a potent and highly-selective humanized mAb of the IgG4/kappa isotype designed to directly block the interaction

between PD-1 and its ligands, PD-L1 and PD-L2. MK-3475 contains the S228P stabilizing mutation and has no antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) activity. MK-3475 strongly enhances T lymphocyte immune responses in cultured blood cells from healthy human donors, cancer patients, and primates. In T- cell activation assays using human donor blood cells, the EC50 was in the range of 0.1 to 0.3 nM. MK-3475 also modulates the level of interleukin-2 (IL-2), tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ), and other cytokines. The antibody potentiates existing immune responses only in the presence of antigen and does not nonspecifically activate T- cells.

[08] The programmed death 1 (PD-1) pathway is a negative feedback system repressing Th1 cytotoxic immune responses that, if unregulated, could damage the host[1-3]. It is upregulated in many tumors and their surrounding microenvironment. Blockade of this pathway with antibodies to PD-1 or its ligands has led to remarkable clinical responses in some patients with many different cancer types, including melanomas, non-small cell lung cancer, renal cell carcinoma, bladder cancer and Hodgkin's lymphoma[4-10]. The expression of ligands to PD-1 (PD-L1 or PD-L2) on the surface of tumor cells or immune cells is important but not a definitive predictive biomarker for response to PD-1 blockade[4,6-8,11].

[09] We were intrigued that, in reports of the effects of PD-1 blockade in human tumors, only one of 33 colorectal cancer (CRC) patients responded to this treatment, in contrast to substantial fractions of patients with melanomas, renal cell cancers, and lung tumors.[10,12]. What was different about this single patient? We hypothesized that this patient had MMR-deficiency, because MMR-deficiency occurs in a small fraction of advanced CRCs,[13,14] somatic mutations found in tumors can be recognized by the patient's own immune system,[15] and MMR-deficient cancers have 10- to 100-fold more somatic mutations than MMR-proficient CRC.[16-18] Moreover, MMR-deficient cancers contain prominent lymphocyte infiltrates, consistent with an immune response[19-22]. And two of the tumor types that were most responsive to PD-1 blockade in a study by Topalian et al.[10] had high numbers of somatic mutations as a result of exposure to cigarette smoke (lung cancers) or UV radiation (melanomas)[23,24]. Our hypothesis was correct: the tumor of the single CRC patient who responded to PD-1

blockade was MMR-deficient[25]. We therefore hypothesized that MMR-deficient tumors are more responsive to PD-1 blockade than are MMR-proficient tumors.

[10] To test this hypothesis, we initiated a phase 2 clinical trial to evaluate immune checkpoint blockade in patients whose tumors had or did not have MMR-deficiency. Since MMR deficiency in tumors arises through two routes[26-28], we recruited patients with Hereditary Non-Polyposis Colorectal Cancer (HNPCC, also known as Lynch Syndrome), which results from an inherited germline defect in one of four MMR genes followed by a second inactivating somatic change in the remaining wild-type allele. We also recruited patients with sporadic MMR-deficient tumors, where both alleles of a MMR gene are inactivated by somatic mutations or by epigenetic silencing[29]. In either case, the neoplasms that arise harbor hundreds or thousands of mutations[16,18].

[11] There is a continuing need in the art to improve cancer treatments so that the lives of patients are not curtailed and so that the quality of life is not diminished.

SUMMARY OF THE INVENTION

[12] According to one embodiment of the invention a method of treating a cancer patient is provided. The cancer patient has a high mutational burden, such as found in microsatellite instable cancer (MSI). An immune checkpoint inhibitory antibody is administered to the cancer patient.

[13] According to another embodiment of the invention a method of treating a cancer patient is provided. A sample from a cancer patient is tested for one or more microsatellite markers selected from the group consisting of BAT-25, BAT-26, MON0-27, NR-21, NR-24, Penta C, and Penta D, and determined to have microsatellite instability. The cancer is selected from the group consisting of: colon, gastric, endometrial, cholangiocarcinoma, pancreatic, and prostate cancers. An anti-PD-1 antibody is administered to the cancer patient.

[14] According to another embodiment of the invention a method is provided for categorizing a tumor of a human. A sample from the human is tested to evaluate stability of one or more microsatellite markers. Microsatellite instability is determined in the sample. The tumor is identified as a good candidate for treatment with an immune checkpoint inhibitory antibody.

[15] According to yet another embodiment of the invention a method is provided for categorizing a tumor of a human. A sample from the human is tested to evaluate stability of one or more microsatellite markers. Microsatellite stability in the sample is determined. The tumor is identified as a bad candidate for treatment with an immune checkpoint inhibitory antibody.

[16] These and other embodiments which will be apparent to those of skill in the art upon reading the specification provide the art with methods for treating microsatellite instable cancers.

BRIEF DESCRIPTION OF THE DRAWINGS

[17] Figs. 1A-1B. Clinical Responses to pembrolizumab. (Fig. 1A) Biochemical Responses. Serum protein biomarker levels were measured with each cycle and the values represent percent change from baseline. Patients were included if baseline tumor marker values were greater than the upper limit of normal. CA-125 was used for a patient with endometrial cancer; CA19-9 was used for one cholangiocarcinoma and one ampullary cancer; and CEA was used for all other patients. Green, red, and black lines represent patients with MMR-deficient CRCs, MMR-proficient CRCs, and MMR-deficient non-CRC, respectively. (Fig. 1B) Radiographic responses. Tumor responses were measured at regular intervals and values show the best fractional change of the sum of longest diameters (SLD) from the baseline measurements of each measurable tumor.

[18] Figs. 2A-2D. Clinical benefit to pembrolizumab according to MMR status. Kaplan-Meier curves are shown for (Fig. 2A) progression-free survival in the colorectal cancer cohorts, (Fig. 2B) overall survival in the colorectal cancer cohorts, (Fig. 2C)

progression-free survival of patients with MMR-deficient cancers other than colorectal (median PFS=5.4 months; 95% CI, 3% to not estimable), and (Fig. 2D) overall survival of patients with MMR-deficient cancers other than colorectal. In both cohorts with MMR-deficient tumors (CRC and non-CRC), median overall survival was not reached. Patients in the cohort with MMR-proficient cancers had a median PFS of 2.2 months (95% CI 1.4 to 2.8%) and a median OS of 5.0 months (95% CI 3.0 to not estimable).

[19] Fig. 3 (Figure S2.) Spider plot of radiographic response. Tumor responses were measured at regular intervals and values show percent change of the sum of longest diameters (SLD) from the baseline measurements of each measurable tumor. Patients were only included if baseline and on study treatment scans were available. Green and red represent patients with MMR-deficient and proficient CRCs, respectively. Blue represents patients with MMR-deficient cancers other than CRC.

[20] Figs. 4A-4B (Figure S3). MMR-proficient and deficient CRCs have comparable time on treatment and duration of metastatic disease prior to study enrollment. Kaplan-Meier estimates of (Fig. 4A) time on therapy immediately prior to study enrollment (HR 0.81, 95% CI 0.38 to 1.752, p=.60) and (Fig. 4B) duration of metastatic disease prior to enrollment (HR 1.13, 95% CI 0.49 to 2.62, p=.78) on this pembrolizumab study were comparable between the MMR-deficient and proficient CRC cohorts. The short duration on prior therapy is expected in a treatment refractory CRC population.

[21] Fig. 5 (Figure S4.) Waterfall plot of biochemical response. Serum protein biomarker levels were measured with each cycle and the values represent best percent change from baseline. Patients were included if baseline tumor marker values were greater than the upper limit of normal. CA-125 was used for a patient with endometrial cancer; CA19-9 was used for 1 cholangiocarcinoma and 1 ampullary cancer; and CEA was used for all other patients. Green and red represent patients with MMR-deficient and proficient CRCs, respectively. Blue represents patients with MMR-deficient cancers other than CRC.

[22] Figs. 6A-6B (Figure S5.) Somatic mutations in MMR-deficient and proficient tumors. Total somatic mutations per tumor identified by exome sequencing of tumor and matched normal DNA (Fig. 6A) and correlation with objective responses (Fig. 6B) (non-parametric Wilcoxon test , p= 0.007 and Jonckheere-Terpstra test for trend, p=0.02).

[23] Fig. 7 (Figure S6). Immunohistochemistry of CD8 and PD-L1 Expression. The invasive front (yellow dashed line) from a MMR-deficient CRC (subject #16, top) and MMR-proficient CRC (subject #3, bottom). The yellow dashed line separates tumor (T) and normal (N) tissue. There is marked expression of PD-L1 (blue arrows) and CD8 (brown dots) in the MMR-deficient tumor (top panels) patient while there is very little expression of either marker in the MMR-proficient tumor (bottom panels). Representative images of tumor infiltrating lymphocytes (TIL) in another MMR-deficient CRC (subject #19, top) and MMR-proficient CRC (subject #3, bottom) immunolabeled with an antibody to CD8 (brown dots). Note the infiltration of CD8 cells in the MMR-deficient tumor. Invasive front original magnification 10x and TIL 20x.

[24] Fig. 8 (Figure S7.) CD8 and PD-L1 Expression in the MMR-deficient and MMR-proficient tumor microenvironment. T cell density units are cells/mm2 of tumor. Invasive front refers to the immune cells (TILs and macrophages) at the junction of the tumor and normal tissue. P-values obtained using an unpaired t-test.

[25] Fig. 9 (Figure S8.) CD8 expression and clinical benefit to pembrolizumab. Correlation between the intratumoral $CD8^+$ T cell density (cells/mm2) and objective response (Jonckheere-Terpstra test for trend, p=0.02).

[26] Fig. 10 (Table S1.) Comparison of immune-related and RECIST response criteria (adapted from Wolchok et al. Clin Can Res 2009;15:7412-20.)

[27] Fig. 11 (Table S2.) Immune-Related response to treatment

[28] Fig. 12 (Table S4.) Correlation of total somatic mutations and mutation associated neoantigens (MANA) with clinical outcomes

[29] Fig. 13 (Table S5.) Correlation of immune markers with clinical outcome

DETAILED DESCRIPTION OF THE INVENTION

[30] The inventors have found that immune checkpoint inhibitors work best in tumors with high mutation burdens. Furthermore, tumors deficient in mismatch repair are particularly susceptible to a particular form of immunotherapy because this phenotype results in ongoing accumulation of mutations at a high frequency. The inventors have developed a treatment for cancer patients that display the microsatellite instability phenotype or other high mutational burden. The treatment involves an inhibitory antibody for an immune checkpoint. Such checkpoints include PD-1, IDO, CTLA-4, PD-L1, and LAG-3. Other immune checkpoints can be used as well. Antibodies can be administered by any means that is convenient, including but not limited to intravenous infusion, oral administration, subcutaneous administration, sublingual administration, ocular administration, nasal administration, etc.

[31] Microsatellite instability (MSI) tumors are deficient in DNA mismatch repair which leads to a high rate of spontaneous mutations and the potential for the expression of neo-antigens. Furthermore, similar to melanoma, in MSI positive colon cancers, there is often prominent lymphocyte infiltration. Any tumors that are MSI or otherwise high mutational burden may be treated according to the invention. They may be tested for the attribute of MSI according to any method known in the art, including but not limited that described in example 1 below. Any of one or more MSI markers can be tested to determine an MSI phenotype. Samples may be tested for high mutational burden by identifying tumors with at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least

1100, at least 1200, at least 1300, at least 1400, at least 1500, or at least 1600 mutations per tumor genome. High mutational burden means a large number of somatic mutations in the tumor relative to normal tissues of the individual. An average number of somatic mutations in a non-MSI tumor is about 70 somatic mutations.

[32] Any type of tumor that displays the MSI phenotype or a high mutational burden may be tested and/or treated according to the invention. These include without limitation cancers of the colon, gastric, endometrial, cholangiocarcinoma, pancreatic, and prostate cancer. Tumors of the ampulla, biliary, brain, including glioma, breast, lung, skin, esophagus, liver, kidney, ovaries, sarcoma, uterus, cervix, bladder, testes, oral cavity, tongue, and small and large bowel may also be tested and/or treated.

[33] Testing of MSI can be accomplished by any means known in the art. One or more of the following markers may be tested: five nearly monomorphic mononucleotide repeat markers (BAT-25, BAT-26, MON0-27, NR-21 and NR-24) and two highly polymorphic pentanucleotide repeat markers (Penta C and Penta D). In one commercial system which can be used, fluorescently labeled primers (marker panel) are used for co-amplification of all seven of the above named markers. Fragments are detected after amplification for assignment of genotype/phenotype.

[34] Samples that can be tested for MSI include tumor tissue as well as body fluids that contain nucleic acids shed from tumors. Testing for tumor DNA in such tissues and body fluids is well known.

[35] Types of antibodies which can be used include any that are developed for the immune checkpoint inhibitors. These can be monoclonal or polyclonal. They may be single chain fragments or other fragments of full antibodies, including those made by enzymatic cleavage or recombinant DNA techniques. They may be of any isotype, including but not limited to IgG, IgM, IgE. The antibodies may be of any species source, including human, goat, rabbit, mouse, cow, chimpanzee. The antibodies may be humanized or chimeric. The antibodies may be conjugated or engineered to be

attached to another moiety, whether a therapeutic molecule or a tracer molecule. The therapeutic molecule may be a toxin, for example.

[36] The data from the small phase 2 trial of pembrolizumab to treat tumors with and without deficiency of MMR supports the hypothesis that MMR-deficient tumors are more responsive to PD-1 blockade than are MMR-proficient tumors. MMR-deficiency occurs in many cancers, including those of the colorectum, uterus, stomach, biliary tract, pancreas, ovary, prostate and small intestine[18,34-42]. Patients with MMR-deficient tumors of these types also benefit from anti-PD-1 therapy, as may patients whose tumors contain other DNA repair deficiencies, such as those with mutations in *POLD, POLE, or MYH.*[18,43,44]

[37] The hypothesis that MMR-deficient tumors stimulate the immune system is not a new idea[45], and has been supported by the dense immune infiltration and Th1-associated cytokine-rich environment observed in MMR-deficient tumors.[19-22,46] A recent study refined these classic observations by showing that the MMR-deficient tumor microenvironment strongly expressed several immune checkpoint ligands including PD-1, PD-L1, CTLA-4, LAG-3 and IDO, indicating that their active immune microenvironment is counterbalanced by immune inhibitory signals that resists tumor elimination[47]. That the immune infiltrate associated with MMR-deficient carcinomas was directed at neoantigens was the most likely explanation for both the old and new findings. The correlation of higher mutational load and higher response rate to anti-CTLA-4 in melanoma[41] and anti-PD-1 in lung cancer[48] provide further support for the idea that MANA recognition is an important component of the endogenous anti-tumor immune response.

[38] Based on the results of the current and previous studies, we suggest that the greatly (>20-fold) increased number of mutation-associated neoantigens resulting from MMR deficiency (Fig.. 12 (Table S4); also available on line at New England Journal of Medicine; incorporated by reference herein) is the basis for the enhanced anti-PD-1 responsiveness of this genetically defined subset of cancers. Though our estimates for the number of mutation-associated neoantigens in tumors is based only on *in silico* predictions of binding-affinity, this suggestion is consistent with the observation that

MMR-proficient tumors have far less infiltration of lymphocytes than MMR-deficient tumors (Fig. 7 (S6), Fig. 8 (S7) and Fig. 13 (Table S5); available on line at New England Journal of Medicine; incorporated by reference herein). Recent studies[49,50] show that only a tiny proportion of predicted neo-epitopes are actually presented on the cell surface with MHC and are targets of endogenous T cell responses. It seems likely, though that the number of predicted mutation-associated neoantigens is proportionate to the number of actual mutation-associated neoantigens, and tumors with a high number of actual mutation-associated neoantigens are more likely to stimulate the immune system to react against the tumor. Alternative mechanisms underlying the difference in anti-PD-1 responsiveness between MMR-deficient and MMR-proficient tumors should also be considered. For example, different signaling pathways activated in MMR-deficient and MMR-proficient tumors may result in differences in secretion of soluble factors that could result in differential activation of the PD-1 pathway within the tumor microenvironment[26-28]. Genetic differences could effect epigenetic differences that alter the expression of tumor-associated self-antigens that in turn could alter the antigenicity of the tumor. Experimental analyses of antigen-specific immune responses as well as changes in immune microenvironments should help to define the relative contribution of these factors to the striking responsiveness of MMR-deficient tumors to PD-1 antibodies.

[39] Several notable observations were made during the course of this study. First, changes in serum protein biomarkers, like CEA, corresponded with clinical benefit after a single dose of therapy. Declines in CEA levels preceded objective radiographic evidence by several months; perhaps other biomarkers such as circulating tumor DNA (ctDNA) may also be beneficial as surrogate markers of early response.[51,52] Second, our results suggest that the evaluation of tumor genomes can help guide immunotherapy. They support the view that the number and type of alterations may prove useful for judging the potential utility of immune checkpoint inhibitors, even in MMR-proficient cancers[41,48,53] Most importantly, our results demonstrate a new approach for the treatment of a specific class of tumors based solely on genetic status: *i.e.,* without regard to underlying tumor type.

[40] The above disclosure generally describes the present invention. All references disclosed herein are expressly incorporated by reference. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLE 1

MSI Testing

[41] MSI testing is already standardized and performed in CLIA-certified laboratories without need for assay development. Archived tumor samples or newly obtained biopsies will be used for determining MSI. MSI status will be performed locally by CLIA certified immunohistochemistry (IHC) or PCR based tests for eligibility. Evaluable patients will be confirmed using the MSI Analysis System from Promega at Johns Hopkins. This test will determine MSI status through the insertion or deletion of repeating units in the five nearly monomorphic mononucleotide repeat markers (BAT-25, BAT-26, MON0-27, NR-21 and NR-24). At least 2 MSI loci are required to be evaluable in Cohorts A and C. Patients may be assigned to a new cohort and/or replaced based on the Promega test results.

EXAMPLE 2

METHODS

[42] Patients

[43] Treatment-refractory progressive metastatic cancer patients for this phase 2 study were recruited from three participating centers (Table 1). Three cohorts were evaluated: Cohort A was composed of patients with MMR-deficient colorectal adenocarcinomas; Cohort B was composed of patients with MMR-proficient colorectal adenocarcinomas; and Cohort C was composed of patients with MMR-deficient cancers of types other than colorectal.

[44] Study Oversight

[45] The protocol, which can be found at NEJM.org, was approved by each site's institutional review boards, and the study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. All the patients provided written informed consent before study entry. The principal investigator (D.L.) and study sponsor (L.A.D.) were responsible for oversight of the study. Merck donated the study drug, reviewed the final drafts of the protocol and of this manuscript. The clinical study was primarily funded through philanthropic support.

[46] Study Design

[47] This phase 2 trial was conducted using a Green-Dahlberg two-stage design and consisted of the three parallel cohorts described above. The study agent, pembrolizumab (Merck), was administered at 10 mg/kg intravenously every 14 days. Pembrolizumab is a humanized monoclonal anti-PD-1 antibody of the IgG4/kappa isotype that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

[48] Safety assessments were performed before each treatment. Assessments of total tumor burden via measurements of serum biomarkers were performed at the start of each cycle. Radiologic assessments were made at 12 weeks and every 8 weeks thereafter. Further details concerning the clinical protocol are provided in the Example 3.

[49] Analysis of mismatch repair status

[50] Tumors with genetic defects in MMR pathways are known to harbor thousands of somatic mutations, especially in regions of repetitive DNA known as microsatellites. The accumulation of mutations in these regions of the genome is termed microsatellite instability (MSI) [26-28]. MMR-status was assessed using the MSI Analysis System from Promega in tumors, through the evaluation of selected microsatellite sequences particularly prone to copying errors when MMR is compromised[26-28]. See Supplementary Appendix for additional details.

[51] Genomic & Bioinformatic Analyses

[52] Primary tumor samples and matched normal peripheral-blood specimens were obtained from a subset of subjects with MMR-deficient and others with MMR-proficient carcinomas where sufficient tumor tissue was available for exome sequencing[30] and HLA haplotyping. To assess the potential for mutant peptide binding, somatic exome data combined with the individual patient's MHC class I HLA haplotype was applied to the an epitope prediction algorithm[31,32]. This algorithm provided an estimate of the total number of mutation-associated neoantigens in each tumor. Additional details are provided in the Supplementary Appendix (available on line at New England Journal of Medicine; incorporated by reference herein).

[53] Statistical Analysis

[54] The primary endpoints for Cohorts A and B were immune-related objective response rate (irORR) and immune-related progression-free survival (irPFS) rate at 20 weeks assessed using immune-related response criteria (irRC)[33].The primary endpoint for Cohort C was irPFS rate at 20 weeks. Immune-related criteria (i.e, criteria used to evaluate immune-based therapies) are based on radiographic responses, and unlike RECIST criteria, capture extent of disease after disease progression; these criteria are defined and compared to RECIST v1.1 in Fig. 10 (Table S1). Response rate and PFS rate at 20 weeks were evaluated and reported in this study using RECIST v1.1 and irRC (Fig. 10 (Table S1)). PFS and overall survival was summarized by Kaplan-Meier method. Details of the hypothesis, the decision rules to reject the null hypotheses and early-stopping rules for efficacy and futility, and statistical methods are provided in the Supplementary Appendix.

EXAMPLE 3

SUPPLEMENTARY METHODS

[55] PATIENTS

[56] To be eligible for participation in this study, patients had to be at least 18 years of age, have histologically confirmed evidence of previously-treated, progressive carcinoma. All patients underwent MMR status testing prior to enrollment. All patients had at

least one measurable lesion as defined by the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, an Eastern Cooperative Oncology Group (ECOG) performance-status score of 0 or 1, and adequate hematologic, hepatic, and renal function. Eligible patients with CRC must have received at least 2 prior cancer therapies and patients with other cancer types must have received at least 1 prior cancer therapy. Patients with untreated brain metastases, history of HIV, hepatitis B, hepatitis C, clinically significant ascites/effusions, or autoimmune disease were excluded.

[57] STUDY OVERSIGHT

[58] Initial drafts of the manuscript were prepared by a subset of the authors and all authors contributed to the final manuscript. All the authors made the decision to submit the manuscript for publication. The principal investigator and study sponsor vouch for the accuracy and completeness of the data reported as well as adherence to the protocol.

[59] HLA TYPING

[60] HLA-A, HLA-B and HLA-C Sequence Based Typing can be divided into three distinct steps, as described below. A generic, A*02 specific, B generic, B group specific, C generic and C*07 specific PCR and sequencing mixes were made in the JHU core facility. Celera's AlleleSEQR HLA-B Sequence Based Typing kit was used for B generic SBT. The HLA-A typing scheme is composed of two PCR reactions, A generic and A*02 specific. A generic amplicon encompasses partial exon 1- partial exon 5. A*02 amplicon encompasses partial intron 1 - partial exon 5. HLA-B typing scheme is composed of two PCR reactions, B generic and B group specific. The B generic PCR is a multiplexed reaction containing two PCR amplicons encompassing exon 2 – exon 3 and exon 4 – exon 7. B group specific amplicon encompasses partial intron 1 - partial exon 5. HLA-C typing scheme is composed of two PCR reactions, C generic and C*07 specific. C generic and C*07 specific amplicons encompasses exons 1 - 7.

[61] The specificity of the HLA-A and B PCR employed AmpliTaq Gold DNA polymerase. The GeneAmp High Fidelity enzyme is used for the HLA-C and C*07 PCR mixes. This enzyme is a mix of two polymerases: AmpliTaq DNA polymerase (non-proofreading polymerase) and a proofreading polymerase. This enzyme mix is necessary to produce efficient and robust amplification of the larger full length HLA-C amplicon.

[62] PCR product purification was performed using Exonuclease I and Shrimp Alkaline Phosphatase The A generic and B generic amplicons were bi-directionally sequenced for exons 2,3,4. The C generic amplicon was bi-directionally sequenced for exons 2,3 and sequenced in a single direction for exons 1,4,5,6,7. A*02 specific, B group specific and C*07 specific amplicons were sequenced in a single direction for exons 2,3. All sequencing reactions were performed with Big Dye Terminator V1.1 from Applied Biosystems and sequenced with an ABI Prism 3500XL Genetic Analyzer. Conexio Genomic's "Assign SBT" allele assignment software was used to process the data files.

[63] MISMATCH REPAIR STATUS TESTING[1,2]

[64] Six slides of tumor and normal (uninvolved lymph node or margin of resection) were cut (5 microns each), deparaffinized (xylene), and one stained with hematoxylin and eosin (H+E). A tumor area containing at least 20% neoplastic cells, designated by a board-certified Anatomic Pathologist was macrodissected using the Pinpoint DNA isolation system (Zymo Research, Irvine, CA), digested in proteinase K for 8 hours and DNA was isolated using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA). MSI was assessed using the MSI Analysis System (Promega, Madison, WI), composed of 5 pseudomonomorphic mononucleotide repeats (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) to detect MSI and 2-pentanucleotide repeat loci (PentaC and PentaD) to confirm identity between normal and tumor samples, per manufacturer's instructions. Following amplification of 50-100 ng DNA, the fluorescent PCR products were sized on an Applied Biosystems 3130xl capillary electrophoresis instrument (Invitrogen, Calsbad, CA). Pentanucleotide loci confirmed identity in all cases. Controls included water as a negative control and a mixture of 80% germline DNA with 20% MSI

cancer DNA as a positive control. The size in bases was determined for each microsatellite locus and tumors were designated as MSI if two or more mononucleotide loci varied in length compared to the germline DNA.

[65] SEQUENCING ANALYSIS

[66] Samples

[67] Samples provided as FFPE blocks or frozen tissue underwent pathological review to determine tumor cellularity. Tumors were macrodissected to remove contaminating normal tissue, resulting in samples containing >20% neoplastic cells. Matched normal samples were provided as blood, saliva or normal tissue obtained from surgery.

[68] Sample Preparation and Next-Generation Sequencing[3]

[69] Sample preparation, library construction, exome capture, next generation sequencing, and bioinformatics analyses of tumor and normal samples were performed at Personal Genome Diagnostics, Inc. (Baltimore, Maryland). In brief, DNA was extracted from frozen or formalin-fixed paraffin embedded (FFPE) tissue, along with matched blood or saliva samples using the Qiagen DNA FFPE tissue kit or Qiagen DNA blood mini kit (Qiagen, CA). Genomic DNA from tumor and normal samples were fragmented and used for Illumina TruSeq library construction (Illumina, San Diego, CA) according to the manufacturer's instructions or as previously described4. Briefly, 50 nanograms (ng) - 3 micrograms (μg) of genomic DNA in 100 microliters (μl) of TE was fragmented in a Covaris sonicator (Covaris, Woburn, MA) to a size of 150-450bp. To remove fragments smaller than 150bp, DNA was purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 0.9 of PCR product to beads twice and washed using 70% ethanol per the manufacturer's instructions. Purified, fragmented DNA was mixed with 36 μl of H2O, 10 μl of End Repair Reaction Buffer, 5 μl of End Repair Enzyme Mix (cat# E6050, NEB, Ipswich, MA). The 100 μl end-repair mixture was incubated at 20°C for 30 min, and purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 1.25 of PCR

product to beads and washed using 70% ethanol per the manufacturer's instructions. To A-tail, 42 µl of end-repaired DNA was mixed with 5 µl of 10X dA Tailing Reaction Buffer and 3 µl of Klenow (exo-)(cat# E6053, NEB, Ipswich, MA). The 50 µl mixture was incubated at 37°C for 30 min and purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 1.0 of PCR product to beads and washed using 70% ethanol per the manufacturer's instructions. For adaptor ligation, 25 µl of A-tailed DNA was mixed with 6.7 µl of H2O, 3.3 µl of PE-adaptor (Illumina), 10 µl of 5X Ligation buffer and 5 µl of Quick T4 DNA ligase (cat# E6056, NEB, Ipswich, MA). The ligation mixture was incubated at 20°C for 15 min and purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 0.95 and 1.0 of PCR product to beads twice and washed using 70% ethanol per the manufacturer's instructions. To obtain an amplified library, twelve PCRs of 25 µl each were set up, each including 15.5 µl of H2O, 5 µl of 5 x Phusion HF buffer, 0.5 µl of a dNTP mix containing 10 mM of each dNTP, 1.25 µl of DMSO, 0.25 µl of Illumina PE primer #1, 0.25 µl of Illumina PE primer #2, 0.25 µl of Hotstart Phusion polymerase, and 2 µl of the DNA. The PCR program used was: 98°C for 2 minutes; 12 cycles of 98°C for 15 seconds, 65°C for 30 seconds, 72°C for 30 seconds; and 72°C for 5 min. DNA was purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 1.0 of PCR product to beads and washed using 70% ethanol per the manufacturer's instructions. Exonic or targeted regions were captured in solution using the Agilent SureSelect v.4 kit according to the manufacturer's instructions (Agilent, Santa Clara, CA). The captured library was then purified with a Qiagen MinElute column purification kit and eluted in 17 µl of 70°C EB to obtain 15 µl of captured DNA library. (5) The captured DNA library was amplified in the following way: Eight 30uL PCR reactions each containing 19 µl of H2O, 6 µl of 5 x Phusion HF buffer, 0.6 µl of 10 mM dNTP, 1.5 µl of DMSO, 0.30 µl of Illumina PE primer #1, 0.30µl of Illumina PE primer #2, 0.30 µl of Hotstart Phusion polymerase, and 2 µl of captured exome library were set up. The PCR program used was: 98°C for 30 seconds; 14 cycles (exome) or 16 cycles (targeted) of 98°C for 10 seconds, 65°C for 30 seconds, 72°C for 30 seconds; and 72°C for 5 min. To purify PCR products, a NucleoSpin Extract II purification kit (Macherey-Nagel, PA) was used following the manufacturer's instructions. Paired-end sequencing, resulting in 100 bases from each end of the fragments for exome libraries and 150 bases from each end of the fragment

for targeted libraries, was performed using Illumina HiSeq 2000/2500 and Illumina MiSeq instrumentation (Illumina, San Diego, CA).

[70] Primary Processing of Next-Generation Sequencing Data and Identification of Putative Somatic Mutations3

[71] Somatic mutations were identified using VariantDx custom software (Personal Genome Diagnostics, Baltimore, Maryland) for identifying mutations in matched tumor and normal samples. Prior to mutation calling, primary processing of sequence data for both tumor and normal samples were performed using Illumina CASAVA software (v1.8), including masking of adapter sequences. Sequence reads were aligned against the human reference genome (version hg18) using ELAND with additional realignment of select regions using the Needleman-Wunsch method 5. Candidate somatic mutations, consisting of point mutations, insertions, and deletions were then identified using VariantDx across the either the whole exome or regions of interest. VariantDx examines sequence alignments of tumor samples against a matched normal while applying filters to exclude alignment and sequencing artifacts. In brief, an alignment filter was applied to exclude quality failed reads, unpaired reads, and poorly mapped reads in the tumor. A base quality filter was applied to limit inclusion of bases with reported phred quality score > 30 for the tumor and > 20 for the normal. A mutation in the tumor was identified as a candidate somatic mutation only when (i) distinct paired reads contained the mutation in the tumor; (ii) the number of distinct paired reads containing a particular mutation in the tumor was at least 10% of read pairs; (iii) the mismatched base was not present in >1% of the reads in the matched normal sample as well as not present in a custom database of common germline variants derived from dbSNP; and (iv) the position was covered in both the tumor and normal at > 150X. Mutations arising from misplaced genome alignments, including paralogous sequences, were identified and excluded by searching the reference genome.

[72] Candidate somatic mutations were further filtered based on gene annotation to identify those occurring in protein-coding regions. Functional consequences were predicted using snpEff and a custom database of CCDS, RefSeq and Ensembl annotations using

the latest transcript versions available on hg18 from UCSC (https://genome.ucsc.edu/). Predictions were ordered to prefer transcripts with canonical start and stop codons and CCDS or Refseq transcripts over Ensembl when available. Finally mutations were filtered to exclude intronic and silent changes, while retaining mutations resulting in missense mutations, nonsense mutations, frameshifts, or splice site alterations. A manual visual inspection step was used to further remove artifactual changes.

[73] MUTANT PEPTIDE MHC BINDING PREDICTION

[74] Somatic frameshift, insertions, deletions, and missense mutations predicted to result in an amino acid change were analyzed for potential MHC class I binding based on the individual patient's HLA haplotype. Our initial analysis focused on HLA-A and HLA-B. Amino acid mutations were linked to their corresponding CCDS accession number and in instances where this was unavailable, either a Refseq or ensemble transcript was used to extract the protein sequence. To identify 8mer, 9mer, and 10mer epitopes, amino acid fragments surrounding each mutation were identified. These 15, 17, and 19 mutant amino acid fragments were analyzed by the epitope prediction program NetMHC 3.4.6 Epitopes with a predicted affinity of <50nm were considered to be strong potential binders and epitopes with a predicted affinity of <500nm were considered to be weak potential binders as suggested by the NetMHC group6.

[75] To further refine the total neoantigen burden, we repeated that same process for the complementary wild-type peptide for each mutant peptide. We then filtered for mutant peptides that were strong potential binders when the complementary wild-type peptide was predicted a weak potential binder. These mutant peptides are referred to as mutation-associated neoantigens (MANA). In the event that a patient had a (e.g., cases 1, 17 and 21) single MHC haplotype not supported by NetMHC 3.4, the individual haplotype was not included in our analysis.

[76] STATISTICAL METHODS

[77] Design of the trial[7]

[78] This trial was conducted using a parallel two-stage design to simultaneously evaluate the efficacy of MK-3475 and MSI as a treatment selection marker for anti-PD-1 therapy. It consisted of two-stage phase 2 studies in parallel in the three cohorts of patients described in the text. The study agent, MK-3475, was administered at 10 mg/kg intravenously every 14 days.

[79] For each of Cohort A and B, the co-primary endpoints were progression-free-survival (irPFS) at 20 weeks and objective response (irOR) assessed using immune related criteria. A step-down gatekeeping procedure was used to preserve the overall type I error. A two-stage Green-Dahlberg design was used to evaluate irPFS, with interim and final analysis after 15 and 25 patients, respectively. At stage 1, $\geq$ 1 of 15 free-of-progression at 20 weeks were required to proceed to the second stage, and $\geq$ 4 of 25 free-of-progression at 20 weeks were then required to proceed to test for irOR, with $\geq$ 4 of 25 responders (irCR or irPR) indicating promising efficacy in that cohort. Each cohort could be terminated for efficacy as soon as $\geq$ 4 free-of-progression at 20 weeks and $\geq$ 4 responses were confirmed, or be terminated for futility as soon as 0 of 15 in stage 1 were free-of-progression at 20 weeks or $\geq$ 22 subjects had disease progression by 20 weeks. This design achieves 90% power to detect a 20-week irPFS rate of 25% and 80% power to detect an irOR rate (irORR) of 21%, with an overall type I error of 0.05 at the null hypothesis of 20-week irPFS rate of 5% and irORR of 5%.

[80] For Cohort C, the primary endpoint was irPFS at 20 weeks. A two-stage Green-Dahlberg two-stage design was used, with an interim and final analysis after 14 and 21 patients; at stage 1, $\geq$ 1 of 14 free-of-progression at 20 weeks were required to proceed to the second stage, with $\geq$ 4 of 21 free-of-progression at 20 weeks at the end indicating adequate efficacy in Cohort C. The cohort could be terminated as soon as $\geq$ 4 free-of-progression at 20 weeks were confirmed. The design has 81% power to detect a 20-week irPFS rate of 25% with a 5% type I error at the null hypothesis of 20-week irPFS rate of 5%.

[81] Statistical analysis

[82] Response and progression were evaluated using RECIST v1.1 and the immune-related response criteria (irRC) adopted from Wolchok et al.8, which uses the sum of the products of bidimensional tumor measurements and incorporates new lesions into the sum. Progression-free survival (PFS) rates and irPFS rate at 20-weeks was estimated as the proportion of patients who were free-of-disease progression and alive at 20 weeks after the initiation of pembrolizumab. Patients who had disease progression prior to 20 weeks or were enrolled for >20 weeks at the time the study data were collated were included in the analysis for estimating 20-week PFS (irPFS) rate. Patients who dropped out early due to toxicities or worsening disease and therefore did not have 20-week tumor assessment were considered as having progressive disease. ORR (irORR) was the proportion of patients who achieved best overall response of CR or PR (irCR or irPR). Patients who were in the study long enough to have tumor response evaluations were included in the analysis for estimating response rates. Among those who responded (CR or PR), duration of response was the time of first RECIST response to the time of disease progression, and was censored at the last evaluable tumor assessment for responders who had not progressed.

[83] PFS and irPFS were defined as the time from the date of initial dose to the date of disease progression or the date of death due to any cause, whichever occurred first. PFS and irPFS were censored on the date of the last evaluable tumor assessment documenting absence of progressive disease for patients who were alive and progression-free. Overall survival (OS) was defined as the time from the date of initial dose to death due to any cause. For patients who were still alive at the time of analysis, the OS time was censored on the last date the patients were known to be alive. Survival times were summarized by the Kaplan-Meier method. As a post hoc analysis, log-rank tests were used to compare Cohort A and B and hazard ratios were estimated based on Cox models.

[84] The association of percent CEA decline after 1 cycle with PFS or OS was assessed using landmark analysis based on Cox regression models. For correlative studies, non-parametric Wilcoxon test was used to compare mutational load between MMR-deficient and MMR-proficient patients. The effects of baseline mutational burden and

immune markers on response and survival times were examined using logistic regression and Cox regression, respectively.

[85] IMMUNOHISTOCHEMISTRY & IMAGE ANALYSIS

[86] The fraction of malignant cells exhibiting a membranous pattern of B7-H1 expression and the percentage at the invasive front were quantified by three pathologists (R.A.A., F.B., and J.M.T.) as previously reported9,10. Image analysis was used to determine the number of CD8 diaminobenzidine (DAB)-stained cells. Using the H&E-stained slide for each case, we identified the following regions: i) tumor, ii) invasive front (the boundary between malignant and non-malignant tissue), and iii) normal tissue. The CD8-stained slides were scanned at 20x equivalent magnification (0.49 micrometers per pixel) on an Aperio ScanScope AT. Regions corresponding to tumor, invasive front and normal tissue (above, from the H&E) were annotated on separate layers using Aperio ImageScope v12.1.0.5029.

[87] CD8-positive lymphocyte density was calculated in each of the above regions using a custom algorithm implemented in PIP11. Results were converted to Deepzoom images using the VIPS library12 and visualized using the OpenSeadragon viewer (http://openseadragon.github.io).

[88] References for Example 3 only.

1. Bacher JW, Flanagan LA, Smalley RL, et al. Development of a fluorescent multiplex assay for detection of MSI-High tumors. Disease markers 2004;20:237-50.

2. Murphy KM, Zhang S, Geiger T, et al. Comparison of the microsatellite instability analysis system and the Bethesda panel for the determination of microsatellite instability in colorectal cancers. The Journal of molecular diagnostics : JMD 2006;8:305-11.

3. Jones S, Anagnostou V, Lytle K, et al. Personalized genomic analyses for cancer mutation discovery and interpretation. Science translational medicine 2015;7:283ra53.

4. Sausen M, Leary RJ, Jones S, et al. Integrated genomic analyses identify ARID1A and ARID1B alterations in the childhood cancer neuroblastoma. Nature genetics 2013;45:12-7.

5. Needleman SB, Wunsch CD. A general method applicable to the search for similarities in the amino acid sequence of two proteins. Journal of molecular biology 1970;48:443-53.

6. Lundegaard C, Lamberth K, Harndahl M, Buus S, Lund O, Nielsen M. NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8-11. Nucleic acids research 2008;36:W509-12.

7. Buyse M, Michiels S, Sargent DJ, Grothey A, Matheson A, de Gramont A. Integrating biomarkers in clinical trials. Expert review of molecular diagnostics 2011;11:171-82.

8. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clinical cancer research : an official journal of the American Association for Cancer Research 2009;15:7412-20.

9. Llosa NJ, Cruise M, Tam A, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov 2015:43-51.

10. Taube JM, Anders RA, Young GD, et al. Colocalization of Inflammatory Response with B7-H1 Expression in Human Melanocytic Lesions Supports an Adaptive Resistance Mechanism of Immune Escape. Science Translational Medicine 2012;4:127ra37.

11. Cuka N, Hempel H, Sfanos K, De Marzo A, Cornish T. PIP: An Open Source Framework for Multithreaded Image Analysis of Whole Slide Images. LABORATORY INVESTIGATION 2014;94:398A-A.

12. Cupitt J, Martinez K. VIPS: an image processing system for large images. Electronic Imaging: Science & Technology; 1996: International Society for Optics and Photonics. p. 19-28.

EXAMPLE 4

[89] Patients

[90] 41 consecutive patients were enrolled and treated between September 2013 and January 2015. (Table 1). Recruitment included patients in pursuit of a clinical trial option who were known to have tumors with mismatch repair, or who had tumors of unknown status who were then tested. One patient in the MMR-deficient CRC cohort was enrolled under an IRB eligibility waiver allowing a grade 3 bilirubin level. A total of 32 CRC patients were enrolled into Cohorts A and B. All CRC patients received ≥2 prior chemotherapy regimens (median=4) except for one MMR-proficient patient who had received one chemotherapeutic and one (non-PD1-based) immunotherapeutic regimen.

[91] Nine subjects diagnosed with MMR-deficient solid tumors other than CRC were enrolled onto Cohort C. All Cohort C patients received ≥1 prior cancer treatments (median=2).

EXAMPLE 5

[92] Primary endpoint evaluation

[93] The irORR and irPFS at 20 weeks (Fig. 11 (Table S2)) for Cohort A were 40% (4 of 10 patients; 95% CI, 12 to 74%) and 78% (7 of 9 patients; 95% CI, 40 to 97%) and for Cohort C were 71% (5 of 7 patients; 95% CI, 29 to 96%) and 67% (4 of 6 patients; 95% CI, 22 to 96%). In Cohort B, comprised of patients with MMR-proficient CRCs, irORR and 20-week irPFS were 0% (95%CI, 0 to 20%) and 11% (2 of 18 patients; 95% CI, 1 to 35%). Both the MMR-deficient cohorts A and C reached their predefined early stopping rule for efficacy when four subjects were free-of-disease progression at 20 weeks and four objective responses were observed based on immune-related response criteria (Fig. 11 (Table S2); available on line at New England Journal of Medicine; incorporated by reference herein; and supplementary methods, above).

[94] The median time of follow-up for patients was 32 weeks (range, 5-51 weeks) for patients with MMR-deficient CRC (Cohort A), 12 weeks (range, 2-56 weeks) for patients with MMR-proficient CRC (Cohort B) and 12 weeks (range, 4-42 weeks) for patients with MMR-deficient non-CRC tumors (Cohort C). All patients evaluable for 20-week irPFS were followed for at least 20 weeks.

EXAMPLE 6

[95] Radiographic evaluation

[96] Of the ten evaluable MMR-deficient CRC patients in Cohort A, four (40%; 95% CI, 12-74%) achieved objective responses by RECIST criteria (Table 2, Fig. 1 and Fig. 3 (S2)). Patients were considered not evaluable unless they underwent a 12-week scan. The disease control rate was defined as the fraction of patients who achieved an objective response or whose disease was stable, and was 90% in Cohort A (9 of 10 patients; 95% CI, 55-100%).

[97] Of the seven evaluable patients with MMR-deficient cancer types other than CRC enrolled in Cohort C, five (71%; 95% CI, 29-96%) achieved objective responses (Table 2, Fig. 3 (S2) and Fig. 1) using RECIST criteria and the disease control rate was 71% (5 of 7 patients; 95% CI, 29-96%).

[98] Patients in Cohort C responded faster than patients in Cohort A (median time to response by RECIST of 12 vs. 28 weeks, p=0.03). Furthermore, all six MMR-deficient tumors that were not associated with Lynch syndrome (100%) achieved an objective response, whereas only three of eleven tumors (27%) associated with Lynch Syndrome responded (Table S3; p=0.009; available on-line at New England Journal of Medicine; and incorporated by reference herein). No other baseline characteristics showed statistically significant association with objective responses.

[99] Of the 18 patients with MMR-proficient CRCs in Cohort B, no objective responses were observed (Table 2, Fig. 3 (S2) and Fig. 1) using RECIST criteria and the disease control rate was 11% (2 of 18 patients; 95%CI, 1 to 35%).

[100] All patients who achieved a response by RECIST criteria (Fig. 11 (Table 2)) also achieved a response by immune-related response criteria (Fig. 11 (Table S2)).

EXAMPLE 7

[101] Survival

[102] In Cohort A, the patients with MMR-deficient CRC, median progression-free survival (PFS) and median overall survival (OS) were not reached (Fig. 2). In contrast, the patients with MMR-proficient cancers in Cohort B achieved a PFS of only 2.2 months (95% CI, 1.4-2.8) and a median OS of 5.0 months (95% CI, 3.0 to not estimable). In Cohort C (MMR-deficient non-CRC), the median PFS was 5.4 months (95% CI, 3 to not estimable) and the median OS was not reached.

[103] A *post hoc* (Fig. 2) comparison of the MMR-deficient and proficient CRC cohorts showed hazard ratios (HR) for disease progression (HR=0.10; 95% CI, 0.03-0.37; $p<0.001$) and overall survival (HR = 0.22; 95% CI, 0.05-1.00; $p=0.05$), favoring patients with MMR-deficient CRC.

[104] To evaluate whether the difference in survival might be due to prognostic differences, we measured the duration of time patients had been diagnosed with metastatic disease and the clinical performance of patients on their previous regimen prior to enrollment. We found that there was no significant difference between MMR-deficient vs. MMR-proficient CRC patients with respect to their duration of metastatic disease ($p=0.77$; Log-rank test) or median PFS ($p=0.60$, Log-rank test) on their prior regimens (Fig. 4 (S3)). We also performed an additional multivariate analysis of PFS and OS to examine the difference in outcomes between MMR-deficient CRC and MMR-proficient tumors adjusting for elapsed time since initial diagnosis. The magnitude of the hazard ratios for PFS (HR 0.04, 95% CI 0.01-0.21, $P<0.001$) and OS (HR 0.18, 95% CI 0.03-1.01, $P=0.05$), representing the different effect of pembrolizumab between MMR-deficient and MMR-proficient tumors, was maintained after adjusting for this potential difference.

EXAMPLE 8

[105] Safety Assessment

[106] Adverse events occurring in > 5% of patients are listed in Table 3. Select adverse events included rash/pruritus (24%), thyroiditis/hypothyroidism/hypophysitis (10%), and asymptomatic pancreatitis (15%). While the numbers were small, thyroid function abnormalities were limited to the MMR-deficient cohorts (Table 3).

EXAMPLE 9

[107] Tumor markers

[108] In the two CRC cohorts, baseline CEA levels were evaluable and above the upper limit of normal (3 mg/dl), in 29 of 32 patients prior to enrollment. Major CEA declines occurred in seven of the ten patients with MMR-deficient CRC and in none of the 19 patients with MMR-proficient CRCin which CEA was evaluable (Fig. 1 and Fig. 5 (S4)). In non-CRC MMR-deficient patients, tumor marker levels (CEA, CA19-9 or CA-125) were elevated above the upper limit of normal in four patients. CA19-9 or CA-125 declines of > 70% occurred in three of these four patients. Tumor marker kinetics of all 3 cohorts are shown in Figure 1. The level of CEA decline after 1 dose (between days 14 and 28) of pembrolizumab was predictive of both progression-free (p=0.01) and overall survival outcomes (p=0.02). The CEA response occurred well in advance of radiographic confirmation of disease control (range, 10 to 35 weeks). In contrast, patients who progressed showed rapid biomarker elevation within 30 days of initiating therapy. Thus, changes in CEA levels significantly preceded and correlated with ultimate radiographic changes.

EXAMPLE 10

[109] Genomic Analysis

[110] Analysis of whole-exome sequences showed an average of 1,782 somatic mutations per tumor in MMR-deficient patients (n = 9) compared with 73 mutations per tumor in MMR-proficient patients (n=6) (non-parametric Wilcoxon test, p=0.007) (Figs. 6A-

6B (S5); see also Table S3 which is available on-line at New England Journal of Medicine; incorporated by reference herein). Most (63%) of these mutations are predicted to alter amino acids.

[111] These mutations were then assessed for their immunogenic potential in the context of each patient's individual MHC haplotype. We thereby identified an average of 578 and 21 potential mutation-associated neoantigens from the tumors of MMR-deficient and MMR-proficient patients, respectively (Table S3; which is available on-line at New England Journal of Medicine; incorporated by reference herein). The fraction of potential mutation-associated neoantigens among all somatic mutations was similar in both cohorts (averaging 32% and 29% in MMR-deficient and -proficient patients, respectively). High numbers of somatic mutations and potential mutation-associated neoantigens were associated with improved progression-free survival and with a trend in favor of objective response (Fig. 13 (S5) and Fig. 12 (Table S4); also available on line at New England Journal of Medicine; incorporated by reference herein).

EXAMPLE 11

[112] Immunohistochemistry

[113] Expression of CD8 and PD-L1 were evaluated by immunohistochemistry within the tumor and at the invasive fronts of the tumor in the 30 cases in which tumor tissue was available (Fig. 7 (S6); also available on line at New England Journal of Medicine; incorporated by reference herein). Tumors from patients in Cohorts A and C contained a greater density of CD8-positive lymphoid cells than did tumors from Cohort B patients (Fig. 8 (S7); p=0.10) and CD8-labeling was associated with a trend favoring objective response and stable disease (Fig. 9 (S8) and Fig. 13 (Table S5); also available on line at New England Journal of Medicine; incorporated by reference herein). This CD8-positive lymphoid infiltrate was especially prominent at the invasive fronts of the tumors (Fig 8 (S7); p=0.04). Significant membranous PD-L1 expression only occurred in MMR-deficient patients and was prominent on tumor infiltrating lymphocytes (TILs) and tumor-associated macrophages located at the tumors' invasive fronts (Fig. 8 (S7); p=0.04). Expression of CD8 and PD-L1 were not statistically associated with PFS or OS (Fig. 13 (Table S5)).

Table 1. Demographic and Baseline Characteristics of Patients

Characteristic	MMR-deficient CRC n=11	MRC-proficient CRC n=21	P values[1]	MMR-deficient non-CRC n=9
Age – years				
median	46	61	0.02	57
range	(24-65)	(32-79)		(34-92)
Sex – no. (%)				
Female	5(45)	8(38)	0.72	4(44)
Male	6(55)	13(62)		5(56)
Race – no. (%)				
white	8(73)	17(81)	0.66	8(89)
black	1(9)	3(14)		0(0)
other	2(18)	1(5)		1(11)
ECOG Performance Status – no. (%)[2]				
0	0(0)	6(29)	0.07	2(22)
1	11(100)	15(71)		7(78)
Diagnosis – no. (%)				
Colon	9(82)	18(86)	>0.99	0(0)
Rectal	2(18)	3(14)		0(0)
Ampullary/Cholangiocarcinoma	0(0)	N/A		4(44)
Endometrial	0(0)	N/A		2(22)
Small bowel	0(0)	N/A		2(22)
Gastric	0(0)	N/A		1(11)
Histology – no. (%)				
Well/moderately differentiated	7(64)	18(86)	0.20	4(44)
Poorly differentiated	4(36)	3(14)		3(33)
Other	0(0)	0(0)		2(22)
Stage IV – no. (%)	(11)100	21(100)	>0.99	9(100)
Liver metastases – no. (%)	6(55)	11(52)	>0.99	6(67)
Time since first diagnosis - months				
median	31	58	0.07	23
range	6-95	27-192		2-105
Prior systemic therapies – no. (%)				
0	0(0)	0(0)	0.89	1(11)
2	3(27)	4(19)		5(56)
3	3(27)	5(24)		1(11)
>4	5(45)	12(57)		2(22)
Detected germline mutation or known Lynch – no. (%)				
Yes	9(82)	0(0)	<0.001	4(44)
No	2(18)	21(100)		4(44)
Unknown	0(0)	0(0)		1(11)
BRAF wild type – no. (%)				
Yes	8(73)	11(52)	0.64	4(44)
No	0(0)	1(5)		0(0)
Unknown	3(27)	9(43)		5(56)
KRAS wild type – no. (%)				
Yes	6(55)	13(62)	0.72	4(44)
No	5(45)	8(38)		1(11)
Unknown	0(0)	0(0)		4(44)

MMR, mismatch repair; CRC, colorectal cancer
[1] MMR-deficient CRC versus MMR-proficient CRC
[2] ECOG, Eastern Cooperative Oncology Group

Table 2. Objective RECIST responses

Type of Response – no. (%)	MMR-deficient CRC n=10	MRC-proficient CRC n=18	MMR-deficient non-CRC n=7
Complete Response	0(0)	0(0)	1(14)[1]
Partial Response	4(40)	0(0)	4(57)[2]
Stable Disease (Week 12)	5(50)	2(11)	0(0)
Progressive Disease	1(10)	11(61)	2(29)
Not Evaluable[3]	0(0)	5(28)	0(0)
Objective Response Rate (%)	40	0	71
95% CI	12-74	0-19	29-96
Disease Control Rate (%)[4]	90	11	71
95% CI	55-100	1-35	29-96
Duration of Response – median weeks	Not reached	N/A[5]	Not reached
Time to Response, median weeks (range)	28 (13-35)	N/A[5]	11 (10-13)

[1] Originally PR at 12 weeks that was converted to CR at 20 weeks
[2] One PR at 12 weeks
[3] Patients were considered not evaluable if they did not undergo a 12 week scan due to clinical progression.
[4] The rate of disease control was defined as the percentage of patients who had a complete response, partial response or stable disease for 12 weeks or more.
[5] No responses recorded for MMR-proficient CRC patients

Table 3. Drug-Related Adverse Events

Event – no (%)[1]	All Grades	Grade 3 or 4
	N=41	N=41
Any	40(98)	17(41)
Blood and Lymphatic		
Anemia	8(20)	7(17)
Lymphopenia	8(20)	8(20)
Cardiac		
Sinus tachycardia	4(10)	0
Dermatologic		
Dry skin	5(12)	0
Rash/pruritis	10(24)	0
Endocrine Disorders		
Thyroiditis/Hypothyroidism/Hypophysitis	4(10)	0
Gastrointestinal		
Abdominal Pain	10(24)	0
Anorexia	4(10)	0
Constipation	8(20)	0
Diarrhea	10(24)	2(5)
Dry mouth	5(12)	0
Nausea	5(12)	0
Bowel Obstruction	3(7)	3(7)
Hepatobiliary		
ALT, elevated	3(7)	2(5)
Pancreatitis[2]	6(15)	0
Metabolism and Nutrition		
Hypoalbuminemia	4(10)	4(10)
Hyponatremia	3(7)	3(7)
Musculoskeletal		
Arthralgia	7(17)	0
Myalgia	6(15)	0
Nervous System		
Dizziness	4(10)	0
Headache	7(17)	0
Psychiatric		
Insomnia	3(7)	0
Respiratory[3]		
Allergic Rhinitis	12(29)	0
Cough	4(10)	0
Dyspnea	6(15)	0
Upper Respiratory Infection	3(7)	0
Other		
Cold intolerance	6(15)	0
Edema	4(10)	0
Fatigue	13(32)	0
Fever	5(12)	0
Pain	14(34)	0

[1] Adverse Events occurring in greater than 5% of patients
[2] All cases of pancreatitis were asymptomatic
[3] One incidence of pneumonitis (2%)

References

The disclosure of each reference cited is expressly incorporated herein.

1. Nishimura H, Okazaki T, Tanaka Y, et al. Autoimmune Dilated Cardiomyopathy in PD-1 Receptor-Deficient Mice. Science 2001;291:319-22.

2. Chen L. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. Nat Rev Immunol 2004;4:336-47.

3. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of Lupus-like Autoimmune Diseases by Disruption of the PD-1 Gene Encoding an ITIM Motif-Carrying Immunoreceptor. Immunity 1999;11:141-51.

4. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 Blockade with Nivolumab in Relapsed or Refractory Hodgkin's Lymphoma. The New England journal of medicine 2015;372:311-9.

5. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. The New England journal of medicine 2013;369:134-44.

6. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014;515:563-7.

7. Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 2014;515:558-62.

8. Topalian SL, Sznol M, McDermott DF, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2014;32:1020-30.

9. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. The New England journal of medicine 2012;366:2455-65.

10. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. The New England journal of medicine 2012;366:2443-54.

11. Taube JM, Klein A, Brahmer JR, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clinical cancer research : an official journal of the American Association for Cancer Research 2014;20:5064-74.

12. Brahmer JR, Drake CG, Wollner I, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:3167-75.

13. Koopman M, Kortman GAM, Mekenkamp L, et al. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. Br J Cancer 0000;100:266-73.

14. Goldstein J, Tran B, Ensor J, et al. Multicenter retrospective analysis of metastatic colorectal cancer (CRC) with high-level microsatellite instability (MSI-H). Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2014;25:1032-8.

15. Segal NH, Parsons DW, Peggs KS, et al. Epitope landscape in breast and colorectal cancer. Cancer research 2008;68:889-92.

16. Timmermann B, Kerick M, Roehr C, et al. Somatic mutation profiles of MSI and MSS colorectal cancer identified by whole exome next generation sequencing and bioinformatics analysis. PloS one 2010;5:e15661.

17. Eshleman JR, Lang EZ, Bowerfind GK, et al. Increased mutation rate at the hprt locus accompanies microsatellite instability in colon cancer. Oncogene 1995;10:33-7.

18. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012;487:330-7.

19. Dolcetti R, Viel A, Doglioni C, et al. High prevalence of activated intraepithelial cytotoxic T lymphocytes and increased neoplastic cell apoptosis in colorectal carcinomas with microsatellite instability. The American journal of pathology 1999;154:1805-13.

20. Alexander J, Watanabe T, Wu TT, Rashid A, Li S, Hamilton SR. Histopathological identification of colon cancer with microsatellite instability. The American journal of pathology 2001;158:527-35.

21. Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. Cancer 2001;91:2417-22.

22. Young J, Simms LA, Biden KG, et al. Features of colorectal cancers with high-level microsatellite instability occurring in familial and sporadic settings: parallel pathways of tumorigenesis. The American journal of pathology 2001;159:2107-16.

23. Berger MF, Hodis E, Heffernan TP, et al. Melanoma genome sequencing reveals frequent PREX2 mutations. Nature 2012;485:502-6.

24. Lee W, Jiang Z, Liu J, et al. The mutation spectrum revealed by paired genome sequences from a lung cancer patient. Nature 2010;465:473-7.

25. Lipson EJ, Sharfman WH, Drake CG, et al. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. Clinical cancer research : an official journal of the American Association for Cancer Research 2013;19:462-8.

26. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology 2010;138:2073-87 e3.

27. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. The New England journal of medicine 2003;348:919-32.

28. Yamamoto H, Imai K, Perucho M. Gastrointestinal cancer of the microsatellite mutator phenotype pathway. Journal of gastroenterology 2002;37:153-63.

29. Herman JG, Umar A, Polyak K, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proceedings of the National Academy of Sciences of the United States of America 1998;95:6870-5.

30. Jones S, Anagnostou V, Lytle K, et al. Personalized genomic analyses for cancer mutation discovery and interpretation. Science translational medicine 2015;7:283ra53.

31. Lundegaard C, Lamberth K, Harndahl M, Buus S, Lund O, Nielsen M. NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8-11. Nucleic acids research 2008;36:W509-12.

32. Lundegaard C, Lund O, Nielsen M. Accurate approximation method for prediction of class I MHC affinities for peptides of length 8, 10 and 11 using prediction tools trained on 9mers. Bioinformatics 2008;24:1397-8.

33. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clinical cancer

research : an official journal of the American Association for Cancer Research 2009;15:7412-20.

34. Maple JT, Smyrk TC, Boardman LA, Johnson RA, Thibodeau SN, Chari ST. Defective DNA mismatch repair in long-term (> or =3 years) survivors with pancreatic cancer. Pancreatology 2005;5:220-7; discussion 7-8.

35. Meltzer SJ, Yin J, Manin B, et al. Microsatellite instability occurs frequently and in both diploid and aneuploid cell populations of Barrett's-associated esophageal adenocarcinomas. Cancer research 1994;54:3379-82.

36. Nakata B, Wang YQ, Yashiro M, et al. Prognostic value of microsatellite instability in resectable pancreatic cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 2002;8:2536-40.

37. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513:202-9.

38. Agaram NP, Shia J, Tang LH, Klimstra DS. DNA mismatch repair deficiency in ampullary carcinoma: a morphologic and immunohistochemical study of 54 cases. American journal of clinical pathology 2010;133:772-80.

39. Kandoth C, Schultz N, Cherniack AD, et al. Integrated genomic characterization of endometrial carcinoma. Nature 2013;497:67-73.

40. Garg K, Leitao MM, Jr., Kauff ND, et al. Selection of endometrial carcinomas for DNA mismatch repair protein immunohistochemistry using patient age and tumor morphology enhances detection of mismatch repair abnormalities. The American journal of surgical pathology 2009;33:925-33.

41. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. The New England journal of medicine 2014;371:2189-99.

42. Williams AS, Huang WY. The analysis of microsatellite instability in extracolonic gastrointestinal malignancy. Pathology 2013;45:540-52.

43. Jones S, Emmerson P, Maynard J, et al. Biallelic germline mutations in MYH predispose to multiple colorectal adenoma and somatic G:C→T:A mutations. Human Molecular Genetics 2002;11:2961-7.

44. Palles C, Cazier J-B, Howarth KM, et al. Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. Nature genetics 2013;45:136-44.

45. Bodmer W, Bishop T, Karran P. Genetic steps in colorectal cancer. Nature genetics 1994;6:217-9.

46. Kim H, Jen J, Vogelstein B, Hamilton SR. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. The American journal of pathology 1994;145:148-56.

47. Llosa NJ, Cruise M, Tam A, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov 2015:43-51. [This disclosure was made by some of the joint inventors.]

48. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015.

49. Gubin MM, Zhang X, Schuster H, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 2014;515:577-81.

50. Linnemann C, van Buuren MM, Bies L, et al. High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma. Nature medicine 2015;21:81-5.

51. Lipson EJ, Velculescu VE, Pritchard TS, et al. Circulating tumor DNA analysis as a real-time method for monitoring tumor burden in melanoma patients undergoing treatment with immune checkpoint blockade. Journal for immunotherapy of cancer 2014;2:42.

52. Diaz LA, Jr., Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2014;32:579-86.

53. Yadav M, Jhunjhunwala S, Phung QT, et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature 2014;515:572-6.

We claim:

1. A method of treating a cancer patient comprising:

administering to a cancer patient an immune checkpoint inhibitory antibody, wherein the cancer patient has a microsatellite instable cancer (MSI).

2. The method of claim 1 wherein the cancer is selected from the group consisting of: colon, gastric, endometrial, cholangiocarcinoma, pancreatic, and prostate cancers.
3. The method of claim 1 wherein the antibody is an anti-PD-1 antibody.
4. The method of claim 1 wherein the antibody is an anti-IDO antibody.
5. The method of claim 1 wherein the antibody is an anti-CTLA-4 antibody.
6. The method of claim 1 wherein the antibody is an anti-PD-L1 antibody.
7. The method of claim 1 wherein the antibody is an anti-LAG-3 antibody.
8. The method of claim 1 wherein a sample from the MSI cancer patient is tested to evaluate stability of one or more microsatellite markers prior to the step of administering.
9. The method of claim 1 wherein the antibody is a humanized monoclonal antibody.
10. The method of claim 1 wherein the antibody is MK-3475.
11. The method of claim 1 wherein the antibody is an IgG4 antibody.
12. The method of claim 8 wherein a plurality of microsatellite markers is tested.
13. The method of claim 8 wherein the one or more microsatellite markers is selected from the group consisting of BAT-25, BAT-26, MON0-27, NR-21, NR-24, Penta C, and Penta D.
14. The method of claim 12 wherein the plurality of microsatellite markers comprises BAT-25, BAT-26, MON0-27, NR-21, NR-24, Penta C, and Penta D.

15. A method of treating a cancer patient comprising:

testing a sample from a cancer patient and determining instability of one or more microsatellite markers selected from the group consisting of BAT-25, BAT-26, MON0-27, NR-21, NR-24, Penta C, and Penta D; and

administering to the cancer patient an anti-PD-1 antibody, wherein the cancer is selected from the group consisting of: colon, gastric, endometrial, cholangiocarcinoma, pancreatic, and prostate cancers.

16. A method of categorizing a tumor of a human, comprising:

testing a sample from the human to evaluate stability of one or more microsatellite markers;

determining microsatellite instability in the sample;

identifying the tumor as a good candidate for treatment with an immune checkpoint inhibitory antibody.

17. The method of claim 16 further comprising the step of:

prescribing an immune checkpoint inhibitory antibody for the human.

18. The method of claim 16 wherein the one or more microsatellite markers is selected from the group consisting of BAT-25, BAT-26, MON0-27, NR-21, NR-24, Penta C, and Penta D.

19. The method of claim 16 wherein the one or more microsatellite markers is selected from the group consisting of BAT-25, BAT-26, MON0-27, NR-21, NR-24, Penta C, and Penta D.

20. A method of categorizing a tumor of a human, comprising:

testing a sample from the human to evaluate stability of one or more microsatellite markers;

determining microsatellite stability in the sample;

identifying the tumor as a bad candidate for treatment with an immune checkpoint inhibitory antibody.

21. The method of claim 20 wherein the human is being treated with an immune checkpoint inhibitory antibody, and further comprising the step of ceasing treatment with an immune checkpoint inhibitory antibody.

22. The method of claim 20 wherein the one or more microsatellite markers is selected from the group consisting of BAT-25, BAT-26, MON0-27, NR-21, NR-24, Penta C, and Penta D.

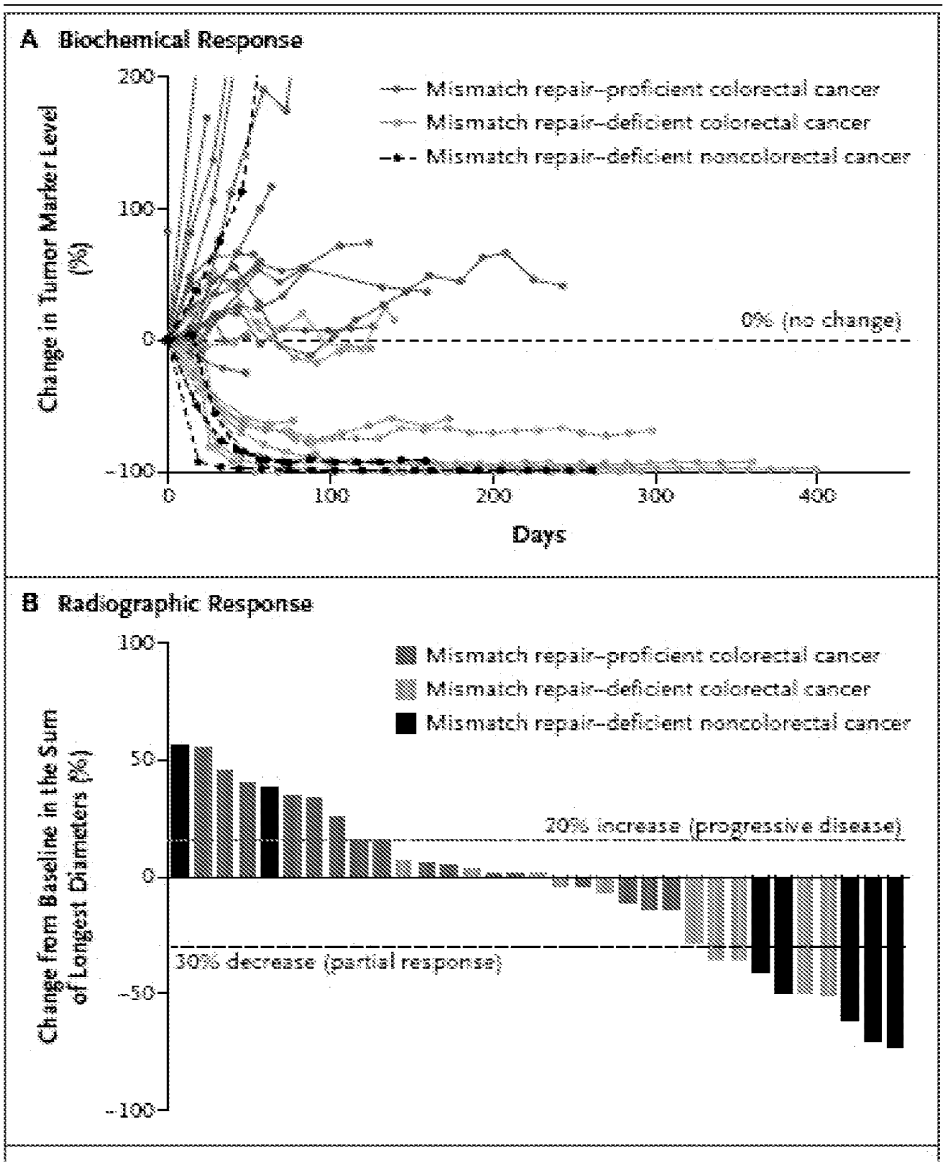
23. The method of claim 20 wherein the one or more microsatellite markers is selected from the group consisting of BAT-25, BAT-26, MON0-27, NR-21, NR-24, Penta C, and Penta D.

24. A method of treating a cancer patient comprising:

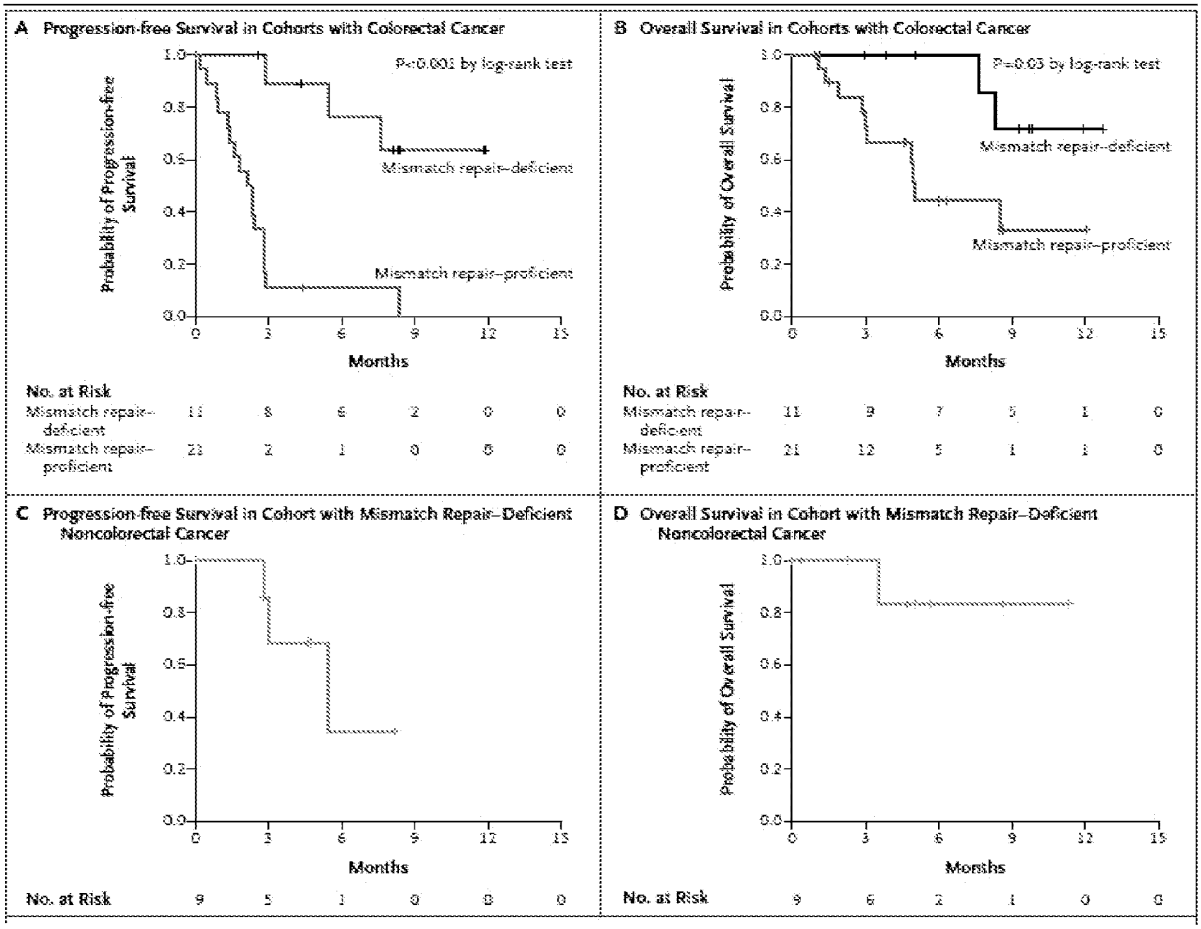
administering to a cancer patient an immune checkpoint inhibitory antibody, wherein the cancer patient has a mutational burden of at least 100 somatic mutations per tumor.

25. The method of claim 24 wherein the antibody is an anti-PD-1 antibody.

26. The method of claim 24 wherein the antibody is an anti-IDO antibody.
27. The method of claim 24 wherein the antibody is an anti-CTLA-4 antibody.
28. The method of claim 24 wherein the antibody is an anti-PD-L1 antibody.
29. The method of claim 24 wherein the antibody is an anti-LAG-3 antibody.
30. The method of claim 24 further comprising determining a high mutational burden in the tumor prior to the step of administering.



Figs. 1A and 1B


A Progression-free Survival in Cohorts with Colorectal Cancer
P<0.001 by log-rank test
Mismatch repair–deficient
Mismatch repair–proficient
Probability of Progression-free Survival
Months
No. at Risk
Mismatch repair–deficient 11 8 6 2 0 0
Mismatch repair–proficient 23 2 1 0 0 0
B Overall Survival in Cohorts with Colorectal Cancer
P=0.03 by log-rank test
Mismatch repair–deficient
Mismatch repair–proficient
Probability of Overall Survival
Months
No. at Risk
Mismatch repair–deficient 11 9 7 5 1 0
Mismatch repair–proficient 21 12 5 1 1 0
C Progression-free Survival in Cohort with Mismatch Repair–Deficient Noncolorectal Cancer
Probability of Progression-free Survival
Months
No. at Risk 9 5 1 0 0 0
D Overall Survival in Cohort with Mismatch Repair–Deficient Noncolorectal Cancer
Probability of Overall Survival
Months
No. at Risk 9 6 2 1 0 0

Figs. 2A-2D

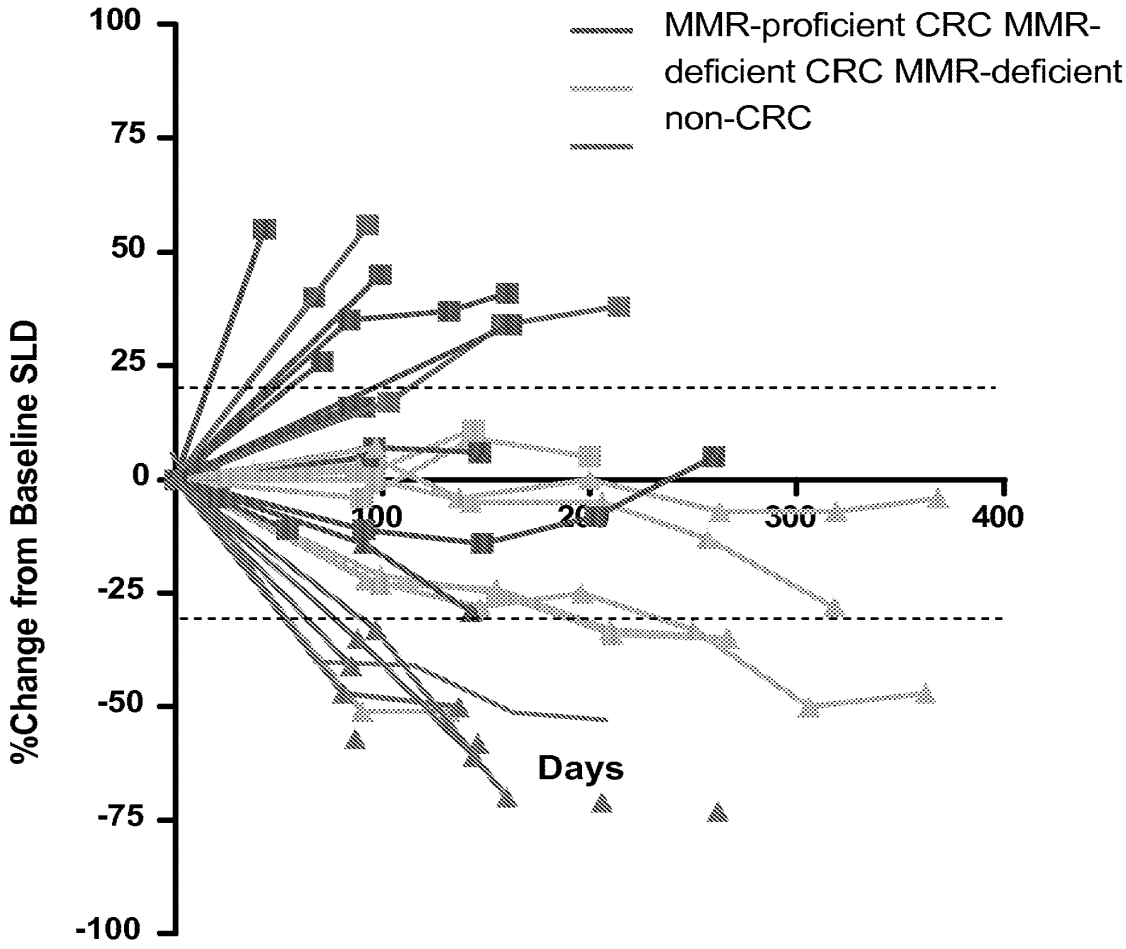
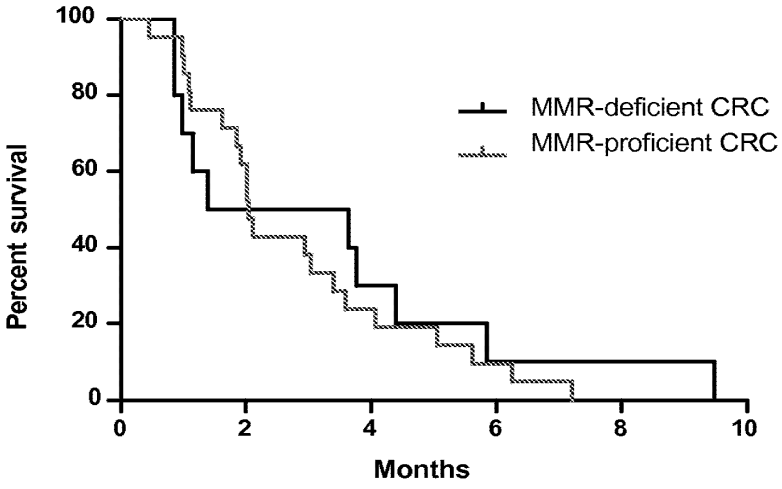
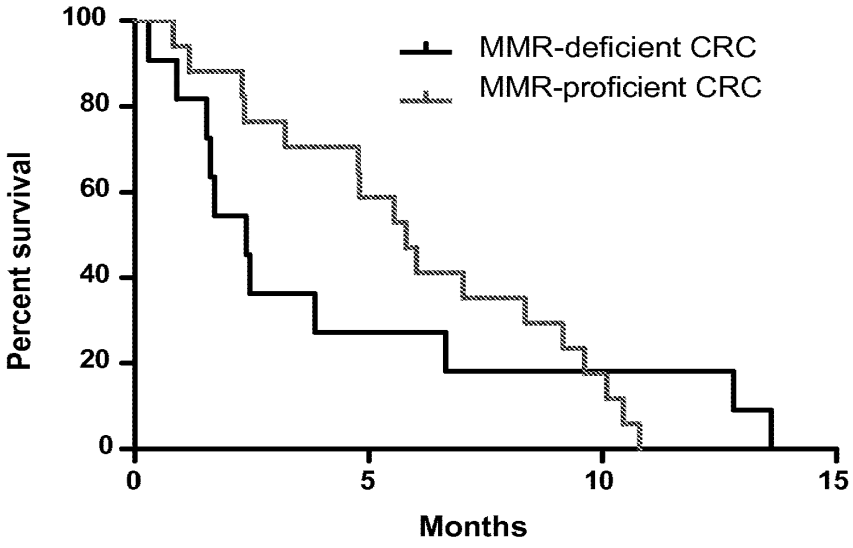

MMR-proficient CRC
MMR-deficient CRC
MMR-deficient non-CRC
100
75
50
25
0
-25
-50
-75
-100
%Change from Baseline SLD
100
200
300
400
Days

Fig. 3



Figs. 4A



Figs. 4B

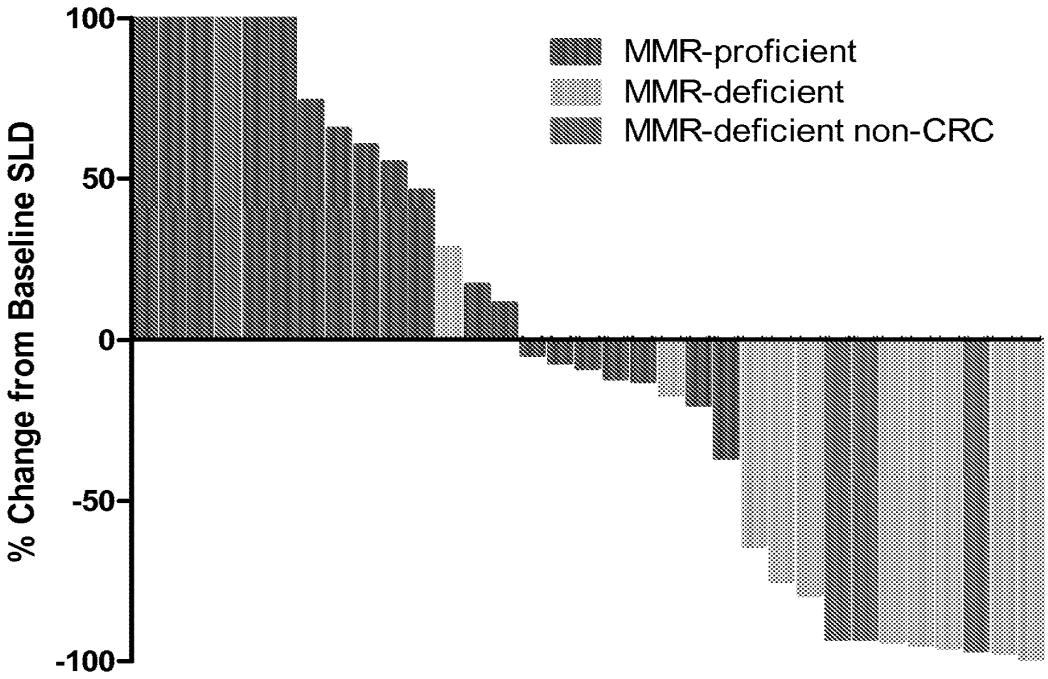

100
50
0
-50
-100
% Change from Baseline SLD
MMR-proficient
MMR-deficient
MMR-deficient non-CRC

Fig. 5

Figs. 6A

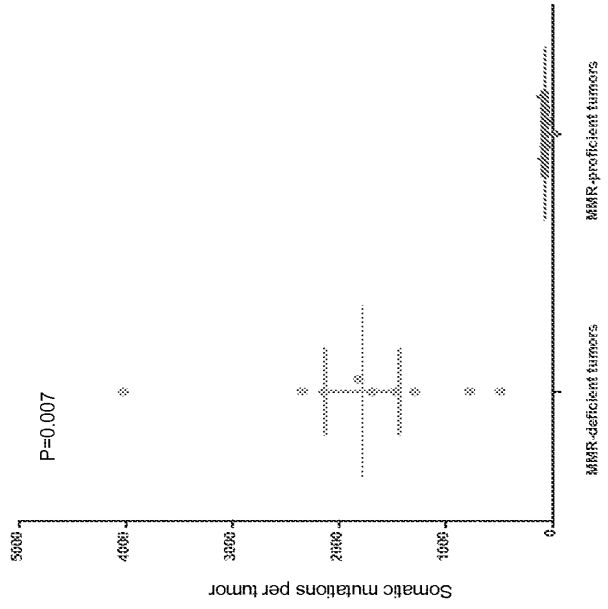
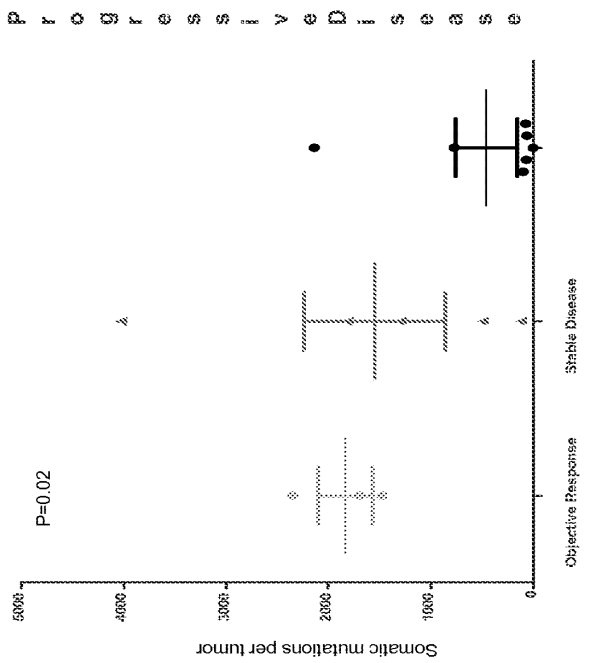


Fig. 6B



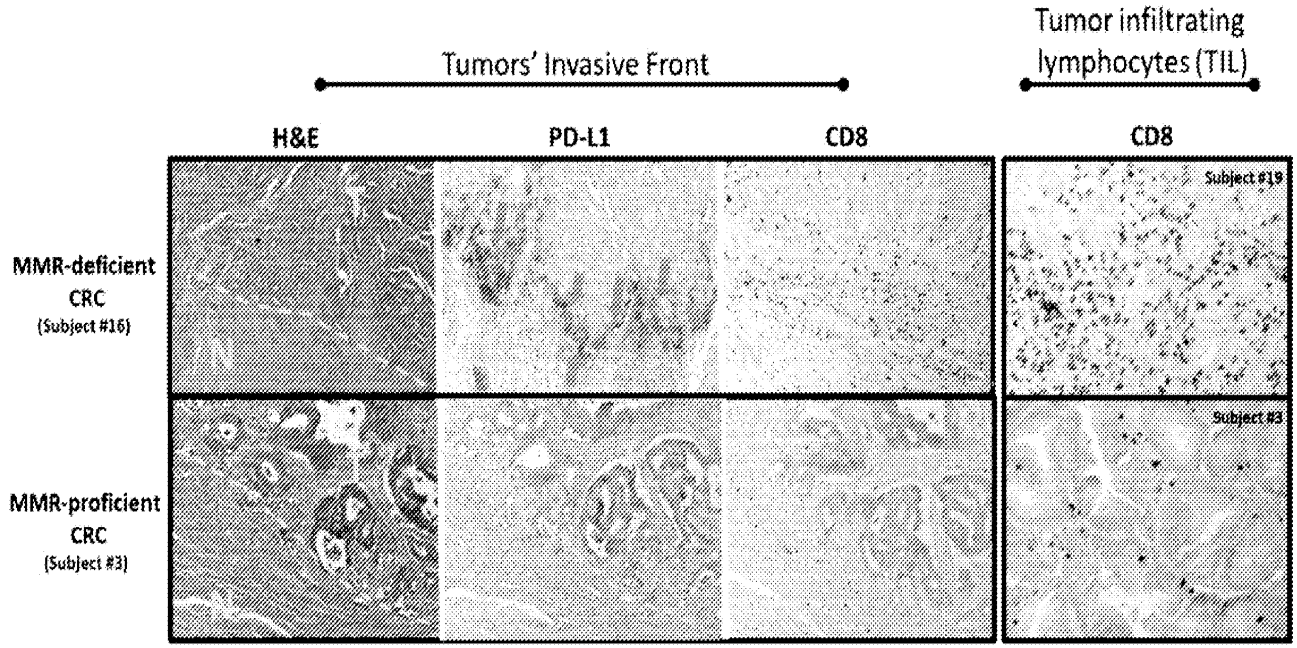

Tumors' Invasive Front
Tumor infiltrating lymphocytes (TIL)
H&E
PD-L1
CD8
CD8
MMR-deficient CRC (Subject #16)
MMR-proficient CRC (Subject #3)
Subject #19
Subject #3

Fig. 7

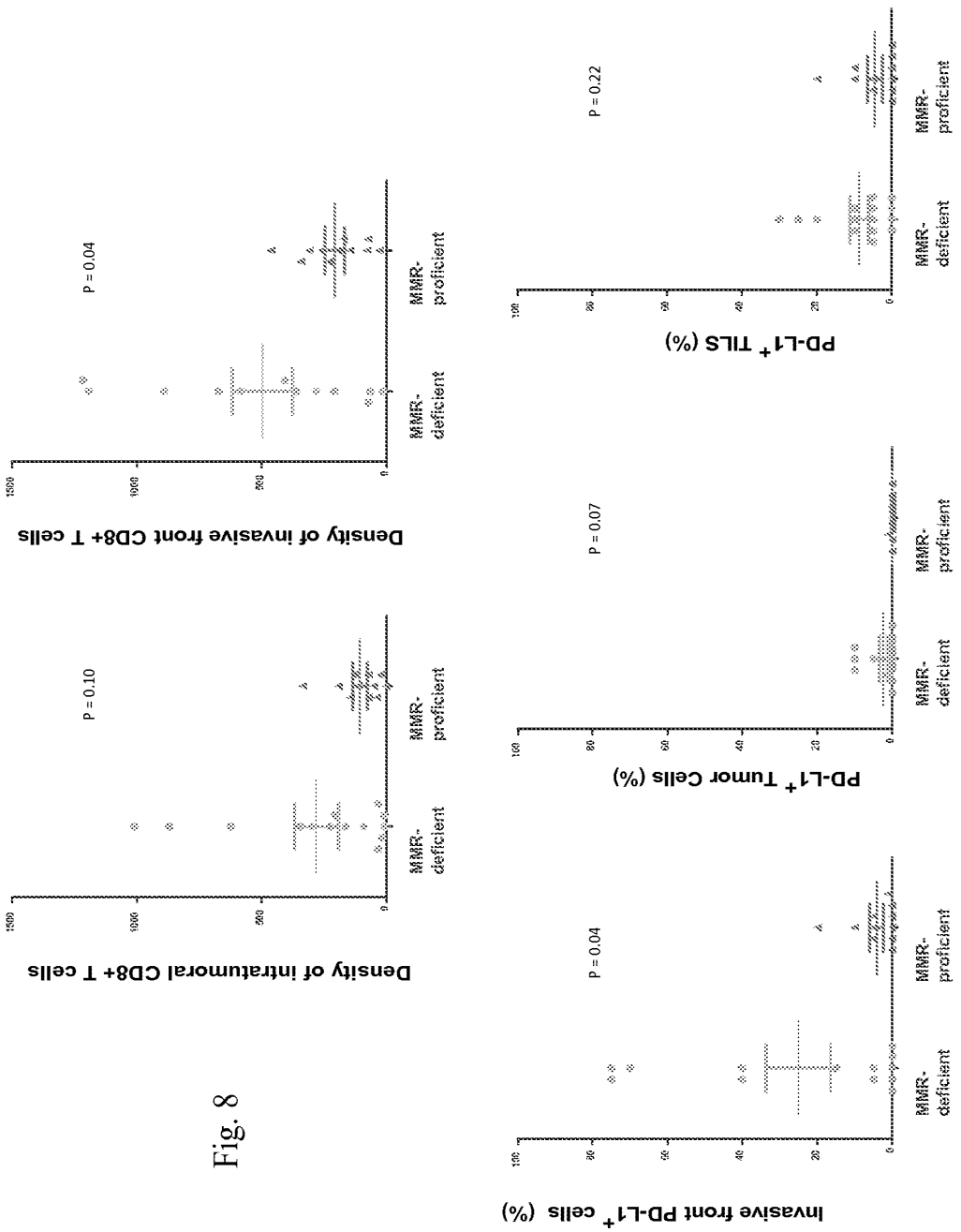


Fig. 8

EX1002 - Page 1350

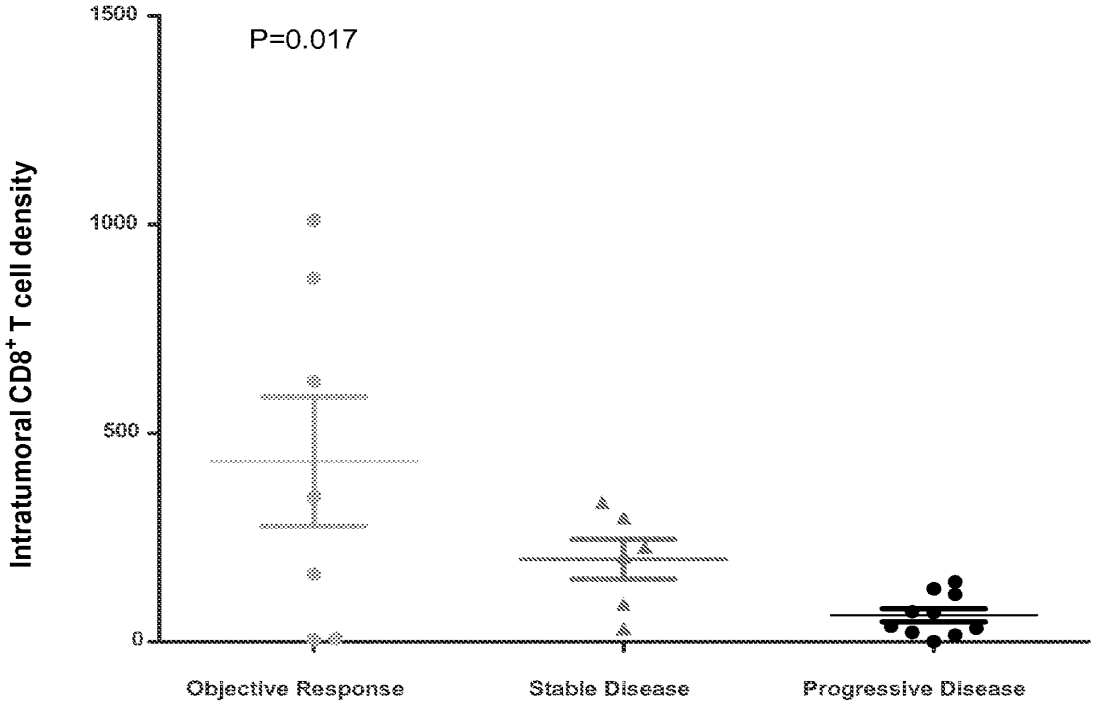

1500
1000
500
0
P=0.017
Intratumoral CD8+ T cell density
Objective Response
Stable Disease
Progressive Disease

Fig. 9

Fig. 10. Comparison of immune-related and RECIST response criteria

	CR	PR	SD	PD
RECIST 1.1	All lesions gone	≥ 30% decrease in sum of target lesion diameters from baseline	Neither R, or PD from nadir	≥ 20% increase in sum of target lesion diameters from nadir Appearance of new lesion

	irCR	irPR	irSD	irPD
irRC	All lesions gone	SPD of index + any new lesions decreases ≥50% from baseline New lesions allowed	SPD of index + any new lesions neither irCR, irPR, or irPD CR, P New lesions allowed	SPD of index + any new lesions increases > 25% from nadir irPD is based on SPD only

RECIST 1.1= Response Evaluation Criteria in Solid Tumors 1.1; irRC= Immune Related Response Criteria
CR= complete response; PR= paritial response; SD= stable disease; PD= progressive disease; SPD= sum of the product of the perpindicular diameters

Fig. 11. Immune-related response to treatment

Type of immune-related responses-no (%)	MMR-deficient CRC n=10	MMR-proficient CRC n=18	MMR-deficient non-CRC n=7
Complete Response Partial Response Stable Disease (Week 12) Progressive Disease Not Evaluable[3]	0 (0) 4 (40) 5 (50) 1 (10) 0 (0)	0 (0) 0 (0) 2 (11) 11 (61) 5 (28)	1 (14)[1] 4 (57)[2] 0 (0) 2 (29) 0 (0)
Immune-related objective response rate (%)	40	0	71
95% CI	12-74	0-19	29-96
Immune-related disease control rate (%)[4]	90	11	71
95% CI	55-100	1-35	29-96
Immune-related PFS at 20 weeks (%)	78	11	67
95% CI	40-97	1-35	22-96

[1] Originally PR at 12 that was converted to CR at 20 weeks

[2] One PR at 12 weeks

[3]Patients were considered not evaluable if they did not undergo a 12 week scan due to clinical progression. [4]The rate of disease control was defined as the percentage of patients who had a complete response,

partial response or stable disease for 12 weeks or more.

Fig. 12. Correlation of total somatic mutations and mutation associated neoantigens (MANA) with clinical outcomes

Trend test to compare CR/PR vs. SD vs. PD

Variable	P value
Total Somatic Mutations	0.024
Mutation-associated neoantigens (MANAs)	0.028

Association of mutations and MANA with response rates

		95% Confidence interval		
Variable (log scale*)	Odds Ratio	Lower	Upper	P value
Total Somatic Mutations	3.312	0.500	21.924	0.214
Mutation-associated neoantigens (MANAs)	3.087	0.617	15.439	0.170

Association of mutations and MANA with PFS

		95% Confidence interval		
Variable (log scale*)	Hazard Ratio	Lower	Upper	P value
Total Somatic Mutations	0.628	0.424	0.931	0.021
Mutation-associated neoantigens (MANAs)	0.599	0.391	0.916	0.018

Association of mutations and MANA with OS

		95% Confidence interval		
Variable (log scale*)	Hazard Ratio	Lower	Upper	P value
Total Somatic Mutations	0.707	0.481	1.038	0.077
Mutation-associated neoantigens (MANAs)	0.676	0.453	1.008	0.055

Fig. 13. Correlation of immune markers with clinical outcome

Trend test to compare CR/PR vs. SD vs. PD

Variable	P value
PD-L1 membranous expression within tumor (≥5% vs. <5%)	0.208
PD-L1 expression by TIL and associated macrophages at invasive front (%)	0.043
PD-L1 expression by TIL and associated macrophages on TILS within tumor (%)	0.024
CD8 density (cells/mm2) within tumor	0.017
CD8 density (cells/mm2) at Invasive front	0.768
CD8 density (cells/mm2) in non-tumor tissue	0.346

* except for PD-L1 Expression on Tumor (>5% vs. <5%), which is represented as a binary variable

Association of immune markers with response rates

		95% Confidence interval		
Variable (log scale*)	Odds Ratio	Lower	Upper	P value
PD-L1 membranous expression within tumor (≥5% vs. <5%)	3.200	0.354	28.945	0.301
PD-L1 expression by TIL and associated macrophages at invasive front (%)	1.075	0.820	1.411	0.600
PD-L1 expression by TIL and associated macrophages on TILS within tumor (%)	1.139	0.862	1.506	0.361
CD8 density (cells/mm2) within tumor	1.337	0.706	2.531	0.372
CD8 density (cells/mm2) at Invasive front	0.515	0.193	1.374	0.185
CD8 density (cells/mm2) in non-tumor tissue	1.219	0.535	2.777	0.637

* except for PD-L1 Expression on Tumor (>5% vs. <5%), which is represented as a binary variable

Association of immune markers with PFS

		95% Confidence interval		
Variable (log scale*)	Hazard Ratio	Lower	Upper	P value
PD-L1 membranous expression within tumor (≥5% vs. <5%)	0.196	0.025	1.518	0.119
PD-L1 expression by TIL and associated macrophages at invasive front (%)	0.899	0.784	1.031	0.126
PD-L1 expression by TIL and associated macrophages on TILS within tumor (%)	0.888	0.765	1.031	0.118
CD8 density (cells/mm2) within tumor	0.900	0.794	1.020	0.100
CD8 density (cells/mm2) at Invasive front	1.017	0.607	1.706	0.948
CD8 density (cells/mm2) in non-tumor tissue	1.144	0.780	1.677	0.492

* except for PD-L1 Expression on Tumor (>5% vs. <5%), which is represented as a binary variable

Association of immune markers with OS

		95% Confidence interval		
Variable (log scale*)	Hazard Ratio	Lower	Upper	P value
PD-L1 membranous expression within tumor (≥5% vs. <5%)	0.513	0.062	4.232	0.535
PD-L1 expression by TIL and associated macrophages at invasive front (%)	0.978	0.816	1.173	0.813
PD-L1 expression by TIL and associated macrophages on TILS within tumor (%)	0.931	0.762	1.138	0.487
CD8 density (cells/mm2) within tumor	0.918	0.813	1.036	0.167
CD8 density (cells/mm2) at Invasive front	0.986	0.502	1.934	0.967
CD8 density (cells/mm2) in non-tumor tissue	1.018	0.635	1.631	0.941

* except for PD-L1 Expression on Tumor (>5% vs. <5%), which is represented as a binary variable

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/060331

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

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

1. ☒ Claims Nos.: 1-15,24-30
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-15 and 24-30 pertain to a method for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.

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

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

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

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

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

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

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

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/060331

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395(2006.01)i, A61P 35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 39/395; C07K 16/28; C12Q 1/68; A61P 35/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: checkpoint blockade, microsatellite instability, cancer, antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2005-064009 A1 (AROS APPLIED BIOTECHNOLOGY APS) 14 July 2005 See abstract; page 64, lines 1-10; claim 1.	16-23
Y	LLOSA, NICOLAS J. et al., 'The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints', Cancer Discovery, Epub. 30 October 2014, Vol. 5, No. 1, pp. 43-51 (NIH Public Access Author Manuscript Version internal pages 1-18). See abstract.	16-23
A	US 2013-0309250 A1 (BRISTOL-MYERS SQUIBB COMPANY) 21 November 2013 See abstract; claim 9.	16-23
A	ZHAO, HUI et al., 'Mismatch repair deficiency endows tumors with a unique mutation signature and sensitivity to DNA double-strand breaks', ELIFE, 1 August 2014, Vol. 3 e02725 (internal pages 1-26). See the whole document.	16-23
PX	LE, DUNG T. et al., 'PD-1 Blockade in Tumors with Mismatch-Repair Deficiency', The New England Journal of Medicine, Epub. 30 May 2015, Vol. 372, No. 26, pp. 2509-2520. See the whole document.	16-23

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 February 2016 (24.02.2016)	**24 February 2016 (24.02.2016)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea
Facsimile No. +82-42-472-7140

Authorized officer
LEE, Dong Wook
Telephone No. +82-42-481-8163

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/060331

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	LAU, ESTHER, 'Mismatch repair deficiency predicts benefit of anti-PD-1 therapy', The Lancet. Oncology, Epub. 7 June 2015, Vol. 16, No. 7, e319. See the whole document.	16-23

Form PCT/ISA/210 (continuation of second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2015/060331

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005-064009 A1	14/07/2005	AT 466958 T	15/05/2010
		CA 2592504 A1	14/07/2005
		DE 602004027086 D1	17/06/2010
		EP 1704248 A1	27/09/2006
		EP 1704248 B1	05/05/2010
		US 2009-0297506 A1	03/12/2009
		WO 2005-064009 A9	05/11/2009
US 2013-0309250 A1	21/11/2013	AU 2013-263076 A1	22/01/2015
		CA 2873402 A1	21/11/2013
		CN 104470949 A	25/03/2015
		EA 201492105 A1	30/06/2015
		EP 2850102 A1	25/03/2015
		HK 1203971 A1	06/11/2015
		IL 235591 A	29/01/2015
		JP 2015-518826 A	06/07/2015
		KR 10-2015-0020189 A	25/02/2015
		MX 2014013565 A	12/02/2015
		SG 11201407190 A	30/12/2014
		US 2015-125463 A1	07/05/2015
		WO 2013-173223 A1	21/11/2013

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14 March 2016 (14.03.2016)

(25) Filing Language: English

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(30) Priority Data:

62/133,231	13 March 2015 (13.03.2015)	US
62/139,596	27 March 2015 (27.03.2015)	US
62/218,883	15 September 2015 (15.09.2015)	US

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(72) Inventors: **WEST, James William**; 724 Laurel Ave. Apt 404, San Mateo, California 94401 (US). **MEI, Li**; 534 Balboa Street, San Francisco, California 94118 (US). **MOORE, Stephen James**; 218 Country Meadows Lane, Danville, California 94506 (US). **NGUYEN, Margaret**; 1176 Pusateri Way, San Jose, California 95121 (US). **HOSTETTER, Daniel**; 3909 Park Blvd, Palo Alto, California 94306 (US). **VASILJEVA, Olga**; 20080 Rodrigues Ave. #D, Cupertino, California 95104 (US). **SAGERT, Jason**; 231 Laurie Meadows Rd Apt 153, San Mateo, California 94403 (US). **TERRETT, Jonathan**; 10678 Maplewood Road, Cupertino, California 95014 (US).

(74) Agents: **ELRIFI, Ivor R.** et al.; COOLEY LLP, 1299 Pennsylvania Avenue, NW, Suite 700, Washington, District of Columbia 20004-2400 (US).

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

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

Declarations under Rule 4.17:

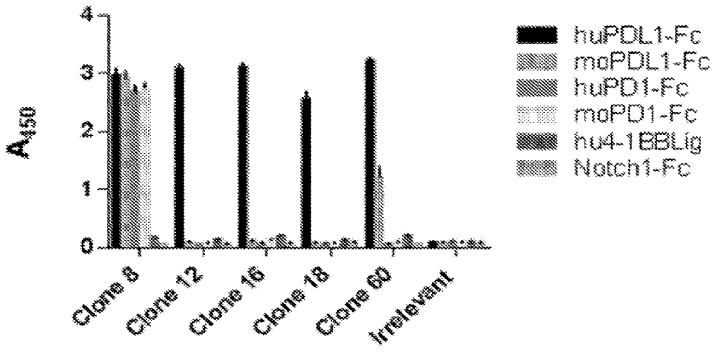
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) Title: ANTI-PDL1 ANTIBODIES, ACTIVATABLE ANTI-PDL1 ANTIBODIES, AND METHODS OF USE THEREOF

FIGURE 1



(57) Abstract: The invention relates generally to antibodies that bind programmed death ligand 1 (PDL1), activatable antibodies that specifically bind to PDL1 and methods of making and using these anti-PDL1 antibodies and anti-PDL1 activatable antibodies in a variety of therapeutic, diagnostic and prophylactic indications.

WO 2016/149201 A2

ANTI-PDL1 ANTIBODIES, ACTIVATABLE ANTI-PDL1 ANTIBODIES, AND METHODS OF USE THEREOF

Related Applications

[0001] This application claims the benefit of U.S. Provisional Application No. 62/133,231, filed March 13, 2015; U.S. Provisional Application No. 62/139,596, filed March 27, 2015; and U.S. Provisional Application No. 62/218,883, filed September 15, 2015; the contents of each of which are incorporated herein by reference in their entirety.

Field of the Invention

[0002] The invention relates generally to antibodies that bind programmed death ligand 1 (PDL1), activatable antibodies that specifically bind to PDL1 and methods of making and using these anti-PDL1 antibodies and anti-PDL1 activatable antibodies in a variety of therapeutic, diagnostic and prophylactic indications.

Background of the Invention

[0003] Antibody-based therapies have proven effective treatments for several diseases but in some cases, toxicities due to broad target expression have limited their therapeutic effectiveness. In addition, antibody-based therapeutics have exhibited other limitations such as rapid clearance from the circulation following administration.

[0004] In the realm of small molecule therapeutics, strategies have been developed to provide prodrugs of an active chemical entity. Such prodrugs are administered in a relatively inactive (or significantly less active) form. Once administered, the prodrug is metabolized *in vivo* into the active compound. Such prodrug strategies can provide for increased selectivity of the drug for its intended target and for a reduction of adverse effects.

[0005] Accordingly, there is a continued need in the field of antibody-based therapeutics for antibodies that mimic the desirable characteristics of the small molecule prodrug.

Summary of the Invention

[0006] The disclosure provides antibodies or antigen-binding fragments thereof that specifically bind programmed death ligand 1 (PDL1), also known as PD-L1, CD274, B7 homolog 1 and/or B7-H1. The use of the term "PDL1" is intended to cover any variation

thereof, such as, by way of non-limiting example, PD-L1 and/or PDL-1, all variations are used herein interchangeably.

[0007] In some embodiments, the antibody includes an antibody or antigen-binding fragment thereof that specifically binds PDL1. In some embodiments, the antibody or antigen binding fragment thereof that binds PDL1 is a monoclonal antibody, domain antibody, single chain, Fab fragment, a $F(ab')_2$ fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, or a single domain light chain antibody. In some embodiments, such an antibody or antigen binding fragment thereof that binds PDL1 is a mouse, other rodent, chimeric, humanized or fully human monoclonal antibody.

[0008] In some embodiments, the antibody is an isolated antibody or antigen binding fragment thereof (AB) that specifically binds to mammalian PDL1, wherein the AB has one or more of the following characteristics: (a) the AB specifically binds to human PDL1 and murine PDL1; (b) the AB specifically binds to human PDL1 and cynomolgus monkey PDL1; (c) the AB specifically binds to human PDL1, murine PDL1, and cynomolgus monkey PDL1; (d) the AB inhibits binding of human B7-1 and human PD1 to human PDL1 with an EC_{50} value less than 10 nM; (e) the AB inhibits binding of murine B7-1 and murine PD1 to murine PDL1 with an EC_{50} value less than 10 nM; and (f) the AB inhibits binding of cynomolgus monkey B7-1 and cynomolgus monkey PD1 to cynomolgus monkey PDL1 with an EC_{50} value less than 10 nM.

[0009] In some embodiments, the antibody or antigen binding fragment thereof specifically binds to the mammalian PDL1 with a dissociation constant is less than or equal to 0.01 nM, less than or equal to 0.05 nM, less than or equal to 0.1 nM, less than or equal to 0.2 nM, less than or equal to 0.3 nM, less than or equal to 0.4 nM, less than or equal to 0.5 nM, less than or equal to 0.75 nM, and less than or equal to 1 nM.

[00010] In some embodiments, the antibody or antigen binding fragment thereof specifically binds to the mammalian PDL1 with a dissociation constant in the range of 0.01 nM to 1 nM, 0.05 nM to 1 nM, 0.1 nM to 1 nM, 0.2 nM to 1 nM, 0.3 nM to 1 nM, 0.4 nM to 1 nM, 0.5 nM to 1 nM, 0.75 nM to 1 nM, 0.01 nM to 0.75 nM, 0.05 nM to 0.75 nM, 0.1 nM to 0.75 nM, 0.2 nM to 0.75 nM, 0.3 nM to 0.75 nM, 0.4 nM to 0.75 nM, 0.5 nM to 0.75 nM, 0.01 nM to 0.5 nM, 0.05 nM to 0.5 nM, 0.1 nM to 0.5 nM, 0.2 nM to 0.5 nM, 0.3 nM to 0.5 nM, 0.4 nM to 0.5 nM, 0.01 nM to 0.4 nM, 0.05 nM to 0.4 nM, 0.1 nM to 0.4 nM, 0.2 nM to 0.4 nM, 0.3 nM to 0.4 nM, 0.01 nM to 0.3 nM, 0.05 nM to

0.3 nM, 0.1 nM to 0.3 nM, 0.2 nM to 0.3 nM, 0.01 nM to 0.2 nM, 0.05 nM to 0.2 nM, 0.1 nM to 0.2 nM, 0.01 nM to 0.1 nM, 0.05 nM to 0.1 nM, or 0.01 nM to 0.05 nM.

[00011] In some embodiments, the mammalian PDL1 is selected from the group consisting of a human PDL1, a murine PDL1, a rat PDL1, and a cynomolgus monkey PDL1.

[00012] In some embodiments, the antibody or antigen binding fragment thereof specifically binds to human PDL1, murine PDL1 or cynomolgus monkey PDL1 with a dissociation constant of less than 1 nM.

[00013] In some embodiments, the mammalian PDL1 is a human PDL1.

[00014] In some embodiments, the antibody or antigen binding fragment thereof has one or more of the following characteristics: (a) the AB specifically binds human PDL1, murine PDL1, and cynomolgus monkey PDL1; (b) the AB inhibits binding of human B7-1 and human PD1 to human PDL1; (c) the AB inhibits binding of murine B7-1 and murine PD1 to murine PDL1; and (d) the AB inhibits binding of cynomolgus monkey B7-1 and cynomolgus monkey PD1 to cynomolgus monkey PDL1.

[00015] In some embodiments, the antibody or antigen binding fragment thereof blocks the ability of a natural ligand to bind to the mammalian PDL1 with an EC_{50} less than or equal to 0.5 nM, less than or equal to 1 nM, less than or equal to 2 nM, less than or equal to 3 nM, less than or equal to 4 nM, less than or equal to 5 nM, less than or equal to 6 nM, less than or equal to 7 nM, less than or equal to 8 nM, less than or equal to 9nM, and/or less than or equal to 10 nM.

[00016] In some embodiments, the antibody or antigen binding fragment thereof blocks the ability of a natural ligand to bind to the mammalian PDL1 with an EC_{50} in the range of 0.5 nM to 10 nM, 0.5 nM to 5 nM, 0.5 nM to 3 nM, 0.5 nM to 2 nM, 0.5 nM to 1 nM, 1 nM to 10 nM, 1 nM to 5 nM, 1 nM to 3 nM, 1 nM to 2 nM,2 nM to 10 nM, 2 nM to 5 nM, 2 nM to 3 nM, 3 nM to 10 nM, 3 nM to 5 nM, or 5 nM to 10 nM. In some embodiments, the natural ligand is a mammalian PD1. In some embodiments, the natural ligand is selected from the group consisting of: a human PD1, a murine PD1, and a cynomolgus monkey PD1.

[00017] In some embodiments, the natural ligand is a mammalian B7-1. In some embodiments, the natural ligand is selected from the group consisting of: a human B7-1, a murine B7-1, and a cynomolgus monkey B7-1.

[00018] In some embodiments, the antibody comprises a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56. In some embodiments, the antibody comprises a light chain amino acid sequence comprising SEQ ID NO: 12 or SEQ ID NO: 58. In some embodiments, the antibody comprises a light chain amino acid sequence comprising SEQ ID NO: 12. In some embodiments, the antibody comprises a light chain amino acid sequence comprising SEQ ID NO: 58.

[00019] In some embodiments, the antibody comprises a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid sequence comprising SEQ ID NO: 58.

[00020] In some embodiments, the antibody comprises a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid sequence comprising SEQ ID NO: 12.

[00021] In some embodiments, the antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56. In some embodiments, the antibody comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, the antibody comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, the antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12.

[00022] In some embodiments, the antibody comprises the heavy chain amino acid sequence of SEQ ID NO: 46 and the light chain amino acid sequence of SEQ ID NO: 58. In some embodiments, the antibody comprises the heavy chain amino acid sequence of SEQ ID NO: 46 and the light chain amino acid sequence of SEQ ID NO: 12.

[00023] In some embodiments, the antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid of SEQ ID NO: 46. In some embodiments, the antibody comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, the antibody comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence selected of SEQ ID NO: 46, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, the antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence selected of SEQ ID NO: 46, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12.

[00024] In some embodiments, the antibody comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence shown in Table 16; a VH CDR2 sequence shown in Table 16; a VH CDR3 sequence shown in Table 16; a VL CDR1 sequence shown in Table 16; a VL CDR2 sequence shown in Table 16; and a VL CDR3 sequence shown in Table 16.

[00025] In some embodiments, the antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence shown in Table 16; a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence shown in Table 16; a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence shown in Table 16; a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence shown in Table 16; a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence shown in Table 16; and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence shown in Table 16.

[00026] In some embodiments, the antibody comprises a heavy chain that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein the combination is a combination of the three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 16.

[00027] In some embodiments, the antibody comprises a light chain that comprise a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the three light chain CDR sequences (VL CDR1, VL CDR2, VL CDR3) shown in a single row in Table 16.

[00028] In some embodiments, the antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 16.

[00029] In some embodiments, the antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

99% or more identical to the corresponding CDR sequence in a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 16.

[00030] In some embodiments, the antibody comprises a heavy chain that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 16.

[00031] In some embodiments, the antibody comprises a light chain that comprise a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of the three light chain CDR sequences (VL CDR1, VL CDR2, VL CDR3) shown in a single row in Table 16.

[00032] In some embodiments, the antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VL CDR1 sequence comprising RASQSISSYLN (SEQ ID NO: 209); a VL CDR2 sequence comprising AASSLQS (SEQ ID NO: 215); a VL CDR3 sequence comprising DNGYPST (SEQ ID NO: 228); a VH CDR1 sequence comprising SYAMS (SEQ ID NO: 212); a VH CDR2 sequence comprising SSIWRNGIVTVYADS (SEQ ID NO: 246); and a VH CDR3 sequence comprising WSAAFDY (SEQ ID NO: 235).

[00033] In some embodiments, the antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR2 sequence comprises SSIWRNGIVTVYADS (SEQ ID NO: 246). In some embodiments, the antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR3 sequence comprises WSAAFDY (SEQ ID NO: 235). In some embodiments, the antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence,

wherein the VH CDR2 sequence comprises SSIWRNGIVTVYADS (SEQ ID NO: 246), and the VH CDR3 sequence comprises WSAAFDY (SEQ ID NO: 235).

[00034] In some embodiments, the antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination comprises a VL CDR1 sequence comprising RASQSISSYLN (SEQ ID NO: 209); a VL CDR2 sequence comprising AASSLQS (SEQ ID NO: 215); a VL CDR3 sequence comprising DNGYPST (SEQ ID NO: 228); a VH CDR1 sequence comprising SYAMS (SEQ ID NO: 212); a VH CDR2 sequence comprising SSIWRNGIVTVYADS (SEQ ID NO: 246); and a VH CDR3 sequence comprising WSAAFDY (SEQ ID NO: 235).

[00035] In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 12 or SEQ ID NO: 58. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 12. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 58 In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 58. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 12. In some embodiments, the antibody is encoded by a nucleic

acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 58. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 12.

[00036] In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence

selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12.

[00037] In some embodiments, the antibody is encoded by a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, 15, 17, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55. In some embodiments, the antibody is encoded by a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 45, 47, 49, 51, 53, and 55. In some embodiments, the antibody is encoded by the light chain nucleic acid sequence of SEQ ID NO: 57. In some embodiments, the antibody is encoded by the light chain nucleic acid sequence of SEQ ID NO: 11. In some embodiments, the antibody is encoded by heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, 15, 17, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55, and a light chain nucleic acid sequence comprising SEQ ID NO: 57. In some embodiments, the antibody is encoded by heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, 15, 17, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55, and a light chain nucleic acid sequence comprising SEQ ID NO: 11. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 45, 47, 49, 51, 53, and 55, and a light chain nucleic acid sequence comprising SEQ

ID NO: 57. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 45, 47, 49, 51, 53, and 55, and a light chain nucleic acid sequence comprising SEQ ID NO: 11.

[00038] In some embodiments, the antibody is encoded by a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, 15, 17, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55. In some embodiments, the antibody is encoded by a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 45, 47, 49, 51, 53, and 55. In some embodiments, the antibody is encoded by a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the light chain nucleic acid sequence of SEQ ID NO: 57. In some embodiments, the antibody is encoded by a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the light chain nucleic acid sequence of SEQ ID NO: 11. In some embodiments, the antibody is encoded by a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, 15, 17, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the light chain nucleic acid sequence comprising SEQ ID NO: 57. In some embodiments, the antibody is encoded by a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, 15, 17, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the light chain nucleic acid sequence comprising SEQ ID NO: 11. In some embodiments, the antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 45, 47, 49, 51, 53, and 55, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to light chain nucleic acid sequence comprising SEQ ID NO: 57. In some embodiments, the antibody is

encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 45, 47, 49, 51, 53, and 55, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to light chain nucleic acid sequence comprising SEQ ID NO: 11.

[00039] In some embodiments, the antibody or antigen-binding fragment thereof is incorporated in a multispecific antibody or antigen-binding fragment thereof, where at least one arm of the multispecific antibody specifically binds PDL1. In some embodiments, the antibody or antigen-binding fragment thereof is incorporated in a bispecific antibody or antigen-binding fragment thereof, where at least one arm of the bispecific antibody specifically binds PDL1.

[00040] In some embodiments, at least one arm of the multispecific antibody, *e.g.*, a bispecific antibody, comprises a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56. In some embodiments, at least one arm of the multispecific antibody, *e.g.*, a bispecific antibody, comprises a light chain amino acid sequence comprising SEQ ID NO: 12 or SEQ ID NO: 58. In some embodiments, at least one arm of the multispecific antibody, *e.g.*, a bispecific antibody, comprises a light chain amino acid sequence comprising SEQ ID NO: 12. In some embodiments, at least one arm of the multispecific antibody, *e.g.*, a bispecific antibody, comprises a light chain amino acid sequence comprising SEQ ID NO: 58.

[00041] In some embodiments, at least one arm of the multispecific antibody, *e.g.*, a bispecific antibody, a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid sequence comprising SEQ ID NO: 58.

[00042] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid sequence comprising SEQ ID NO: 12.

[00043] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38,

40, 42, 44, 46, 48, 50, 52, 54, and 56. In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12.

[00044] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises the heavy chain amino acid sequence of SEQ ID NO: 46 and the light chain amino acid sequence of SEQ ID NO: 58. In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises the heavy chain amino acid sequence of SEQ ID NO: 46 and the light chain amino acid sequence of SEQ ID NO: 12.

[00045] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid of SEQ ID NO: 46. In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID

NO: 12. In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence selected of SEQ ID NO: 46, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence selected of SEQ ID NO: 46, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12.

[00046] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence shown in Table 16; a VH CDR2 sequence shown in Table 16; a VH CDR3 sequence shown in Table 16; a VL CDR1 sequence shown in Table 16; a VL CDR2 sequence shown in Table 16; and a VL CDR3 sequence shown in Table 16.

[00047] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence shown in Table 16; a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence shown in

Table 16; a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence shown in Table 16; a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence shown in Table 16; a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence shown in Table 16; and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence shown in Table 16.

[00048] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 16.

[00049] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a heavy chain that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein the combination is a combination of the three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 16.

[00050] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 16.

[00051] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a heavy chain that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a

combination of three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 16.

[00052] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VL CDR1 sequence comprising RASQSISSYLN (SEQ ID NO: 209); a VL CDR2 sequence comprising AASSLQS (SEQ ID NO: 215); a VL CDR3 sequence comprising DNGYPST (SEQ ID NO: 228); a VH CDR1 sequence comprising SYAMS (SEQ ID NO: 212); a VH CDR2 sequence comprising SSIWRNGIVTVYADS (SEQ ID NO: 246); and a VH CDR3 sequence comprising WSAAFDY (SEQ ID NO: 235).

[00053] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR2 sequence comprises SSIWRNGIVTVYADS (SEQ ID NO: 246). In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR3 sequence comprises WSAAFDY (SEQ ID NO: 235). In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR2 sequence comprises SSIWRNGIVTVYADS (SEQ ID NO: 246), and the VH CDR3 sequence comprises WSAAFDY (SEQ ID NO: 235).

[00054] In some embodiments, at least one arm of the multispecific antibody, e.g., a bispecific antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination comprises a VL CDR1 sequence comprising RASQSISSYLN (SEQ ID NO: 209); a VL CDR2 sequence comprising AASSLQS (SEQ ID NO: 215); a VL CDR3 sequence comprising DNGYPST (SEQ ID NO: 228); a VH CDR1 sequence comprising SYAMS (SEQ ID NO: 212); a VH CDR2 sequence comprising SSIWRNGIVTVYADS (SEQ ID NO: 246); and a VH CDR3 sequence comprising WSAAFDY (SEQ ID NO: 235).

[00055] The disclosure also provides activatable antibodies that include an antibody or antigen-binding fragment thereof that specifically binds PDL1 coupled to a masking moiety (MM), such that coupling of the MM reduces the ability of the antibody or antigen-binding fragment thereof to bind PDL1. In some embodiments, the MM is coupled via a sequence that includes a substrate for a protease, for example, a protease that is active in diseased tissue and/or a protease that is co-localized with PDL1 at a treatment site in a subject. The activatable anti-PDL1 antibodies provided herein, also referred to herein as anti-PDL1 activatable antibodies or PDL1 activatable antibodies, are stable in circulation, activated at intended sites of therapy and/or diagnosis but not in normal, *e.g.*, healthy tissue or other tissue not targeted for treatment and/or diagnosis, and, when activated, exhibit binding to PDL1 that is at least comparable to the corresponding, unmodified antibody.

[00056] In some embodiments, the activatable antibody that, in an activated state, specifically binds to mammalian PDL1, includes an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian PDL1; a masking moiety (MM) that inhibits the binding of the AB to mammalian PDL1 when the activatable antibody is in an uncleaved state; and a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

[00057] In some embodiments, the activatable antibody that, in an activated state, has one or more of the following characteristics: (a) specifically binds to mammalian PDL1; and(b) specifically blocks a natural ligand of PDL1 from binding to the mammalian PDL1, wherein the activatable antibody comprises: an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian PDL1; a masking moiety (MM) that inhibits the binding of the AB to mammalian PDL1 when the activatable antibody is in an uncleaved state; and a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

[00058] In some embodiments, the activatable antibody in an uncleaved state specifically binds to the mammalian PDL1 with a dissociation constant less than or equal to 1 nM, less than or equal to 2 nM, less than or equal to 3 nM, less than or equal to 4 nM, less than or equal to 5 nM, less than or equal to 10 nM, less than or equal to 15 nM, less than or equal to 20 nM, and/or less than or equal to 25 nM.

[00059] In some embodiments, the activatable antibody in an uncleaved state specifically binds to the mammalian PDL1 with a dissociation constant greater than or equal to 1 nM, greater than or equal to 2 nM, greater than or equal to 3 nM, greater than or equal

to 4 nM, greater than or equal to 5 nM, greater than or equal to 10 nM, greater than or equal to 15 nM, greater than or equal to 20 nM, and/or greater than or equal to 25 nM.

[00060] In some embodiments, the activatable antibody in an uncleaved state specifically binds to the mammalian PDL1 with a dissociation constant in the range of 1 to 2 nM, 1 to 5 nM, 1 to 10 nM, 1 to 15 nM, 1 to 20 nM, 1 to 25 nM, 2 nM to 5 nM, 2 nM to 10 nM, 2 nM to 15 nM, 2 to 20 nM, 2 to 25 nM, 5 nM to 10 nM, 5 nM to 15 nM, 5 to 20 nM, 5 to 25 nM, 10 nM to 15 nM, 10 to 20 nM, 10 to 25 nM, 15 to 20 nM, 15 to 25 nM, or 20 to 25 nM.

[00061] In some embodiments, the activatable antibody in an activated state specifically binds to the mammalian PDL1 with a dissociation constant is less than or equal to 0.01 nM, less than or equal to 0.05 nM, less than or equal to 0.1 nM, less than or equal to 0.2 nM, less than or equal to 0.3 nM, less than or equal to 0.4 nM, less than or equal to 0.5 nM, less than or equal to 0.75 nM, and less than or equal to 1 nM.

[00062] In some embodiments, the activatable antibody in an activated state specifically binds to the mammalian PDL1 with a dissociation constant in the range of 0.01 nM to 1 nM, 0.05 nM to 1 nM, 0.1 nM to 1 nM, 0.2 nM to 1 nM, 0.3 nM to 1 nM, 0.4 nM to 1 nM, 0.5 nM to 1 nM, 0.75 nM to 1 nM, 0.01 nM to 0.75 nM, 0.05 nM to 0.75 nM, 0.1 nM to 0.75 nM, 0.2 nM to 0.75 nM, 0.3 nM to 0.75 nM, 0.4 nM to 0.75 nM, 0.5 nM to 0.75 nM, 0.01 nM to 0.5 nM, 0.05 nM to 0.5 nM, 0.1 nM to 0.5 nM, 0.2 nM to 0.5 nM, 0.3 nM to 0.5 nM, 0.4 nM to 0.5 nM, 0.01 nM to 0.4 nM, 0.05 nM to 0.4 nM, 0.1 nM to 0.4 nM, 0.2 nM to 0.4 nM, 0.3 nM to 0.4 nM, 0.01 nM to 0.3 nM, 0.05 nM to 0.3 nM, 0.1 nM to 0.3 nM, 0.2 nM to 0.3 nM, 0.01 nM to 0.2 nM, 0.05 nM to 0.2 nM, 0.1 nM to 0.2 nM, 0.01 nM to 0.1 nM, 0.05 nM to 0.1 nM, or 0.01 nM to 0.05 nM.

[00063] In some embodiments, the AB specifically binds to the mammalian PDL1 with a dissociation constant is less than or equal to 0.01 nM, less than or equal to 0.05 nM, less than or equal to 0.1 nM, less than or equal to 0.2 nM, less than or equal to 0.3 nM, less than or equal to 0.4 nM, less than or equal to 0.5 nM, less than or equal to 0.75 nM, and less than or equal to 1 nM.

[00064] In some embodiments, the AB specifically binds to the mammalian PDL1 with a dissociation constant in the range of 0.01 nM to 1 nM, 0.05 nM to 1 nM, 0.1 nM to 1 nM, 0.2 nM to 1 nM, 0.3 nM to 1 nM, 0.4 nM to 1 nM, 0.5 nM to 1 nM, 0.75 nM to 1 nM, 0.01 nM to 0.75 nM, 0.05 nM to 0.75 nM, 0.1 nM to 0.75 nM, 0.2 nM to 0.75 nM, 0.3 nM to 0.75 nM, 0.4 nM to 0.75 nM, 0.5 nM to 0.75 nM, 0.01 nM to 0.5 nM, 0.05 nM to 0.5 nM,

0.1 nM to 0.5 nM, 0.2 nM to 0.5 nM, 0.3 nM to 0.5 nM, 0.4 nM to 0.5 nM, 0.01 nM to 0.4 nM, 0.05 nM to 0.4 nM, 0.1 nM to 0.4 nM, 0.2 nM to 0.4 nM, 0.3 nM to 0.4 nM, 0.01 nM to 0.3 nM, 0.05 nM to 0.3 nM, 0.1 nM to 0.3 nM, 0.2 nM to 0.3 nM, 0.01 nM to 0.2 nM, 0.05 nM to 0.2 nM, 0.1 nM to 0.2 nM, 0.01 nM to 0.1 nM, 0.05 nM to 0.1 nM, or 0.01 nM to 0.05 nM.

[00065] In some embodiments, the mammalian PDL1 is selected from the group consisting of a human PDL1, a murine PDL1, a rat PDL1, and a cynomolgus monkey PDL1. In some embodiments, the AB specifically binds to human PDL1, murine PDL1 or cynomolgus monkey PDL1 with a dissociation constant of less than 1 nM. In some embodiments, the mammalian PDL1 is a human PDL1.

[00066] In some embodiments, the AB has one or more of the following characteristics: (a) the AB specifically binds to human PDL1 and murine PDL1; (b) the AB specifically binds to human PDL1 and cynomolgus monkey PDL1; and (c) the AB specifically binds to human PDL1, murine PDL1, and cynomolgus monkey PDL1.

[00067] In some embodiments, the AB has one or more of the following characteristics: (a) the AB specifically binds human PDL1, murine PDL1, and cynomolgus monkey PDL1; (b) the AB inhibits binding of human B7-1 and human PD1 to human PDL1; (c) the AB inhibits binding of murine B7-1 and murine PD1 to murine PDL1; and (d) the AB inhibits binding of cynomolgus monkey B7-1 and cynomolgus monkey PD1 to cynomolgus monkey PDL1.

[00068] In some embodiments, the AB blocks the ability of a natural ligand to bind to the mammalian PDL1 with an EC_{50} less than or equal to 0.5 nM, less than or equal to 1 nM, less than or equal to 2 nM, less than or equal to 3 nM, less than or equal to 4 nM, less than or equal to 5 nM, less than or equal to 6 nM, less than or equal to 7 nM, less than or equal to 8 nM, less than or equal to 9nM, and/or less than or equal to 10 nM.

[00069] In some embodiments, the AB blocks the ability of a natural ligand to bind to the mammalian PDL1 with an EC_{50} of 0.5 nM to 10 nM, 0.5 nM to 5 nM, 0.5 nM to 3 nM, 0.5 nM to 2 nM, 0.5 nM to 1 nM, 1 nM to 10 nM, 1 nM to 5 nM, 1 nM to 3 nM, 1 nM to 2 nM, 2 nM to 10 nM, 2 nM to 5 nM, 2 nM to 3 nM, 3 nM to 10 nM, 3 nM to 5 nM, or 5 nM to 10 nM.

[00070] In some embodiments, natural ligand is a mammalian PD1. In some embodiments, the natural ligand is selected from the group consisting of: a human PD1, a murine PD1, and a cynomolgus monkey PD1.

[00071] In some embodiments, the natural ligand is a mammalian B7-1. In some embodiments, the natural ligand is selected from the group consisting of: a human B7-1, a murine B7-1, and a cynomolgus monkey B7-1.

[00072] In some embodiments, the activatable antibody has one or more of the following characteristics (a) the AB induces type 1 diabetes in a non-obese diabetic (NOD) mouse; and (b) the activatable antibody in an uncleaved state inhibits the induction of type 1 diabetes in a NOD mouse.

[00073] In some embodiments, the activatable antibody inhibits the induction of type 1 diabetes in the NOD mouse after administration of the activatable antibody at a single dose of 0.1 mg/kg to 3 mg/kg, 0.5 mg/kg to 3 mg/kg, 1 mg/kg to 3 mg/kg, 2 mg/kg to 3 mg/kg, 0.1 mg/kg to 2 mg/kg, 0.5 mg/kg to 2 mg/kg, 1 mg/kg to 2 mg/kg, 0.1 mg/kg to 1 mg/kg, 0.5 mg/kg to 1 mg/kg, or 0.1 mg/kg to 0.5 mg/kg.

[00074] In some embodiments, the activatable antibody induces type 1 diabetes in a subject after administration of the activatable antibody at a single dose of 1 mg/kg. In some embodiments, the subject is a NOD mouse.

[00075] In some embodiments, the activatable antibody induces type 1 diabetes in a subject after administration of the activatable antibody at single dose less than or equal to 3 mg/kg, less than or equal to 2 mg/kg, less than or equal to 1 mg/kg, less than or equal to 0.5 mg/kg, and/or less than or equal to 0.1 mg/kg.

[00076] In some embodiments, the activatable antibody induces type 1 diabetes in a subject after administration of the activatable antibody at single dose in the range selected from the group consisting of 0.1 mg/kg to 3 mg/kg, 0.5 mg/kg to 3 mg/kg, 1 mg/kg to 3 mg/kg, 2 mg/kg to 3 mg/kg, 0.1 mg/kg to 2 mg/kg, 0.5 mg/kg to 2 mg/kg, 1 mg/kg to 2 mg/kg, 0.1 mg/kg to 1 mg/kg, 0.5 mg/kg to 1 mg/kg, and 0.1 mg/kg to 0.5 mg/kg. In some embodiments, the subject is a NOD mouse.

[00077] In some embodiments, the activatable antibody has one or more of the following characteristics (a) the activatable antibody in an uncleaved state does not induce type 1 diabetes in greater than 50% of a population of non-obese diabetic (NOD) mice, and the AB induces type 1 diabetes in greater than 50% of a population of NOD mice.

[00078] In some embodiments, the activatable antibody does not induce type 1 diabetes in greater than 50% of the population of NOD mice after administration to each mouse in the population a single dose less than or equal to 3 mg/kg, less than or equal to

2 mg/kg, less than or equal to 1 mg/kg, less than or equal to 0.5 mg/kg, and/or less than or equal to 0.1 mg/kg.

[00079] In some embodiments, the activatable antibody does not induce type 1 diabetes in greater than 50% of the population of NOD mice after administration to each mouse in the population a single dose of the activatable antibody at a dosage of: 0.1 mg/kg to 3 mg/kg, 0.5 mg/kg to 3 mg/kg, 1 mg/kg to 3 mg/kg, 2 mg/kg to 3 mg/kg, 0.1 mg/kg to 2 mg/kg, 0.5 mg/kg to 2 mg/kg, 1 mg/kg to 2 mg/kg, 0.1 mg/kg to 1 mg/kg, 0.5 mg/kg to 1 mg/kg, or 0.1 mg/kg to 0.5 mg/kg.

[00080] In some embodiments, the activatable antibody has one or more of the following characteristics (a) the activatable antibody in an uncleaved state does not induce type 1 diabetes in greater than 50% of a population of non-obese diabetic (NOD) mice when administered at a single dose of 1 mg/kg; and (b) the AB induces type 1 diabetes in greater than 50% of a population of NOD mice, when administered at a single dose of 1 mg/kg.

[00081] In some embodiments, the AB induces type 1 diabetes in greater than 50% of the population of the NOD mice after administration to each mouse in the population a single dose of the AB at a dosage less than or equal to 3 mg/kg, less than or equal to 2 mg/kg, less than or equal to 1 mg/kg, less than or equal to 0.5 mg/kg, and/or less than or equal to 0.1 mg/kg.

[00082] In some embodiments, the AB induces type 1 diabetes in greater than 50% of the population of the NOD mice after administration to each mouse in the population a single dose of the AB at a dosage of: 0.1 mg/kg to 3 mg/kg, 0.5 mg/kg to 3 mg/kg, 1 mg/kg to 3 mg/kg, 2 mg/kg to 3 mg/kg, 0.1 mg/kg to 2 mg/kg, 0.5 mg/kg to 2 mg/kg, 1 mg/kg to 2 mg/kg, 0.1 mg/kg to 1 mg/kg, 0.5 mg/kg to 1 mg/kg, or 0.1 mg/kg to 0.5 mg/kg.

[00083] In some embodiments, he NOD mouse is a female NOD/ShiLtJ mouse substrain.

[00084] In some embodiments, the activatable antibody inhibits the induction of type 1 diabetes in a NOD mouse by at least 3-fold compared to the AB.

[00085] In some embodiments, the activatable antibody exhibits a safety margin that is at least a three-fold safety margin relative to the AB.

[00086] In some embodiments, the activatable antibody in an uncleaved state binds to a smaller percentage of a population of peripheral blood CD4+ CD8+ T lymphocytes than does the AB.

[00087] In some embodiments, the activatable antibody does not bind to greater than 50% of the population of peripheral blood CD4+ CD8+ T lymphocytes after administration of a single dose of the activatable antibody at a dosage less than or equal to 5 mg/kg, less than or equal to 4 mg/kg, less than or equal to 3 mg/kg, less than or equal to 2 mg/kg, less than or equal to 1 mg/kg, less than or equal to 0.5 mg/kg, and/or less than or equal to 0.1 mg/kg.

[00088] In some embodiments, the activatable antibody does not bind to greater than 50% of the population of peripheral blood CD4+ CD8+ T lymphocytes after administration of a single dose of the activatable antibody at a dosage of: 0.1 mg/kg to 5 mg/kg, 0.5 mg/kg to 5 mg/kg, 1 mg/kg to 5 mg/kg, 2 mg/kg to 5 mg/kg, 3 mg/kg to 5 mg/kg, 0.1 mg/kg to 3 mg/kg, 0.5 mg/kg to 3 mg/kg, 1 mg/kg to 3 mg/kg, 2 mg/kg to 3 mg/kg, 0.1 mg/kg to 2 mg/kg, 0.5 mg/kg to 2 mg/kg, 1 mg/kg to 2 mg/kg, 0.1 mg/kg to 1 mg/kg, 0.5 mg/kg to 1 mg/kg, or 0.1 mg/kg to 0.5 mg/kg.

[00089] In some embodiments, the AB binds to greater than 50% of the population of peripheral blood CD4+ CD8+ T lymphocytes after administration of a single dose of the AB at a dosage of: 0.1 mg/kg to 5 mg/kg, 0.5 mg/kg to 5 mg/kg, 1 mg/kg to 5 mg/kg, 2 mg/kg to 5 mg/kg, 3 mg/kg to 5 mg/kg, 0.1 mg/kg to 3 mg/kg, 0.5 mg/kg to 3 mg/kg, 1 mg/kg to 3 mg/kg, 2 mg/kg to 3 mg/kg, 0.1 mg/kg to 2 mg/kg, 0.5 mg/kg to 2 mg/kg, 1 mg/kg to 2 mg/kg, 0.1 mg/kg to 1 mg/kg, 0.5 mg/kg to 1 mg/kg, or 0.1 mg/kg to 0.5 mg/kg.

[00090] In some embodiments, the peripheral blood CD4+ CD8+ T lymphocytes are murine. In some embodiments, the murine peripheral blood CD4+ CD8+ T lymphocytes are derived from a tumor-bearing mouse.

[00091] In some embodiments, the percentage of the population of peripheral blood CD4+CD8+ T lymphocytes to which the AB binds is less than 60% when the activatable antibody is administered at a dose between 1 mg/kg and 5 mg/kg. In some embodiments, the percentage of the population of peripheral blood CD4+CD8+ T lymphocytes to which the AB binds is less than 50% when the activatable antibody is administered at a dose between 1 mg/kg and 5 mg/kg. In some embodiments, the percentage of the population of peripheral blood CD4+CD8+ T lymphocytes to which the AB binds is between 30 and 60% when the activatable antibody is administered at a dose between 1 mg/kg and 5 mg/kg. In some embodiments, the percentage of the population of peripheral blood CD4+CD8+ T lymphocytes to which the AB binds is between 30 and 50% when the activatable antibody is administered at a dose between 1 mg/kg and 5 mg/kg.

[00092] The invention also provides methods of treating, preventing and/or delaying the onset or progression of, or alleviating a symptom associated with aberrant expression and/or activity of PDL1 in a subject using activatable antibodies that bind PDL1, particularly activatable antibodies that bind and neutralize or otherwise inhibit at least one biological activity of PDL1 and/or PDL1-mediated signaling.

[00093] The activatable antibodies in an activated state bind PDL1 and include (i) an antibody or an antigen binding fragment thereof (AB) that specifically binds to PDL1; (ii) a masking moiety (MM) that, when the activatable antibody is in an uncleaved state, inhibits the binding of the AB to PDL1; and (c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

[00094] In some embodiments, the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM.

[00095] In some embodiments, the activatable antibody comprises a linking peptide between the MM and the CM.

[00096] In some embodiments, the activatable antibody comprises a linking peptide between the CM and the AB.

[00097] In some embodiments, the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM. In some embodiments, the two linking peptides need not be identical to each other.

[00098] In some embodiments, at least one of LP1 or LP2 comprises an amino acid sequence selected from the group consisting of $(GS)_n$, $(GGS)_n$, $(GSGGS)_n$ (SEQ ID NO: 191) and $(GGGS)_n$ (SEQ ID NO: 192), where n is an integer of at least one.

[00099] In some embodiments, at least one of LP1 or LP2 comprises an amino acid sequence selected from the group consisting of GGSG (SEQ ID NO: 193), GGSGG (SEQ ID NO: 194), GSGSG (SEQ ID NO: 195), GSGGG (SEQ ID NO: 196), GGGSG (SEQ ID NO: 197), and GSSSG (SEQ ID NO: 198).

[000100] In some embodiments, LP1 comprises the amino acid sequence GSSGGSGGSGGSG (SEQ ID NO: 199), GSSGGSGGSGG (SEQ ID NO: 200), GSSGGSGGSGGS (SEQ ID NO: 201), GSSGGSGGSGGSGGGS (SEQ ID NO: 202), GSSGGSGGSG (SEQ ID NO: 203), or GSSGGSGGSGS (SEQ ID NO: 204).

[000101] In some embodiments, LP2 comprises the amino acid sequence GSS, GGS, GGGS (SEQ ID NO: 205), GSSGT (SEQ ID NO: 206) or GSSG (SEQ ID NO: 207).

[000102] In some embodiments, the AB has a dissociation constant of about 100 nM or less for binding to PDL1.

[000103] In some embodiments, the activatable antibody includes an antibody or antigen-binding fragment thereof (AB) that specifically binds PDL1. In some embodiments, the antibody or antigen binding fragment thereof that binds PDL1 is a monoclonal antibody, domain antibody, single chain, Fab fragment, a $F(ab')_2$ fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, or a single domain light chain antibody. In some embodiments, such an antibody or antigen binding fragment thereof that binds PDL1 is a mouse, other rodent, chimeric, humanized or fully human monoclonal antibody.

[000104] In some embodiments, the activatable antibody comprises a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56. In some embodiments, the activatable antibody comprises a light chain amino acid sequence comprising SEQ ID NO: 12 or SEQ ID NO: 58. In some embodiments, the activatable antibody comprises a light chain amino acid sequence comprising SEQ ID NO: 12. In some embodiments, the activatable antibody comprises a light chain amino acid sequence comprising SEQ ID NO: 58.

[000105] In some embodiments, the activatable antibody comprises a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid sequence comprising SEQ ID NO: 58.

[000106] In some embodiments, the activatable antibody comprises a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid sequence comprising SEQ ID NO: 12.

[000107] In some embodiments, the activatable antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56. In some embodiments, the activatable antibody comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid

sequence of SEQ ID NO: 58. In some embodiments, the activatable antibody comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the activatable antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, the activatable antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12.

[000108] In some embodiments, the AB of the activatable anti-PDL1 antibody comprises a heavy chain amino acid sequence selected from the group consisting of the heavy chain sequences shown in Table 15. In some embodiments, the AB of the activatable anti-PDL1 antibody comprises a light chain amino acid sequence selected from the group consisting of the light chain sequences shown in Table 15. In some embodiments, the AB of the activatable anti-PDL1 antibody comprises a heavy chain amino acid sequence selected from the group consisting of the heavy chain sequences shown in Table 15 and a light chain amino acid sequence selected from the group consisting of the light chain sequences shown in Table 15.

[000109] In some embodiments, the AB of the activatable anti-PDL1 antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of the heavy chain sequences shown in Table 15. In some embodiments, the AB of the activatable anti-PDL1 antibody comprises a light chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of the light chain sequences shown in Table 15. In some embodiments, the AB of the activatable anti-PDL1 antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of the heavy chain sequences shown in Table 15 and a light chain amino acid sequence that is at

least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of the light chain sequences shown in Table 15.

[000110] In some embodiments, the activatable antibody comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence shown in Table 16; a VH CDR2 sequence shown in Table 16; a VH CDR3 sequence shown in Table 16; a VL CDR1 sequence shown in Table 16; a VL CDR2 sequence shown in Table 16; and a VL CDR3 sequence shown in Table 16.

[000111] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence shown in Table 16; a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence shown in Table 16; a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence shown in Table 16; a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence shown in Table 16; a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence shown in Table 16; and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence shown in Table 16.

[000112] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence,

a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 16.

[000113] In some embodiments, the activatable antibody comprises a heavy chain that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein the combination is a combination of the three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 16.

[000114] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 16.

[000115] In some embodiments, the activatable antibody comprises a heavy chain that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 16.

[000116] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VL CDR1 sequence comprising RASQSISSYLN (SEQ ID NO: 209); a VL CDR2 sequence comprising AASSLQS (SEQ ID NO: 215); a VL CDR3 sequence comprising DNGYPST (SEQ ID NO: 228); a VH CDR1 sequence comprising SYAMS (SEQ ID NO: 212); a VH CDR2 sequence comprising SSIWRNGIVTVYADS (SEQ ID NO: 246); and a VH CDR3 sequence comprising WSAAFDY (SEQ ID NO: 235).

[000117] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR2 sequence comprises SSIWRNGIVTVYADS (SEQ ID NO: 246). In some embodiments, the

activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR3 sequence comprises WSAAFDY (SEQ ID NO: 235). In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR2 sequence comprises SSIWRNGIVTVYADS (SEQ ID NO: 246), and the VH CDR3 sequence comprises WSAAFDY (SEQ ID NO: 235).

[000118] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination comprises a VL CDR1 sequence comprising RASQSISSYLN (SEQ ID NO: 209); a VL CDR2 sequence comprising AASSLQS (SEQ ID NO: 215); a VL CDR3 sequence comprising DNGYPST (SEQ ID NO: 228); a VH CDR1 sequence comprising SYAMS (SEQ ID NO: 212); a VH CDR2 sequence comprising SSIWRNGIVTVYADS (SEQ ID NO: 246); and a VH CDR3 sequence comprising WSAAFDY (SEQ ID NO: 235).

[000119] In some embodiments, the activatable antibody comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence shown in Table 17; a VH CDR2 sequence shown in Table 17; a VH CDR3 sequence shown in Table 17; a VL CDR1 sequence shown in Table 17; a VL CDR2 sequence shown in Table 17; and a VL CDR3 sequence shown in Table 17.

[000120] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is

at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence shown in Table 17; a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence shown in Table 17; a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence shown in Table 17; a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence shown in Table 17; a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence shown in Table 17; and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence shown in Table 17.

[000121] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination shown in Table 17.

[000122] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination shown in Table 17.

[000123] In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201. In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56. In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141,

143, 145, 147, 149, 151, 153, 155, 157, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201, and an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56.

[000124] In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201. In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56. In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201, and an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56.

[000125] In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, and 1201. In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56. In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 100, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, and 1201, and an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56.

[000126] In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino

acid sequence selected from the group consisting of SEQ ID NOs: 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201. In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56. In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201, and an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56.

[000127] In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201. In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56. In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201, and an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%

identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56.

[000128] In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1200, and 1201. In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56. In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, and 1201, and an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56.

[000129] In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201, and the heavy chain amino acid sequence of SEQ ID NO: 46.

[000130] In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201, and the heavy chain amino acid sequence of SEQ ID NO: 46.

[000131] In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, and 1201, and the heavy chain amino acid sequence of SEQ ID NO: 46.

[000132] In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201, and an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the heavy chain amino acid sequence of SEQ ID NO: 46.

[000133] In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201, and an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the heavy chain amino acid sequence of SEQ ID NO: 46.

[000134] In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, and 1201, and an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the heavy chain amino acid sequence of SEQ ID NO: 46.

[000135] In some embodiments, the activatable antibody comprises the amino acid sequence of SEQ ID NO: 428. In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 430, 432, 434, and 1202. In some embodiments, the activatable antibody comprises the amino acid sequence of SEQ ID NO: 428, and an amino acid sequence selected from the group consisting of SEQ ID NO: 430, 432, and 434.

[000136] In some embodiments, the activatable antibody comprises the amino acid sequence of SEQ ID NO: 1008. In some embodiments, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 430, 432, 434, and 1202. In some embodiments, the activatable antibody comprises the amino acid sequence of SEQ ID NO: 1008, and an amino acid sequence selected from the group consisting of SEQ ID NO: 430, 432, 434, and 1202.

[000137] In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 428. In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 430, 432, 434, and 1202. In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 428, and an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 430, 432, 434, and 1202.

[000138] In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 1008. In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 430, 432, 434, and 1202. In some embodiments, the activatable antibody includes an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 1008, and an amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 430, 432, 434, and 1202.

[000139] In some embodiments, the activatable antibody comprises a masking moiety (MM) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 59-81, 208, and 426; a cleavable moiety (CM) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 341, 352, 359, 364, 372, 377, 379, 383, 394, 437, 883-893, 901-920, and 921; and a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination comprises a VL CDR1 sequence comprising RASQSISSYLN (SEQ ID NO: 209); a VL CDR2 sequence comprising AASSLQS (SEQ ID NO: 215); a VL CDR3 sequence comprising DNGYPST (SEQ ID NO: 228); a VH CDR1 sequence comprising SYAMS (SEQ ID NO: 212); a VH CDR2 sequence comprising SSIWRNGIVTVYADS (SEQ ID NO: 246); and a VH CDR3 sequence comprising WSAAFDY (SEQ ID NO: 235).

[000140] In some embodiments, the activatable antibody comprises a masking moiety (MM) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 60, 62, 63, 66-68, 71, 75, and 76; a cleavable moiety (CM) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 341, 364, 377, 394, 437, 883-893, 901-911, and 920; and a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination comprises a VL CDR1 sequence comprising RASQSISSYLN (SEQ ID NO: 209); a VL CDR2 sequence comprising AASSLQS (SEQ ID NO: 215); a VL CDR3 sequence comprising DNGYPST (SEQ ID NO: 228); a VH CDR1 sequence comprising SYAMS (SEQ ID NO: 212); a VH CDR2 sequence comprising SSIWRNGIVTVYADS (SEQ ID NO: 246); and a VH CDR3 sequence comprising WSAAFDY (SEQ ID NO: 235).

[000141] In some embodiments, the activatable antibody comprises a masking moiety (MM) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 59-81, 208, and 426; a cleavable moiety (CM) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 341, 352, 359, 364, 372, 377, 379, 383, 394, 437, 883-893, 901-920, and 921; and of a variable light chain comprising the amino acid sequence of SEQ ID NO: 58 and a variable heavy chain comprising the amino acid sequence of SEQ ID NO: 46.

[000142] In some embodiments, the activatable antibody comprises a masking moiety (MM) comprising an amino acid sequence selected from the group consisting of SEQ ID

NOs: 60, 62, 63, 66-68, 71, 75, and 76; a cleavable moiety (CM) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 341, 364, 377, 394, 437, 883-893, 901-911, and 920; and of a variable light chain comprising the amino acid sequence of SEQ ID NO: 58 and a variable heavy chain comprising the amino acid sequence of SEQ ID NO: 46.

[000143] In some embodiments, the activatable antibody comprises a masking moiety (MM) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 59-81, 208, and 426; a cleavable moiety (CM) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 341, 352, 359, 364, 372, 377, 379, 383, 394, 437, 883-893, 901-920, and 921; and of a variable light chain comprising the amino acid sequence of SEQ ID NO: 12 and a variable heavy chain comprising the amino acid sequence of SEQ ID NO: 46.

[000144] In some embodiments, the activatable antibody comprises a masking moiety (MM) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 60, 62, 63, 66-68, 71, 75, and 76; a cleavable moiety (CM) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 341, 364, 377, 394, 437, 883-893, 901-11, and 920; and of a variable light chain comprising the amino acid sequence of SEQ ID NO: 12 and a variable heavy chain comprising the amino acid sequence of SEQ ID NO: 46.

[000145] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 58. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 12. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42,

44, 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 58. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 12. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 58. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising SEQ ID NO: 12.

[000146] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain

amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12.

[000147] In some embodiments, the activatable antibody is encoded by a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, 15, 17, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55. In some embodiments, the activatable antibody is encoded by a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 45, 47, 49, 51, 53, and 55. In some embodiments, the activatable antibody is encoded by the light chain nucleic acid sequence of SEQ ID NO: 57.

In some embodiments, the activatable antibody is encoded by the light chain nucleic acid sequence of SEQ ID NO: 11. In some embodiments, the activatable antibody is encoded by heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, 15, 17, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55, and a light chain nucleic acid sequence comprising SEQ ID NO: 57. In some embodiments, the activatable antibody is encoded by heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, 15, 17, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55, and a light chain nucleic acid sequence comprising SEQ ID NO: 11. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 45, 47, 49, 51, 53, and 55, and a light chain nucleic acid sequence comprising SEQ ID NO: 57. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 45, 47, 49, 51, 53, and 55, and a light chain nucleic acid sequence comprising SEQ ID NO: 11.

[000148] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, 15, 17, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 45, 47, 49, 51, 53, and 55. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the light chain nucleic acid sequence of SEQ ID NO: 57. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the light chain nucleic acid sequence of SEQ ID NO: 11. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, 15, 17, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the light chain nucleic acid sequence comprising SEQ ID NO: 57.

In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, 15, 17, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the light chain nucleic acid sequence comprising SEQ ID NO: 11. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 45, 47, 49, 51, 53, and 55, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to light chain nucleic acid sequence comprising SEQ ID NO: 57. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NO: 45, 47, 49, 51, 53, and 55, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to light chain nucleic acid sequence comprising SEQ ID NO: 11.

[000149] In some embodiments, the MM has a dissociation constant for binding to the AB that is greater than the dissociation constant of the AB to PDL1.

[000150] In some embodiments, the MM has a dissociation constant for binding to the AB that is no more than the dissociation constant of the AB to PDL1.

[000151] In some embodiments, the MM has a dissociation constant for binding to the AB that is less than the dissociation constant of the AB to PDL1.

[000152] In some embodiments, the MM has a dissociation constant for binding to the AB that is approximately equal to the dissociation constant of the AB to PDL1.

[000153] In some embodiments, the MM does not interfere or compete with the AB for binding to PDL1 when the activatable antibody is in a cleaved state.

[000154] In some embodiments, the MM is a polypeptide of about 2 to 40 amino acids in length. In some embodiments, the MM is a polypeptide of up to about 40 amino acids in length.

[000155] In some embodiments, the MM polypeptide sequence is different from that of PDL1. In some embodiments, the MM polypeptide sequence is no more than 50% identical to any natural binding partner of the AB. In some embodiments, the MM

polypeptide sequence is different from that of PDL1 and is no more than 40%, 30%, 25%, 20%, 15%, or 10% identical to any natural binding partner of the AB.

[000156] In some embodiments, the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 59-81, 208, and 426. In some embodiments, the MM comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 59-81, 208, and 426.

[000157] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind PDL1 such that the dissociation constant (K_d) of the AB when coupled to the MM towards PDL1 is at least two times greater than the K_d of the AB when not coupled to the MM towards PDL1.

[000158] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind PDL1 such that the dissociation constant (K_d) of the AB when coupled to the MM towards PDL1 is at least five times greater than the K_d of the AB when not coupled to the MM towards PDL1.

[000159] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind PDL1 such that the dissociation constant (K_d) of the AB when coupled to the MM towards PDL1 is at least 10 times greater than the K_d of the AB when not coupled to the MM towards PDL1.

[000160] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind PDL1 such that the dissociation constant (K_d) of the AB when coupled to the MM towards PDL1 is at least 20 times greater than the K_d of the AB when not coupled to the MM towards PDL1.

[000161] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind PDL1 such that the dissociation constant (K_d) of the AB when coupled to the MM towards PDL1 is at least 40 times greater than the K_d of the AB when not coupled to the MM towards PDL1.

[000162] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind PDL1 such that the dissociation constant (K_d) of the AB when coupled to the MM towards PDL1 is at least 100 times greater than the K_d of the AB when not coupled to the MM towards PDL1.

[000163] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind PDL1 such that the dissociation constant (K_d) of the AB when coupled to

the MM towards PDL1 is at least 1000 times greater than the K_d of the AB when not coupled to the MM towards PDL1.

[000164] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind PDL1 such that the dissociation constant (K_d) of the AB when coupled to the MM towards PDL1 is at least 10,000 times greater than the K_d of the AB when not coupled to the MM towards PDL1.

[000165] In some embodiments, in the presence of PDL1, the MM reduces the ability of the AB to bind PDL1 by at least 90% when the CM is uncleaved, as compared to when the CM is cleaved when assayed *in vitro* using a target displacement assay such as, for example, the assay described in PCT Publication No. WO 2010/081173, the contents of which are hereby incorporated by reference in their entirety.

[000166] In some embodiments, the protease that cleaves the CM is active, e.g., up-regulated, in diseased tissue, and the protease cleaves the CM in the activatable antibody when the activatable antibody is exposed to the protease.

[000167] In some embodiments, the protease is co-localized with PDL1 in a tissue, and the protease cleaves the CM in the activatable antibody when the activatable antibody is exposed to the protease.

[000168] In some embodiments, the protease is present at relatively higher levels in or in close proximity to target-containing tissue of a treatment site or diagnostic site than in tissue of non-treatment sites (for example in healthy tissue), and the protease cleaves the CM in the activatable antibody when the activatable antibody is exposed to the protease.

[000169] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to PDL1 is reduced to occur with a dissociation constant that is at least twofold greater than the dissociation constant of an unmodified AB binding to PDL1, whereas in the cleaved state (i.e., when the activatable antibody is in the cleaved state), the AB binds PDL1.

[000170] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to PDL1 is reduced to occur with a dissociation constant that is at least fivefold greater than the dissociation constant of an unmodified AB binding to PDL1, whereas in the cleaved state (i.e., when the activatable antibody is in the cleaved state), the AB binds PDL1.

[000171] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to PDL1 is reduced to occur with a dissociation constant that is at least 10-fold greater than the dissociation constant of an unmodified AB binding to PDL1, whereas in the cleaved state (i.e., when the activatable antibody is in the cleaved state), the AB binds PDL1.

[000172] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to PDL1 is reduced to occur with a dissociation constant that is at least 20-fold greater than the dissociation constant of an unmodified AB binding to PDL1, whereas in the cleaved state (i.e., when the activatable antibody is in the cleaved state), the AB binds PDL1.

[000173] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to PDL1 is reduced to occur with a dissociation constant that is at least 40-fold greater than the dissociation constant of an unmodified AB binding to PDL1, whereas in the cleaved state, the AB binds PDL1.

[000174] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to PDL1 is reduced to occur with a dissociation constant that is at least 50-fold greater than the dissociation constant of an unmodified AB binding to PDL1, whereas in the cleaved state, the AB binds PDL1.

[000175] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to PDL1 is reduced to occur with a dissociation constant that is at least 100-fold greater than the dissociation constant of an unmodified AB binding to PDL1, whereas in the cleaved state, the AB binds PDL1.

[000176] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to PDL1 is reduced to occur with a dissociation constant that is at least 200-fold greater than the dissociation constant of an unmodified AB binding to PDL1, whereas in the cleaved state, the AB binds PDL1.

[000177] In some embodiments, the CM is a polypeptide of up to 15 amino acids in length.

[000178] In some embodiments, the CM is a polypeptide that includes a first cleavable moiety (CM1) that is a substrate for at least one matrix metalloprotease (MMP) and a second cleavable moiety (CM2) that is a substrate for at least one serine protease (SP). In some embodiments, each of the CM1 substrate sequence and the CM2 substrate sequence of the CM1-CM2 substrate is independently a polypeptide of up to 15 amino acids in length.

[000179] In some embodiments, the CM is a substrate for at least one protease that is or is believed to be up-regulated in cancer. In some embodiments, the CM is a substrate for at least one protease that is or is believed to be up-regulated in inflammation. In some embodiments, the CM is a substrate for at least one protease that is or is believed to be up-regulated in autoimmunity.

[000180] In some embodiments, the CM is a substrate for at least one protease selected from the group consisting of a matrix metalloprotease (MMP), a thrombin, a neutrophil elastase, a cysteine protease, a legumain, and a serine protease, such as a matriptase (MT-SP1), and a urokinase (uPA). Without being bound by theory, it is believed that these proteases are up-regulated in at least one of cancer, inflammation, and/or autoimmunity.

[000181] Exemplary substrates include but are not limited to substrates cleavable by one or more of the following enzymes or proteases listed in Table 12.

[000182] In some embodiments, the CM is selected for use with a specific protease, for example a protease that is known to be co-localized with the target of the activatable antibody.

[000183] In some embodiments, the CM is selected for use with a specific protease, for example a protease that is known to be in close proximity to the target of the activatable antibody.

[000184] In some embodiments, the CM is a substrate for at least one MMP. Examples of MMPs include the MMPs listed in the Table 12. In some embodiments, the CM is a substrate for a protease selected from the group consisting of MMP 9, MMP14, MMP1, MMP3, MMP13, MMP17, MMP11, and MMP19. In some embodiments the CM is a substrate for MMP9. In some embodiments, the CM is a substrate for MMP14.

[000185] In some embodiments, the CM is a substrate that includes the sequence TGRGPSWV (SEQ ID NO: 338); SARGPSRW (SEQ ID NO: 339); TARGPSFK (SEQ ID NO: 340); LSGRSDNH (SEQ ID NO: 341); GGWHTGRN (SEQ ID NO: 342);

HTGRSGAL (SEQ ID NO: 343); PLTGRSGG (SEQ ID NO: 344); AARGPAIH (SEQ ID NO: 345); RGPAFNPM (SEQ ID NO: 346); SSRGPAYL (SEQ ID NO: 347); RGPATPIM (SEQ ID NO: 348); RGPA (SEQ ID NO: 349); GGQPSGMWGW (SEQ ID NO: 350); FPRPLGITGL (SEQ ID NO: 351); VHMPLGFLGP (SEQ ID NO: 352); SPLTGRSG (SEQ ID NO: 353); SAGFSLPA (SEQ ID NO: 354); LAPLGLQRR (SEQ ID NO: 355); SGGPLGVR (SEQ ID NO: 356); PLGL (SEQ ID NO: 357); LSGRSGNH (SEQ ID NO: 883); SGRSANPRG (SEQ ID NO: 884); LSGRSDDH (SEQ ID NO: 885); LSGRSDIH (SEQ ID NO: 886); LSGRSDQH (SEQ ID NO: 887); LSGRSDTH (SEQ ID NO: 888); LSGRSDYH (SEQ ID NO: 889); LSGRSDNP (SEQ ID NO: 890); LSGRSANP (SEQ ID NO: 891); LSGRSANI (SEQ ID NO: 892); and/or LSGRSDNI (SEQ ID NO: 893).

[000186] In some embodiments, the CM comprises the amino acid sequence LSGRSDNH (SEQ ID NO: 341). In some embodiments, the CM comprises the amino acid sequence TGRGPSWV (SEQ ID NO: 338). In some embodiments, the CM comprises the amino acid sequence PLTGRSGG (SEQ ID NO: 344). In some embodiments, the CM comprises the amino acid sequence GGQPSGMWGW (SEQ ID NO: 350). In some embodiments, the CM comprises the amino acid sequence FPRPLGITGL (SEQ ID NO: 351). In some embodiments, the CM comprises the amino acid sequence VHMPLGFLGP (SEQ ID NO: 352). In some embodiments, the CM comprises the amino acid sequence PLGL (SEQ ID NO: 357). In some embodiments, the CM comprises the amino acid sequence SARGPSRW (SEQ ID NO: 339). In some embodiments, the CM comprises the amino acid sequence TARGPSFK (SEQ ID NO: 340). In some embodiments, the CM comprises the amino acid sequence GGWHTGRN (SEQ ID NO: 342). In some embodiments, the CM comprises the amino acid sequence HTGRSGAL (SEQ ID NO: 343). In some embodiments, the CM comprises the amino acid sequence AARGPAIH (SEQ ID NO: 345). In some embodiments, the CM comprises the amino acid sequence RGPAFNPM (SEQ ID NO: 346). In some embodiments, the CM comprises the amino acid sequence SSRGPAYL (SEQ ID NO: 347). In some embodiments, the CM comprises the amino acid sequence RGPATPIM (SEQ ID NO: 348). In some embodiments, the CM comprises the amino acid sequence RGPA (SEQ ID NO: 349). In some embodiments, the CM comprises the amino acid sequence LSGRSGNH (SEQ ID NO: 883). In some embodiments, the CM comprises the amino acid sequence SGRSANPRG (SEQ ID NO: 884). In some embodiments, the CM comprises the amino acid sequence LSGRSDDH (SEQ ID NO: 885).

In some embodiments, the CM comprises the amino acid sequence LSGRSDIH (SEQ ID NO: 886). In some embodiments, the CM comprises the amino acid sequence LSGRSDQH (SEQ ID NO: 887). In some embodiments, the CM comprises the amino acid sequence LSGRSDTH (SEQ ID NO: 888). In some embodiments, the CM comprises the amino acid sequence LSGRSDYH (SEQ ID NO: 889). In some embodiments, the CM comprises the amino acid sequence LSGRSDNP (SEQ ID NO: 890). In some embodiments, the CM comprises the amino acid sequence LSGRSANP (SEQ ID NO: 891). In some embodiments, the CM comprises the amino acid sequence LSGRSANI (SEQ ID NO: 892). In some embodiments, the CM comprises the amino acid sequence LSGRSDNI (SEQ ID NO: 893)

[000187] In some embodiments, the CM is a substrate for an MMP and includes the sequence ISSGLSS (SEQ ID NO: 358); QNQALRMA (SEQ ID NO: 359); AQNLLGMV (SEQ ID NO: 360); STFPFGMF (SEQ ID NO: 361); PVGYTSSL (SEQ ID NO: 362); DWLYWPGI (SEQ ID NO: 363), ISSGLLSS (SEQ ID NO: 364), LKAAPRWA (SEQ ID NO: 365); GPSHLVLT (SEQ ID NO: 366); LPGGLSPW (SEQ ID NO: 367); MGLFSEAG (SEQ ID NO: 368); SPLPLRVP (SEQ ID NO: 369); RMHLRSLG (SEQ ID NO: 370); LAAPLGLL (SEQ ID NO: 371); AVGLLAPP (SEQ ID NO: 372); LLAPSHRA (SEQ ID NO: 373); PAGLWLDP (SEQ ID NO: 374); MIAPVAYR (SEQ ID NO: 894); RPSPMWAY (SEQ ID NO: 895); WATPRPMR (SEQ ID NO: 896); FRLLDWQW (SEQ ID NO: 897); ISSGL (SEQ ID NO: 898); ISSGLLS (SEQ ID NO: 899); and/or ISSGLL (SEQ ID NO: 900).

[000188] In some embodiments, the CM comprises the amino acid sequence ISSGLSS (SEQ ID NO: 358). In some embodiments, the CM comprises the amino acid sequence QNQALRMA (SEQ ID NO: 359). In some embodiments, the CM comprises the amino acid sequence AQNLLGMV (SEQ ID NO: 360). In some embodiments, the CM comprises the amino acid sequence STFPFGMF (SEQ ID NO: 361). In some embodiments, the CM comprises the amino acid sequence PVGYTSSL (SEQ ID NO: 362). In some embodiments, the CM comprises the amino acid sequence DWLYWPGI (SEQ ID NO: 363). In some embodiments, the CM comprises the amino acid sequence ISSGLLSS (SEQ ID NO: 364). In some embodiments, the CM comprises the amino acid sequence LKAAPRWA (SEQ ID NO: 365). In some embodiments, the CM comprises the amino acid sequence GPSHLVLT (SEQ ID NO: 366). In some embodiments, the CM comprises the amino acid sequence LPGGLSPW (SEQ ID NO: 367). In some embodiments, the CM comprises the amino acid sequence MGLFSEAG (SEQ ID NO: 368). In some embodiments, the CM comprises the

amino acid sequence SPLPLRVP (SEQ ID NO: 369). In some embodiments, the CM comprises the amino acid sequence RMHLRSLG (SEQ ID NO: 370). In some embodiments, the CM comprises the amino acid sequence LAAPLGLL (SEQ ID NO: 371). In some embodiments, the CM comprises the amino acid sequence AVGLLAPP (SEQ ID NO: 372). In some embodiments, the CM comprises the amino acid sequence LLAPSHRA (SEQ ID NO: 373). In some embodiments, the CM comprises the amino acid sequence PAGLWLDP (SEQ ID NO: 374). In some embodiments, the CM comprises the amino acid sequence MIAPVAYR (SEQ ID NO: 894). In some embodiments, the CM comprises the amino acid sequence RPSPMWAY (SEQ ID NO: 895). In some embodiments, the CM comprises the amino acid sequence WATPRPMR (SEQ ID NO: 896). In some embodiments, the CM comprises the amino acid sequence FRLLDWQW (SEQ ID NO: 897). In some embodiments, the CM comprises the amino acid sequence ISSGL (SEQ ID NO: 898). In some embodiments, the CM comprises the amino acid sequence ISSGLLS (SEQ ID NO: 899). In some embodiments, the CM comprises the amino acid sequence ISSGLL (SEQ ID NO: 900).

[000189] In some embodiments, the CM is a substrate for thrombin. In some embodiments, the CM is a substrate for thrombin and includes the sequence GPRSFGL (SEQ ID NO: 375) or GPRSFG (SEQ ID NO: 376). In some embodiments, the CM comprises the amino acid sequence GPRSFGL (SEQ ID NO: 375). In some embodiments, the CM comprises the amino acid sequence GPRSFG (SEQ ID NO: 376).

[000190] In some embodiments, the CM comprises an amino acid sequence selected from the group consisting of NTLSGRSENHSG (SEQ ID NO: 435); NTLSGRSGNHGS (SEQ ID NO: 436); TSTSGRSANPRG (SEQ ID NO: 437); TSGRSANP (SEQ ID NO: 438); VAGRSMRP (SEQ ID NO: 439); VVPEGRRS (SEQ ID NO: 440); ILPRSPAF (SEQ ID NO: 441); MVLGRSLL (SEQ ID NO: 442); QGRAITFI (SEQ ID NO: 443); SPRSIMLA (SEQ ID NO: 444); and SMLRSMPL (SEQ ID NO: 445).

[000191] In some embodiments, the CM comprises the amino acid sequence NTLSGRSENHSG (SEQ ID NO: 435). In some embodiments, the CM comprises the amino acid sequence NTLSGRSGNHGS (SEQ ID NO: 436). In some embodiments, the CM comprises the amino acid sequence TSTSGRSANPRG (SEQ ID NO: 437). In some embodiments, the CM comprises the amino acid sequence TSGRSANP (SEQ ID NO: 438). In some embodiments, the CM comprises the amino acid sequence VAGRSMRP (SEQ ID NO: 439). In some embodiments, the CM comprises the amino acid sequence VVPEGRRS

(SEQ ID NO: 440). In some embodiments, the CM comprises the amino acid sequence ILPRSPAF (SEQ ID NO: 441). In some embodiments, the CM comprises the amino acid sequence MVLGRSLL (SEQ ID NO: 442). In some embodiments, the CM comprises the amino acid sequence QGRAITFI (SEQ ID NO: 443). In some embodiments, the CM comprises the amino acid sequence SPRSIMLA (SEQ ID NO: 444). In some embodiments, the CM comprises the amino acid sequence SMLRSMPL (SEQ ID NO: 445).

[000192] In some embodiments, the CM is a substrate for a neutrophil elastase. In some embodiments, the CM is a substrate for a serine protease. In some embodiments, the CM is a substrate for uPA. In some embodiments, the CM is a substrate for legumain. In some embodiments, the CM is a substrate for matriptase. In some embodiments, the CM is a substrate for a cysteine protease. In some embodiments, the CM is a substrate for a cysteine protease, such as a cathepsin.

[000193] In some embodiments, the CM is a CM1-CM2 substrate and includes the sequence ISSGLLSGRSDNH (SEQ ID NO: 377); ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 378); AVGLLAPPGGTSTSGRSANPRG (SEQ ID NO: 379); TSTSGRSANPRGGGAVGLLAPP (SEQ ID NO: 380); VHMPLGFLGPGGTSTSGRSANPRG (SEQ ID NO: 381); TSTSGRSANPRGGGVHMPLGFLGP (SEQ ID NO: 382); AVGLLAPPGGLSGRSDNH (SEQ ID NO: 383); LSGRSDNHGGAVGLLAPP (SEQ ID NO: 384); VHMPLGFLGPGGLSGRSDNH (SEQ ID NO: 385); LSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 386); LSGRSDNHGGSGGSISSGLLSS (SEQ ID NO: 387); LSGRSGNHGGSGGSISSGLLSS (SEQ ID NO: 388); ISSGLLSSGGSGGSLSGRSGNH (SEQ ID NO: 389); LSGRSDNHGGSGGSQNQALRMA (SEQ ID NO: 390); QNQALRMAGGSGGSLSGRSDNH (SEQ ID NO: 391); LSGRSGNHGGSGGSQNQALRMA (SEQ ID NO: 392); QNQALRMAGGSGGSLSGRSGNH (SEQ ID NO: 393); ISSGLLSGRSGNH (SEQ ID NO: 394); ISSGLLSGRSANPRG (SEQ ID NO: 901); AVGLLAPPTSGRSANPRG (SEQ ID NO: 902); AVGLLAPPSGRSANPRG (SEQ ID NO: 903); ISSGLLSGRSDDH (SEQ ID NO: 904); ISSGLLSGRSDIH (SEQ ID NO: 905); ISSGLLSGRSDQH (SEQ ID NO: 906); ISSGLLSGRSDTH (SEQ ID NO: 907); ISSGLLSGRSDYH (SEQ ID NO: 908); ISSGLLSGRSDNP (SEQ ID NO: 909); ISSGLLSGRSANP (SEQ ID NO: 910); ISSGLLSGRSANI (SEQ ID NO: 911); AVGLLAPPGGLSGRSDDH (SEQ ID NO: 912); AVGLLAPPGGLSGRSDIH (SEQ ID NO: 913); AVGLLAPPGGLSGRSDQH (SEQ ID

NO: 914); AVGLLAPPGGLSGRSDTH (SEQ ID NO: 915); AVGLLAPPGGLSGRSDYH (SEQ ID NO: 916); AVGLLAPPGGLSGRSDNP (SEQ ID NO: 917); AVGLLAPPGGLSGRSANP (SEQ ID NO: 918); AVGLLAPPGGLSGRSANI (SEQ ID NO: 919); ISSGLLSGRSDNI (SEQ ID NO: 920); AVGLLAPPGGLSGRSDNI (SEQ ID NO: 921); GLSGRSDNHGGAVGLLAPP (SEQ ID NO: 1009); and/or GLSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 1010).

[000194] In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDNH (SEQ ID NO: 377), which is also referred to herein as substrate 2001. In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 378), which is also referred to herein as substrate 1001/LP′/0001, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GGSGGS (SEQ ID NO: 922). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGTSTSGRSANPRG (SEQ ID NO: 379), which is also referred to herein as substrate 2015 and/or substrate 1004/LP′/0003, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GG. In some embodiments, the CM1-CM2 substrate includes the sequence TSTSGRSANPRGGGAVGLLAPP (SEQ ID NO: 380), which is also referred to herein as substrate 0003/LP′/1004, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GG. In some embodiments, the CM1-CM2 substrate includes the sequence VHMPLGFLGPGGTSTSGRSANPRG (SEQ ID NO: 381), which is also referred to herein as substrate 1003/LP′/0003, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GG. In some embodiments, the CM1-CM2 substrate includes the sequence TSTSGRSANPRGGGVHMPLGFLGP (SEQ ID NO: 382), which is also referred to herein as substrate 0003/LP′/1003, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GG. In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDNH (SEQ ID NO: 383), which is also referred to herein as substrate 3001 and/or substrate 1004/LP′/0001, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GG. In some embodiments, the CM1-CM2 substrate includes the sequence LSGRSDNHGGAVGLLAPP (SEQ ID NO: 384), which is also referred to herein as substrate 0001/LP′/1004, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GG. In some embodiments, the CM1-CM2 substrate includes the sequence VHMPLGFLGPGGLSGRSDNH (SEQ ID NO: 385), which is also referred to herein as substrate 1003/LP'/0001, wherein LP′ as used in this CM1-CM2 substrate is the amino acid sequence GG. In some embodiments, the CM1-CM2

substrate includes the sequence LSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 386), which is also referred to herein as substrate 0001/LP′/1003, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GG. In some embodiments, the CM1-CM2 substrate includes the sequence LSGRSDNHGGSGGSISSGLLSS (SEQ ID NO: 387), which is also referred to herein as substrate 0001/LP′/1001, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GGSGGS (SEQ ID NO: 922). In some embodiments, the CM1-CM2 substrate includes the sequence LSGRSGNHGGSGGSISSGLLSS (SEQ ID NO: 388), which is also referred to herein as substrate 0002/LP′/1001, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GGSGGS (SEQ ID NO: 922). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSSGGSGGSLSGRSGNH (SEQ ID NO: 389), which is also referred to herein as substrate 1001/LP′/0002, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GGSGGS (SEQ ID NO: 922). In some embodiments, the CM1-CM2 substrate includes the sequence LSGRSDNHGGSGGSQNQALRMA (SEQ ID NO: 390), which is also referred to herein as substrate 0001/LP′/1002, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GGSGGS (SEQ ID NO: 922). In some embodiments, the CM1-CM2 substrate includes the sequence QNQALRMAGGSGGSLSGRSDNH (SEQ ID NO: 391), which is also referred to herein as substrate 1002/LP′/0001, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GGSGGS (SEQ ID NO: 922). In some embodiments, the CM1-CM2 substrate includes the sequence LSGRSGNHGGSGGSQNQALRMA (SEQ ID NO: 392), which is also referred to herein as substrate 0002/LP′/1002, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GGSGGS (SEQ ID NO: 922). In some embodiments, the CM1-CM2 substrate includes the sequence QNQALRMAGGSGGSLSGRSGNH (SEQ ID NO: 393), which is also referred to herein as substrate 1002/LP′/0002, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GGSGGS (SEQ ID NO: 922). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSGNH (SEQ ID NO: 394), which is also referred to herein as substrate 2002. In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSANPRG (SEQ ID NO: 901), which is also referred to herein as substrate 2003. In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPTSGRSANPRG (SEQ ID NO: 902), which is also referred to herein as substrate 2004. In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPSGRSANPRG (SEQ ID NO: 903), which is also

referred to herein as substrate 2005. In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDDH (SEQ ID NO: 904), which is also referred to herein as substrate 2006. In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDIH (SEQ ID NO: 905), which is also referred to herein as substrate 2007. In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDQH (SEQ ID NO: 906), which is also referred to herein as substrate 2008. In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDTH (SEQ ID NO: 907), which is also referred to herein as substrate 2009. In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDYH (SEQ ID NO: 908), which is also referred to herein as substrate 2010. In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDNP (SEQ ID NO: 909), which is also referred to herein as substrate 2011. In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSANP (SEQ ID NO: 910), which is also referred to herein as substrate 2012. In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSANI (SEQ ID NO: 911), which is also referred to herein as substrate 2013. In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDDH (SEQ ID NO: 912), which is also referred to herein as substrate 3006. In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDIH (SEQ ID NO: 913), which is also referred to herein as substrate 3007. In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDQH (SEQ ID NO: 914), which is also referred to herein as substrate 3008. In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDTH (SEQ ID NO: 915), which is also referred to herein as substrate 3009. In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDYH (SEQ ID NO: 916), which is also referred to herein as substrate 3010. In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDNP (SEQ ID NO: 917), which is also referred to herein as substrate 3011. In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSANP (SEQ ID NO: 918), which is also referred to herein as substrate 3012. In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSANI (SEQ ID NO: 919), which is also referred to herein as substrate 3013. In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDNI (SEQ ID NO: 920), which is also referred to herein as substrate 2014. In

some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDNI (SEQ ID NO: 921), which is also referred to herein as substrate 3014. In some embodiments, the CM1-CM2 substrate includes the sequence GLSGRSDNHGGAVGLLAPP (SEQ ID NO: 1009), which is also referred to herein as substrate 0001/LP′/1004, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GG. In some embodiments, the CM1-CM2 substrate includes the sequence GLSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 1010), which is also referred to herein as substrate 0001/LP′/1003, where LP′ as used in this CM1-CM2 substrate is the amino acid sequence GG.

[000195] In some embodiments, the CM is a substrate for at least two proteases. In some embodiments, each protease is selected from the group consisting of those shown in Table 12. In some embodiments, the CM is a substrate for at least two proteases, wherein one of the proteases is selected from the group consisting of a MMP, a thrombin, a neutrophil elastase, a cysteine protease, a uPA, a legumain and a matriptase and other serine proteases and the other protease is selected from the group consisting of those shown in Table 12. In some embodiments, the CM is a substrate for at least two proteases selected from the group consisting of a MMP, a thrombin, a neutrophil elastase, a cysteine protease, a uPA, a legumain and a matriptase and other serine proteases.

[000196] In some embodiments, the activatable antibody includes at least a first CM and a second CM. In some embodiments, the first CM and the second CM are each polypeptides of no more than 15 amino acids long. In some embodiments, the first CM and the second CM in the activatable antibody in the uncleaved state have the structural arrangement from N-terminus to C-terminus as follows: MM-CM1-CM2-AB or AB-CM2-CM1-MM. In some embodiments, at least one of the first CM and the second CM is a polypeptide that functions as a substrate for a protease selected from the group consisting of a MMP, a thrombin, a neutrophil elastase, a cysteine protease, a uPA, a legumain, and a matriptase and other serine proteases. In some embodiments, the first CM is cleaved by a first cleaving agent selected from the group consisting of a MMP, a thrombin, a neutrophil elastase, a cysteine protease, a uPA, a legumain, and a matriptase and other serine proteases in a target tissue and the second CM is cleaved by a second cleaving agent in a target tissue. In some embodiments, the other protease is selected from the group consisting of those shown in Table 12. In some embodiments, the first cleaving agent and the second cleaving agent are the same protease selected from the group consisting of a MMP, a thrombin, a

neutrophil elastase, a cysteine protease, a uPA, a legumain, and a matriptase and other serine proteases, and the first CM and the second CM are different substrates for the enzyme. In some embodiments, the first cleaving agent and the second cleaving agent are the same protease selected from the group consisting of those shown in Table 12. In some embodiments, the first cleaving agent and the second cleaving agent are different proteases. In some embodiments, the first cleaving agent and the second cleaving agent are co-localized in the target tissue. In some embodiments, the first CM and the second CM are cleaved by at least one cleaving agent in the target tissue.

[000197] In some embodiments, the activatable antibody is exposed to and cleaved by a protease such that, in the activated or cleaved state, the activated antibody includes a light chain amino acid sequence that includes at least a portion of LP2 and/or CM sequence after the protease has cleaved the CM.

[000198] In some embodiments, the activatable antibody also includes an agent conjugated to the AB. In some embodiments, the agent conjugated to the AB or the AB of an activatable antibody is a therapeutic agent. In some embodiments, the agent is an antineoplastic agent. In some embodiments, the agent is a toxin or fragment thereof. As used herein, a fragment of a toxin is a fragment that retains toxic activity. In some embodiments, the agent is conjugated to the AB via a cleavable linker. In some embodiments, the agent is conjugated to the AB via a linker that includes at least one CM1-CM2 substrate sequence. In some embodiments, the agent is conjugated to the AB via a noncleavable linker. In some embodiments, the agent is conjugated to the AB via a linker that is cleavable in an intracellular or lysosomal environment. In some embodiments, the agent is a microtubule inhibitor. In some embodiments, the agent is a nucleic acid damaging agent, such as a DNA alkylator, a DNA cleaving agent, a DNA cross-linker, or DNA intercalator, or other DNA damaging agent. In some embodiments, the agent is an agent selected from the group listed in Table 11. In some embodiments, the agent is a dolastatin. In some embodiments, the agent is an auristatin or derivative thereof. In some embodiments, the agent is auristatin E or a derivative thereof. In some embodiments, the agent is monomethyl auristatin E (MMAE). In some embodiments, the agent is monomethyl auristatin D (MMAD). In some embodiments, the agent is a maytansinoid or maytansinoid derivative. In some embodiments, the agent is DM1 or DM4. In some embodiments, the agent is a duocarmycin or derivative thereof. In some embodiments, the agent is a

calicheamicin or derivative thereof. In some embodiments, the agent is a pyrrolobenzodiazepine. In some embodiments, the agent is a pyrrolobenzodiazepine dimer.

[000199] In some embodiments, the activatable antibody is conjugated to one or more equivalents of an agent. In some embodiments, the activatable antibody is conjugated to one equivalent of the agent. In some embodiments, the activatable antibody is conjugated to two, three, four, five, six, seven, eight, nine, ten, or greater than ten equivalents of the agent. In some embodiments, the activatable antibody is part of a mixture of activatable antibodies having a homogeneous number of equivalents of conjugated agents. In some embodiments, the activatable antibody is part of a mixture of activatable antibodies having a heterogeneous number of equivalents of conjugated agents. In some embodiments, the mixture of activatable antibodies is such that the average number of agents conjugated to each activatable antibody is between zero to one, between one to two, between two and three, between three and four, between four and five, between five and six, between six and seven, between seven and eight, between eight and nine, between nine and ten, and ten and greater. In some embodiments, the mixture of activatable antibodies is such that the average number of agents conjugated to each activatable antibody is one, two, three, four, five, six, seven, eight, nine, ten, or greater.

[000200] In some embodiments, the activatable antibody comprises one or more site-specific amino acid sequence modifications such that the number of lysine and/or cysteine residues is increased or decreased with respect to the original amino acid sequence of the activatable antibody, thus in some embodiments correspondingly increasing or decreasing the number of agents that can be conjugated to the activatable antibody, or in some embodiments limiting the conjugation of the agents to the activatable antibody in a site-specific manner. In some embodiments, the modified activatable antibody is modified with one or more non-natural amino acids in a site-specific manner, thus in some embodiments limiting the conjugation of the agents to only the sites of the non-natural amino acids.

[000201] In some embodiments, the agent is an anti-inflammatory agent.

[000202] In some embodiments, the activatable antibody also includes a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent.

[000203] In some embodiments, the activatable antibody also includes a signal peptide. In some embodiments, the signal peptide is conjugated to the activatable antibody via a spacer. In some embodiments, the spacer is conjugated to the activatable antibody in the absence of a signal peptide. In some embodiments, the spacer is joined directly to the

MM of the activatable antibody. In some embodiments, the spacer is joined directly to the MM of the activatable antibody in the structural arrangement from N-terminus to C-terminus of spacer-MM-CM-AB. An example of a spacer joined directly to the N-terminus of MM of the activatable antibody is selected from the group consisting of QGQSGS (SEQ ID NO: 923); GQSGS (SEQ ID NO: 1192); QSGS (SEQ ID NO: 1193); SGS (SEQ ID NO: 1194); GS (SEQ ID NO: 1195); S; QGQSGQG (SEQ ID NO: 924); GQSGQG (SEQ ID NO: 395); QSGQG (SEQ ID NO: 925); SGQG (SEQ ID NO: 926); GQG (SEQ ID NO: 927); QG (SEQ ID NO: 928); G; QGQSGQ (SEQ ID NO: 1196); GQSGQ (SEQ ID NO: 1197); QSGQ (SEQ ID NO: 1198); SGQ (SEQ ID NO: 1198); GQ (SEQ ID NO: 1199); and Q. In some embodiments, the spacer includes at least the amino acid sequence QGQSGS (SEQ ID NO: 923). In some embodiments, the spacer includes at least the amino acid sequence GQSGS (SEQ ID NO: 1192). In some embodiments, the spacer includes at least the amino acid sequence QSGS (SEQ ID NO: 1193). In some embodiments, the spacer includes at least the amino acid sequence SGS (SEQ ID NO: 1194). In some embodiments, the spacer includes at least the amino acid sequence GS (SEQ ID NO: 1195). In some embodiments, the spacer includes at least the amino acid sequence S. In some embodiments, the spacer includes at least the amino acid sequence QGQSGQG (SEQ ID NO: 924). In some embodiments, the spacer includes at least the amino acid sequence GQSGQG (SEQ ID NO: 395). In some embodiments, the spacer includes at least the amino acid sequence QSGQG (SEQ ID NO: 925). In some embodiments, the spacer includes at least the amino acid sequence SGQG (SEQ ID NO: 926). In some embodiments, the spacer includes at least the amino acid sequence GQG (SEQ ID NO: 927). In some embodiments, the spacer includes at least the amino acid sequence QG (SEQ ID NO: 928). In some embodiments, the spacer includes at least the amino acid sequence G. In some embodiments, the spacer includes at least the amino acid sequence QGQSGQ (SEQ ID NO: 1196). In some embodiments, the spacer includes at least the amino acid sequence GQSGQ (SEQ ID NO: 1197). In some embodiments, the spacer includes at least the amino acid sequence QSGQ (SEQ ID NO: 1198). In some embodiments, the spacer includes at least the amino acid sequence SGQ (SEQ ID NO: 1198). In some embodiments, the spacer includes at least the amino acid sequence GQ (SEQ ID NO: 1199). In some embodiments, the spacer includes at least the amino acid sequence Q. In some embodiments, the activatable antibody does not include a spacer sequence.

[000204] In some embodiments, the AB of the activatable antibody naturally contains one or more disulfide bonds. In some embodiments, the AB can be engineered to include one or more disulfide bonds.

[000205] In some embodiments, the serum half-life of the activatable antibody is longer than that of the corresponding antibody; e.g., the pK of the activatable antibody is longer than that of the corresponding antibody. In some embodiments, the serum half-life of the activatable antibody is similar to that of the corresponding antibody. In some embodiments, the serum half-life of the activatable antibody is at least 15 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 12 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 11 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 10 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 9 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 8 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 7 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 6 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 5 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 4 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 3 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 2 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 24 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 20 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 18 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 16 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 14 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 12 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least

10 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 8 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 6 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 4 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 3 hours when administered to an organism.

[000206] In some embodiments, the activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody is monospecific. In some embodiments, the activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody is multispecific, *e.g.*, by way of non-limiting example, bispecific or trifunctional. In some embodiments, the activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody is formulated as part of a pro-Bispecific T Cell Engager (BITE) molecule, i.e., the BITE includes a masking moiety and a cleavable moiety. In some embodiments, the activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody is formulated as part of a pro-Chimeric Antigen Receptor (CAR) -modified T cell, -modified NK cell, or other -modified immune effector cell. In some embodiments, an activatable anti-PDL1 antibody is formulated as part of another engineered receptor on an immune effector cell; i.e., the pro-CAR or other pro-engineered receptor includes a masking moiety and a cleavable moiety.

[000207] In some embodiments, the activatable antibody or antigen-binding fragment thereof is incorporated in a multispecific activatable antibody or antigen-binding fragment thereof, where at least one arm of the multispecific activatable antibody specifically binds PDL1. In some embodiments, the activatable antibody or antigen-binding fragment thereof is incorporated in a bispecific antibody or antigen-binding fragment thereof, where at least one arm of the bispecific activatable antibody specifically binds PDL1.

[000208] In some embodiments, at least one arm of the multispecific activatable antibody, *e.g.*, a bispecific activatable antibody, comprises a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56. In some embodiments, at least one arm of the multispecific activatable antibody, *e.g.*, a bispecific activatable antibody, comprises a light chain amino acid sequence comprising SEQ ID NO: 12 or SEQ ID NO: 58. In some embodiments, at least one arm of the multispecific activatable antibody, *e.g.*, a bispecific activatable antibody, comprises a light chain amino acid sequence

comprising SEQ ID NO: 12. In some embodiments, at least one arm of the multispecific activatable antibody, *e.g.*, a bispecific activatable antibody, comprises a light chain amino acid sequence comprising SEQ ID NO: 58.

[000209] In some embodiments, at least one arm of the multispecific activatable antibody, *e.g.*, a bispecific activatable antibody, a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid sequence comprising SEQ ID NO: 58.

[000210] In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid sequence comprising SEQ ID NO: 12.

[000211] In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56. In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 48, 50, 52, 54, and 56,

and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12.

[000212] In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises the heavy chain amino acid sequence of SEQ ID NO: 46 and the light chain amino acid sequence of SEQ ID NO: 58. In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises the heavy chain amino acid sequence of SEQ ID NO: 46 and the light chain amino acid sequence of SEQ ID NO: 12.

[000213] In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid of SEQ ID NO: 46. In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence selected of SEQ ID NO: 46, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence selected of SEQ ID NO: 46, and a light chain amino acid that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12.

[000214] In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH

CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence shown in Table 16; a VH CDR2 sequence shown in Table 16; a VH CDR3 sequence shown in Table 16; a VL CDR1 sequence shown in Table 16; a VL CDR2 sequence shown in Table 16; and a VL CDR3 sequence shown in Table 16.

[000215] In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence shown in Table 16; a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence shown in Table 16; a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence shown in Table 16; a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence shown in Table 16; a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence shown in Table 16; and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence shown in Table 16.

[000216] In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 16.

[000217] In some embodiments, the antibody comprises a heavy chain that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein the combination is a combination of the three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 16.

[000218] In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 16.

[000219] In some embodiments, the antibody comprises a heavy chain that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 16.

[000220] In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VL CDR1 sequence comprising RASQSISSYLN (SEQ ID NO: 209); a VL CDR2 sequence comprising AASSLQS (SEQ ID NO: 215); a VL CDR3 sequence comprising DNGYPST (SEQ ID NO: 228); a VH CDR1 sequence comprising SYAMS (SEQ ID NO: 212); a VH CDR2 sequence comprising SSIWRNGIVTVYADS (SEQ ID NO: 246); and a VH CDR3 sequence comprising WSAAFDY (SEQ ID NO: 235).

[000221] In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR2 sequence comprises SSIWRNGIVTVYADS (SEQ ID NO: 246). In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a

combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR3 sequence comprises WSAAFDY (SEQ ID NO: 235). In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR2 sequence comprises SSIWRNGIVTVYADS (SEQ ID NO: 246), and the VH CDR3 sequence comprises WSAAFDY (SEQ ID NO: 235).

[000222] In some embodiments, at least one arm of the multispecific activatable antibody, e.g., a bispecific activatable antibody, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination comprises a VL CDR1 sequence comprising RASQSISSYLN (SEQ ID NO: 209); a VL CDR2 sequence comprising AASSLQS (SEQ ID NO: 215); a VL CDR3 sequence comprising DNGYPST (SEQ ID NO: 228); a VH CDR1 sequence comprising SYAMS (SEQ ID NO: 212); a VH CDR2 sequence comprising SSIWRNGIVTVYADS (SEQ ID NO: 246); and a VH CDR3 sequence comprising WSAAFDY (SEQ ID NO: 235).

[000223] In some embodiments, the anti-PDL1 antibodies, conjugated anti-PDL1 antibodies, activatable anti-PDL1 antibodies and/or conjugated activatable anti-PDL1 antibodies described herein are used as sole active agents. In some embodiments, the anti-PDL1 antibodies, conjugated anti-PDL1 antibodies, activatable anti-PDL1 antibodies and/or conjugated activatable anti-PDL1 antibodies described herein are used in conjunction with one or more additional agents or a combination of additional agents. Suitable additional agents include current pharmaceutical and/or surgical therapies for an intended application, such as, for example, cancer. For example, the anti-PDL1 antibodies, conjugated anti-PDL1 antibodies, activatable anti-PDL1 antibodies and/or conjugated activatable anti-PDL1 antibodies can be used in conjunction with an additional chemotherapeutic or anti-neoplastic agent.

[000224] In some embodiments, the additional agent(s) is a chemotherapeutic agent, such as a chemotherapeutic agent selected from the group consisting of docetaxel, paclitaxel, abraxane (i.e., albumin-conjugated paclitaxel), doxorubicin, oxaliplatin, carboplatin, cisplatin, irinotecan, and gemcitabine.

[000225] In some embodiments, the additional agent(s) is a checkpoint inhibitor, a kinase inhibitor, an agent targeting inhibitors in the tumor microenvironment, and/or a T cell or NK agonist. In some embodiments, the additional agent(s) is radiation therapy, alone or in combination with another additional agent(s) such as a chemotherapeutic or anti-neoplastic agent. In some embodiments, the additional agent(s) is a vaccine, an oncovirus, and/or a DC-activating agent such as, by way of non-limiting example, a toll-like receptor (TLR) agonist and/or α-CD40. In some embodiments, the additional agent(s) is a tumor-targeted antibody designed to kill the tumor via ADCC or via direct conjugation to a toxin (e.g., an antibody drug conjugate (ADC).

[000226] In some embodiments, the checkpoint inhibitor is an inhibitor of a target selected from the group consisting of CTLA-4, LAG-3, PD1 (also referred to as PD-1), PDL1, TIGIT, TIM-3, B7H4, and Vista. In some embodiments, the kinase inhibitor is selected from the group consisting of B-RAFi, MEKi, and Btk inhibitors, such as ibrutinib. In some embodiments, the kinase inhibitor is crizotinib. In some embodiments, the tumor microenvironment inhibitor is selected from the group consisting of an IDO inhibitor, an α-CSF1R inhibitor, an α-CCR4 inhibitor, a TGF-beta, a myeloid-derived suppressor cell, or a T-regulatory cell. In some embodiments, the agonist is selected from the group consisting of Ox40, GITR, CD137, ICOS, CD27, and HVEM.

[000227] In some embodiments, the inhibitor is a CTLA-4 inhibitor. In some embodiments, the inhibitor is a LAG-3 inhibitor. In some embodiments, the inhibitor is a PD1 inhibitor. In some embodiments, the inhibitor is a PDL1 inhibitor. In some embodiments, the inhibitor is a TIGIT inhibitor. In some embodiments, the inhibitor is a TIM-3 inhibitor. In some embodiments, the inhibitor is a B7H4 inhibitor. In some embodiments, the inhibitor is a Vista inhibitor. In some embodiments, the inhibitor is a B-RAFi inhibitor. In some embodiments, the B-RAFi inhibitor is vemurafenib. In some embodiments, the inhibitor is a MEKi inhibitor. In some embodiments, the inhibitor is a Btk inhibitor. In some embodiments, the inhibitor is ibrutinib. In some embodiments, the inhibitor is crizotinib. In some embodiments, the inhibitor is an IDO inhibitor. In some embodiments, the inhibitor is an α-CSF1R inhibitor. In some embodiments, the inhibitor is an α-CCR4 inhibitor. In some embodiments, the inhibitor is a TGF-beta. In some embodiments, the inhibitor is a myeloid-derived suppressor cell. In some embodiments, the inhibitor is a T-regulatory cell.

[000228] In some embodiments, the agonist is Ox40. In some embodiments, the agonist is GITR. In some embodiments, the agonist is CD137. In some embodiments, the agonist is ICOS. In some embodiments, the agonist is CD27. In some embodiments, the agonist is HVEM.

[000229] In some embodiments, the anti-PDL1 antibody, conjugated antibody, activatable antibody and/or conjugated activatable antibody is administered before and/or during and/or after treatment in combination with one or more additional agents such as, for example, a chemotherapeutic agent, an anti-inflammatory agent, and/or an immunosuppressive agent. In some embodiments, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and the additional agent are formulated into a single therapeutic composition, and the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and additional agent are administered simultaneously. Alternatively, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and additional agent are separate from each other, *e.g.*, each is formulated into a separate therapeutic composition, and the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and the additional agent are administered simultaneously, or the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and the additional agent are administered at different times during a treatment regimen. For example, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody is administered prior to the administration of the additional agent, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody is administered subsequent to the administration of the additional agent, or the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and the additional agent are administered in an alternating fashion. As described herein, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and additional agent are administered in single doses or in multiple doses.

[000230] In some embodiments, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and the additional agent(s) are administered simultaneously. For example, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and the additional agent(s) can be formulated in a single composition or administered as two or more separate compositions. In some embodiments, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and the additional agent(s) are administered sequentially, or the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and the additional agent are administered at different times during a treatment regimen.

[000231] In some embodiments, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody is administered before and/or during and/or after treatment in combination with one or more additional agents such as, by way of non-limiting example, a chemotherapeutic agent, an anti-inflammatory agent, and/or an immunosuppressive agent, such as an alkylating agent, an anti-metabolite, an anti-microtubule agent, a topoisomerase inhibitor, a cytotoxic antibiotic, and/or any other nucleic acid damaging agent. In some embodiments, the additional agent is a taxane, such as paclitaxel (e.g., Abraxane®). In some embodiments, the additional agent is an anti-metabolite, such as gemcitabine. In some embodiments, the additional agent is an alkylating agent, such as platinum-based chemotherapy, such as carboplatin or cisplatin. In some embodiments, the additional agent is a targeted agent, such as a kinase inhibitor, e.g., sorafenib or erlotinib. In some embodiments, the additional agent is a targeted agent, such as another antibody, e.g., a monoclonal antibody (e.g., bevacizumab), a bispecific antibody, or a multispecific antibody. In some embodiments, the additional agent is a proteosome inhibitor, such as bortezomib or carfilzomib. In some embodiments, the additional agent is an immune modulating agent, such as lenolidominde or IL-2. In some embodiments, the additional agent is radiation. In some embodiments, the additional agent is an agent considered standard of care by those skilled in the art. In some embodiments, the additional agent is a chemotherapeutic agent well known to those skilled in the art.

[000232] In some embodiments, the additional agent is another antibody or antigen-binding fragment thereof, another conjugated antibody or antigen-binding fragment thereof,

another activatable antibody or antigen-binding fragment thereof and/or another conjugated activatable antibody or antigen-binding fragment thereof. In some embodiments the additional agent is another antibody or antigen-binding fragment thereof, another conjugated antibody or antigen-binding fragment thereof, another activatable antibody or antigen-binding fragment thereof and/or another conjugated activatable antibody or antigen-binding fragment thereof against the same target as the first antibody or antigen-binding fragment thereof, the first conjugated antibody or antigen-binding fragment thereof, activatable antibody or antigen-binding fragment thereof and/or a conjugated activatable antibody or antigen-binding fragment thereof, e.g., against PDL1. In some embodiments the additional agent is another antibody or antigen-binding fragment thereof, another conjugated antibody or antigen-binding fragment thereof, another activatable antibody or antigen-binding fragment thereof and/or another conjugated activatable antibody or antigen-binding fragment thereof against a target different than the target of the first antibody or antigen-binding fragment thereof, the first conjugated antibody or antigen-binding fragment thereof, activatable antibody or antigen-binding fragment thereof and/or a conjugated activatable antibody or antigen-binding fragment thereof (i.e., target other than PDL1). In some embodiments, the additional agent is a chimeric antigen receptor-modified T cell, -modified NK cell or other -modified immune effector cell or is another engineered receptor-modified immune effector cell. In some embodiments, the additional agent is a pro-chimeric antigen receptor-modified T cell, -modified NK cell, or other -modified immune effector cell or is another pro-engineered receptor-modified immune effector cell.

[000233] As a non-limiting example, the antibody or antigen-binding fragment and/or the AB of an activatable antibody is a binding partner for any target listed in Table 22. In some embodiments that additional agent is ipilimumab, a CTLA4-binding fragment of ipilimumab, and/or an ipilimumab activatable antibody.

Table 22: Exemplary Targets

1-92-LFA-3	CD52	DL44	HVEM	LIF-R	STEAP1
Alpha-4 integrin	CD56	DLK1	Hyaluronidase	Lewis X	STEAP2
Alpha-V integrin	CD64	DLL4	ICOS	LIGHT	TAG-72
alpha4beta1 integrin	CD70	DPP-4	IFNalpha	LRP4	TAPA1
alpha4beta7 integrin	CD71	DSG1	IFNbeta	LRRC26	TGFbeta

AGR2	CD74	EGFR	IFNgamma	MCSP	TIGIT
Anti-Lewis-Y		EGFRviii	IgE	Mesothelin	TIM-3
Apelin J receptor	CD80	Endothelin B receptor (ETBR)	IgE Receptor (FceRI)	MRP4	TLR2
APRIL	CD81	ENPP3	IGF	MUC1	TLR4
B7-H4	CD86	EpCAM	IGF1R	Mucin-16 (MUC16, CA-125)	TLR6
BAFF	CD95	EPHA2	IL1B	Na/K ATPase	TLR7
BTLA	CD117	EPHB2	IL1R	Neutrophil elastase	TLR8
C5 complement	CD125	ERBB3	IL2	NGF	TLR9
C-242	CD132 (IL-2RG)	F protein of RSV	IL11	Nicastrin	TMEM31
CA9	CD133	FAP	IL12	Notch Receptors	TNFalpha
CA19-9 (Lewis a)	CD137	FGF-2	IL12p40	Notch 1	TNFR
Carbonic anhydrase 9	CD138	FGF8	IL-12R, IL-12Rbeta1	Notch 2	TNFRS12 A
CD2	CD166	FGFR1	IL13	Notch 3	TRAIL-R1
CD3	CD172A	FGFR2	IL13R	Notch 4	TRAIL-R2
CD6	CD248	FGFR3	IL15	NOV	Transferrin
CD9	CDH6	FGFR4	IL17	OSM-R	Transferrin receptor
CD11a	CEACAM5 (CEA)	Folate receptor	IL18	OX-40	TRK-A
CD19	CEACAM6 (NCA-90)	GAL3ST1	IL21	PAR2	TRK-B
CD20	CLAUDIN-3	G-CSF	IL23	PDGF-AA	uPAR
CD22	CLAUDIN-4	G-CSFR	IL23R	PDGF-BB	VAP1
CD24	cMet	GD2	IL27/IL27R (wsx1)	PDGFRalpha	VCAM-1
CD25	Collagen	GITR	IL29	PDGFRbeta	VEGF
CD27	Cripto	GLUT1	IL-31R	PD-1	VEGF-A
CD28	CSFR	GLUT4	IL31/IL31R	PD-L1	VEGF-B
CD30	CSFR-1	GM-CSF	IL2R	PD-L2	VEGF-C
CD33	CTLA-4	GM-CSFR	IL4	Phosphatidyl-serine	VEGF-D
CD38	CTGF	GP IIb/IIIa receptors	IL4R	P1GF	VEGFR1
CD40	CXCL10	Gp130	IL6, IL6R	PSCA	VEGFR2
CD40L	CXCL13	GPIIB/IIIA	Insulin Receptor	PSMA	VEGFR3
CD41	CXCR1	GPNMB	Jagged Ligands	RAAG12	VISTA

CD44	CXCR2	GRP78	Jagged 1	RAGE	WISP-1
CD44v6		HER2/neu	Jagged 2	SLC44A4	WISP-2
CD47	CXCR4	HGF	LAG-3	Sphingosine 1 Phosphate	WISP-3
CD51	CYR61	hGH			

[000234] As a non-limiting example, the antibody or antigen-binding fragment and/or the AB of an activatable antibody is or is derived from an antibody listed in Table 23.

Table 23: Exemplary sources for Abs

Antibody Trade Name (antibody name)	Target
Avastin™ (bevacizumab)	VEGF
Lucentis™ (ranibizumab)	VEGF
Erbitux™ (cetuximab)	EGFR
Vectibix™ (panitumumab)	EGFR
Remicade™ (infliximab)	TNFα
Humira™ (adalimumab)	TNFα
Tysabri™ (natalizumab)	Integrinα4
Simulect™ (basiliximab)	IL2R
Soliris™ (eculizumab)	Complement C5
Raptiva™ (efalizumab)	CD11a
Bexxar™ (tositumomab)	CD20
Zevalin™ (ibritumomab tiuxetan)	CD20
Rituxan™ (rituximab)	CD20
(Ocrelizumab)	CD20
Arzerra™ (ofatumumab)	CD20
Gazyva™ (Obinutuzumab)	CD20
Zenapax™ (daclizumab)	CD25
Adcetris™ (brentuximab vedotin)	CD30
Myelotarg™ (gemtuzumab)	CD33
Mylotarg™ (gemtuzumab ozogamicin)	CD33
Campath™ (alemtuzumab)	CD52
ReoPro™ (abiciximab)	Glycoprotein receptor IIb/IIIa
Xolair™ (omalizumab)	IgE
Herceptin™ (trastuzumab)	Her2
Kadcyla™ (trastuzumab emtansine)	Her2
Synagis™ (palivizumab)	F protein of RSV
(ipilimumab)	CTLA-4
(tremelimumab)	CTLA-4
Hu5c8	CD40L
(pertuzumab)	Her2-neu
(ertumaxomab)	CD3/Her2-neu
Orencia™ (abatacept)	CTLA-4
(tanezumab)	NGF
(bavituximab)	Phosphatidylserine
(zalutumumab)	EGFR
(mapatumumab)	EGFR

(matuzumab)	EGFR
(nimotuzumab)	EGFR
ICR62	EGFR
mAb 528	EGFR
CH806	EGFR
MDX-447	EGFR/CD64
(edrecolomab)	EpCAM
RAV12	RAAG12
huJ591	PSMA
Enbrel™ (etanercept)	TNF-R
Amevive™ (alefacept)	1-92-LFA-3
Antril™, Kineret™ (ankinra)	IL-1Ra
GC1008	TGFbeta
	Notch, e.g., Notch 1
	Jagged 1 or Jagged 2
(adecatumumab)	EpCAM
(figitumumab)	IGF1R
(tocilizumab)	IL-6 receptor
Stelara™ (ustekinumab)	IL-12/IL-23
Prolia™ (denosumab)	RANKL

[000235] In some embodiments, the additional antibody or antigen binding fragment thereof, conjugated antibody or antigen binding fragment thereof, activatable antibody or antigen binding fragment thereof, and/or conjugated activatable antibody or antigen binding fragment thereof is a monoclonal antibody, domain antibody, single chain, Fab fragment, a $F(ab')_2$ fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, or a single domain light chain antibody. In some embodiments, the additional antibody or antigen binding fragment thereof, conjugated antibody or antigen binding fragment thereof, activatable antibody or antigen binding fragment thereof, and/or conjugated activatable antibody or antigen binding fragment thereof is a mouse, other rodent, chimeric, humanized or fully human monoclonal antibody.

[000236] The disclosure also provides methods of producing an anti-PDL1 antibody and/or activatable anti-PDL1 antibody polypeptide by culturing a cell under conditions that lead to expression of the polypeptide, wherein the cell comprises an isolated nucleic acid molecule encoding an antibody and/or an activatable antibody described herein, and/or vectors that include these isolated nucleic acid sequences. The disclosure provides methods of producing an antibody and/or activatable antibody by culturing a cell under conditions that lead to expression of the antibody and/or activatable antibody, wherein the cell comprises an isolated nucleic acid molecule encoding an antibody and/or an activatable antibody described herein, and/or vectors that include these isolated nucleic acid sequences.

[000237] The invention also provides a method of manufacturing activatable antibodies that in an activated state binds PDL1 by (a) culturing a cell comprising a nucleic acid construct that encodes the activatable antibody under conditions that lead to expression of the activatable antibody, wherein the activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM), and an antibody or an antigen binding fragment thereof (AB) that specifically binds PDL1, (i) wherein the CM is a polypeptide that functions as a substrate for a protease; and (ii) wherein the CM is positioned in the activatable antibody such that, when the activatable antibody is in an uncleaved state, the MM interferes with specific binding of the AB to PDL1 and in a cleaved state the MM does not interfere or compete with specific binding of the AB to PDL1; and (b) recovering the activatable antibody. Suitable AB, MM, and/or CM include any of the AB, MM, and/or CM disclosed herein.

[000238] In some embodiments, the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM. In some embodiments, the activatable antibody comprises a linking peptide between the MM and the CM. In some embodiments, the activatable antibody comprises a linking peptide between the CM and the AB. In some embodiments, the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM. In some embodiments, the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: spacer-MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM-spacer. In some embodiments, the two linking peptides need not be identical to each other.

[000239] In some embodiments, at least one of LP1 or LP2 comprises an amino acid sequence selected from the group consisting of $(GS)_n$, $(GGS)_n$, $(GSGGS)_n$ (SEQ ID NO: 191) and $(GGGS)_n$ (SEQ ID NO: 192), where n is an integer of at least one.

[000240] In some embodiments, at least one of LP1 or LP2 comprises an amino acid sequence selected from the group consisting of GGSG (SEQ ID NO: 193), GGSGG (SEQ ID NO: 194), GSGSG (SEQ ID NO: 195), GSGGG (SEQ ID NO: 196), GGGSG (SEQ ID NO: 197), and GSSSG (SEQ ID NO: 198).

[000241] In some embodiments, LP1 comprises the amino acid sequence GSSGGSGGSGGSG (SEQ ID NO: 199), GSSGGSGGSGG (SEQ ID NO: 200),

GSSGGSGGSGGS (SEQ ID NO: 201), GSSGGSGGSGGSGGGS (SEQ ID NO: 202), GSSGGSGGSG (SEQ ID NO: 203), or GSSGGSGGSGS (SEQ ID NO: 204).

[000242] In some embodiments, LP2 comprises the amino acid sequence GSS, GGS, GGGS (SEQ ID NO: 205), GSSGT (SEQ ID NO: 206) or GSSG (SEQ ID NO: 207).

[000243] The invention provides methods of preventing, delaying the progression of, treating, alleviating a symptom of, or otherwise ameliorating an PDL1 mediated disease in a subject by administering a therapeutically effective amount of an anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody described herein to a subject in need thereof.

[000244] The invention also provides methods of preventing, delaying the progression of, treating, alleviating a symptom of, or otherwise ameliorating cancer in a subject by administering a therapeutically effective amount of an anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody described herein to a subject in need thereof. PDL1 is known to be expressed in a variety of cancers, such as, by way of non-limiting example, melanoma, non-small cell lung cancer, nasopharyngeal cancer, glioblastoma/mixed glioma, colon adenocarcinoma, hepatocellular carcinoma, urothelial cancer, multiple myeloma, ovarian cancer, gastric carcinoma, esophageal cancer, pancreatic cancer, renal cell carcinoma (RCC), breast cancer, lymphomas, and leukemias. (See e.g., Chen et al., "Molecular Pathways: Next-Generation Immunotherapy – Inhibiting Programmed Death-Ligand 1 and Programmed Death-1," Clin. Can. Res., vol. 18: 6580-6587 (2012), the contents of which are hereby incorporated by reference in their entirety).

[000245] In some embodiments, the cancer is a bladder cancer, a bone cancer, a breast cancer, a carcinoid, a cervical cancer, a colon cancer, an endometrial cancer, a glioma, a head and neck cancer, a liver cancer, a lung cancer, a lymphoma, a melanoma, an ovarian cancer, a pancreatic cancer, a prostate cancer, a renal cancer, a sarcoma, a skin cancer, a stomach cancer, a testis cancer, a thyroid cancer, a urogenital cancer, and/or a urothelial cancer.

[000246] In some embodiments, the cancer is selected from the group consisting of melanoma (MEL), renal cell carcinoma (RCC), squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer (CRC), castration-resistant prostate cancer (CRPC), hepatocellular carcinoma (HCC), squamous cell carcinoma of the head and neck, carcinomas of the esophagus, ovary, gastrointestinal tract and breast, or a hematologic

malignancy such as multiple myeloma, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma/primary mediastinal B-cell lymphoma, and chronic myelogenous leukemia. In some embodiments, the cancer is due to a PDL1-expressing tumor.

[000247] The invention also provides methods of treating cancer patients with an autoimmune or inflammatory disease by administering a therapeutically effective amount of an anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody described herein to a subject in need thereof. In some embodiments, the autoimmune disease is colitis, RA, pancreatitis, diabetes, or pneumonitis.

[000248] An anti-PDL1 antibody, a conjugated anti-PDL1 antibody, an activatable anti-PDL1 antibody and/or a conjugated activatable anti-PDL1 antibody used in any of the embodiments of these methods and uses can be administered at any stage of the disease. For example, such an anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody can be administered to a patient suffering cancer of any stage, from early to metastatic. The terms subject and patient are used interchangeably herein.

[000249] In some embodiments, the subject is a mammal, such as a human, non-human primate, companion animal (*e.g.*, cat, dog, horse), farm animal, work animal, or zoo animal. In some embodiments, the subject is a human. In some embodiments, the subject is a companion animal. In some embodiments, the subject is an animal in the care of a veterinarian.

[000250] The anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and therapeutic formulations thereof are administered to a subject suffering from or susceptible to a disease or disorder associated with aberrant PDL1 expression and/or activity. A subject suffering from or susceptible to a disease or disorder associated with aberrant PDL1 expression and/or activity is identified using any of a variety of methods known in the art. For example, subjects suffering from cancer or other neoplastic condition are identified using any of a variety of clinical and/or laboratory tests such as, physical examination and blood, urine and/or stool analysis to evaluate health status. For example, subjects suffering from inflammation and/or an inflammatory disorder are identified using any of a variety of clinical and/or laboratory tests such as physical examination and/or bodily fluid analysis, e.g., blood, urine and/or stool analysis, to evaluate health status.

[000251] Administration of an anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody to a patient suffering from a disease or disorder associated with aberrant PDL1 expression and/or activity is considered successful if any of a variety of laboratory or clinical objectives is achieved. For example, administration of an anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody to a patient suffering from a disease or disorder associated with aberrant PDL1 expression and/or activity is considered successful if one or more of the symptoms associated with the disease or disorder is alleviated, reduced, inhibited or does not progress to a further, *i.e.*, worse, state. Administration of an anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody to a patient suffering from a disease or disorder associated with aberrant PDL1 expression and/or activity is considered successful if the disease or disorder enters remission or does not progress to a further, *i.e.*, worse, state.

[000252] In some embodiments, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody is administered before and/or during and/or after treatment in combination with one or more additional agents such as, for example, a chemotherapeutic agent, an anti-inflammatory agent, and/or an immunosuppressive agent. In some embodiments, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and the additional agent(s) are administered simultaneously. For example, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and the additional agent(s) can be formulated in a single composition or administered as two or more separate compositions. In some embodiments, the anti-PDL1 antibody, conjugated anti-PDL1 antibody, activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody and the additional agent(s) are administered sequentially.

[000253] The invention also provides methods and kits for using the activatable anti-PDL1 antibodies and/or conjugated activatable anti-PDL1 antibodies in a variety of diagnostic and/or prophylactic indications. For example, the invention provides methods and kits for detecting the presence or absence of a cleaving agent and a target of interest in a subject or a sample by (i) contacting a subject or sample with an anti-PDL1 activatable antibody, wherein the anti-PDL1 activatable antibody comprises a masking moiety (MM), a

cleavable moiety (CM) that is cleaved by the cleaving agent, and an antigen binding domain or fragment thereof (AB) that specifically binds the target of interest, wherein the anti-PDL1 activatable antibody in an uncleaved, non-activated state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to PDL1, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB and is not a modified form of a natural binding partner of the AB; and (b) wherein, when the AB is in an uncleaved, non-activated state, the MM interferes with specific binding of the AB to PDL1, and when the AB is in a cleaved, activated state the MM does not interfere or compete with specific binding of the AB to PDL1; and (ii) measuring a level of activated anti-PDL1 activatable antibody in the subject or sample, wherein a detectable level of activated anti-PDL1 activatable antibody in the subject or sample indicates that the cleaving agent and PDL1 are present in the subject or sample and wherein no detectable level of activated anti-PDL1 activatable antibody in the subject or sample indicates that the cleaving agent, PDL1 or both the cleaving agent and PDL1 are absent in the subject or sample.

[000254] In some embodiments, the activatable anti-PDL1 antibody is an activatable anti-PDL1 antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable anti-PDL1 antibody is not conjugated to an agent. In some embodiments, the activatable anti-PDL1 antibody comprises a detectable label. In some embodiments, the detectable label is positioned on the AB. In some embodiments, measuring the level of activatable anti-PDL1 antibody in the subject or sample is accomplished using a secondary reagent that specifically binds to the activated antibody, wherein the reagent comprises a detectable label. In some embodiments, the secondary reagent is an antibody comprising a detectable label.

[000255] In some embodiments of these methods and kits, the activatable anti-PDL1 antibody includes a detectable label. In some embodiments of these methods and kits, the detectable label includes an imaging agent, a contrasting agent, an enzyme, a fluorescent label, a chromophore, a dye, one or more metal ions, or a ligand-based label. In some embodiments of these methods and kits, the imaging agent comprises a radioisotope. In some embodiments of these methods and kits, the radioisotope is indium or technetium. In some embodiments of these methods and kits, the contrasting agent comprises iodine, gadolinium or iron oxide. In some embodiments of these methods and kits, the enzyme comprises horseradish peroxidase, alkaline phosphatase, or β-galactosidase. In some

embodiments of these methods and kits, the fluorescent label comprises yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), green fluorescent protein (GFP), modified red fluorescent protein (mRFP), red fluorescent protein tdimer2 (RFP tdimer2), HCRED, or a europium derivative. In some embodiments of these methods and kits, the luminescent label comprises an N- methylacrydium derivative. In some embodiments of these methods, the label comprises an Alexa Fluor® label, such as Alex Fluor® 680 or Alexa Fluor® 750. In some embodiments of these methods and kits, the ligand-based label comprises biotin, avidin, streptavidin or one or more haptens.

[000256] In some embodiments of these methods and kits, the subject is a mammal. In some embodiments of these methods, the subject is a human. In some embodiments, the subject is a non-human mammal, such as a non-human primate, companion animal (e.g., cat, dog, horse), farm animal, work animal, or zoo animal. In some embodiments, the subject is a rodent.

[000257] In some embodiments of these methods and kits, the method is an *in vivo* method. In some embodiments of these methods, the method is an *in situ* method. In some embodiments of these methods, the method is an *ex vivo* method. In some embodiments of these methods, the method is an *in vitro* method.

[000258] In some embodiments of the methods and kits, the method is used to identify or otherwise refine a patient population suitable for treatment with an anti-PDL1 activatable antibody of the disclosure, followed by treatment by administering that activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody to a subject in need thereof. For example, patients that test positive for both the target (e.g., PDL1) and a protease that cleaves the substrate in the cleavable moiety (CM) of the anti-PDL1 activatable antibody being tested in these methods are identified as suitable candidates for treatment with such an anti-PDL1 activatable antibody comprising such a CM, and the patient is then administered a therapeutically effective amount of the activatable anti-PDL1 antibody and/or conjugated activatable anti-PDL1 antibody that was tested. Likewise, patients that test negative for either or both of the target (*e.g.*, PDL1) and the protease that cleaves the substrate in the CM in the activatable antibody being tested using these methods might be identified as suitable candidates for another form of therapy. In some embodiments, such patients can be tested with other anti-PDL1 activatable antibodies until a suitable anti-PDL1 activatable antibody for treatment is identified (e.g., an anti-PDL1 activatable antibody comprising a CM that is cleaved by the patient at the site of disease). In

some embodiments, the patient is then administered a therapeutically effective amount of the activatable anti-PDL1 antibody and/or conjugated for which the patient tested positive. Suitable AB, MM, and/or CM include any of the AB, MM, and/or CM disclosed herein.

[000259] Pharmaceutical compositions according to the invention can include an antibody of the invention and a carrier. These pharmaceutical compositions can be included in kits, such as, for example, diagnostic kits.

Brief Description of the Drawings

[000260] Figure 1 is graph depicting by ELISA-based binding that PDL1 c60 ScFv-phage binds specifically to human and mouse PDL1.

[000261] Figure 2 is a graph depicting that hPD1 inhibits the binding of clones 12, 18 and 60 to hPDL1 in an ELISA format, and, furthermore mPD1 inhibits the binding of clone 60 to mPDL1.

[000262] Figure 3 is a graph depicting saturation binding data and demonstrates that anti-PDL1 clone 60:IgG binds to human PDL1 with a binding affinity ~ 1 nM, and to mouse PDL1 at 30 nM.

[000263] Figures 4A and 4B are a series of graphs depicting H2 specificity and cross reactivity (Figure 4A) and H3W specificity and cross reactivity (Figure 4B).

[000264] Figures 5A and 5B are a series of graphs depicting that combining the matured heavy variable CDR3 domains with the heavy variable CDR2 domains results in antibodies with enhanced affinity towards human PDL1.

[000265] Figures 6A, 6B, 6C, and 6D are a series of graphs depicting that anti-PDL1 antibody C5H9 binds to human and mouse PDL1 with near equal affinity and that anti-PDL1 antibodies C5B10 and C5E10 only bind human PDL1.

[000266] Figure 7 is a graph depicting the ability of anti-PDL1 antibody C5H9 to bind with high and equal affinity to human, mouse and cyno PDL1.

[000267] Figures 8A and 8B are a series of graphs depicting that anti-PDL1 antibody C5H9v2 has lower immunogenicity potential and enhanced manufacturability. These graphs demonstrate decreased predicted immunogenicity, increased expression levels (3X), and increased monomer percentage in human IgG1 format.

[000268] Figures 9A, 9B, and 9C are a series of graphs depicting ability of anti-PDL1 antibody C5H9v2 to bind with high affinity to (A) mouse, (B) cyno, and (C) human PDL1. Figures 9A-9C show that both anti-PDL1 antibodies C5H9 and C5H9v2 bind human, mouse and cyno PDL1 with near equal affinities.

[000269] Figure 10 is a graph depicting C5H9v2 specificity for a panel of human and mouse proteins. Figure 10 demonstrates that anti-PDL1 antibody C5H9v2 binds only to human and mouse PDL1 demonstrating specificity toward PDL1.

[000270] Figure 11 is a series of graphs depicting B7-1 and PD1 blockade. Figure 11 demonstrates that both anti-PDL1 antibody C5H9 and anti-PDL1 antibody C5H9v2 are potent blockers of either B7-1 or PD1 binding to PDL1, and that blockade includes all three species human, cyno and mouse with single digit nM EC_{50}'s.

[000271] Figure 12 is a graph depicting that anti-PDL1 antibody C5H9 accelerates the onset of diabetes in NOD mice similar to the positive control anti-PDL1 antibody 10F9G2.

[000272] Figure 13 is a graph depicting that anti-PDL1 antibody C5H9v2 accelerates the onset of diabetes in NOD mice in a dose dependent matter.

[000273] Figure 14 is a graph depicting that an anti-PDL1 antibody C5H9v2 inhibits the growth of MC38 syngeneic tumors similar to positive control anti-PDL1 antibody 10F9G2.

[000274] Figure 15 is a graph depicting binding isotherms for anti-PDL1 activatable antibodies that include the anti-PDL1 antibody C5H9v2.

[000275] Figure 16 is a graph depicting binding isotherms for anti-PDL1 activatable antibodies that include the anti-PDL1 antibody C5H9v2.

[000276] Figure 17 is a graph depicting binding isotherms for anti-PDL1 activatable antibodies that include the anti-PDL1 antibody C5H9v2.

[000277] Figures 18A and 18B are a series of graphs depicting that anti-PDL1 activatable antibody PL15-0003-C5H9v2, when administered at a dose of 1 mg/kg, has an absence of diabetes induction and anti-PDL1 activatable antibody PL18-0003-C5H9v2, when administered at a dose of 1 mg/kg, has a delay in diabetes induction compared to the anti-PDL1 antibody C5H9v2 administered at a dose of 1 mg/kg in NOD mice (Figure 18A); in addition, anti-PDL1 activatable antibody PL15-0003-C5H9v2, when administered at a dose of 3 mg/kg induced diabetes in 2 of 8 NOD mice (75% non-diabetic), compared to 1 mg/kg anti-PDL1 antibody C5H9v2 which led to 38% non-diabetic NOD mice (Figure 18B).

[000278] Figure 19 is a graph depicting that anti-PDL1 activatable antibodies PL15-0003-C5H9v2 and PL18-0003-C5H9v2 inhibit the growth of MC38 syngeneic tumors similar to anti-PDL1 antibody C5H9v2.

[000279] Figures 20A, 20B, 20C, and 20D are a series of graphs depicting the percent of anti-PDL1 antibody C5H9v2 and anti-PDL1 activatable antibody PL15-0003-C5H9v2 bound to CD4+ and CD8+ T cells from peripheral blood (Figures 20A, 20B) or spleen (Figures 20C, 20D). In all graphs, the circle represents isotype, the square represents anti-PDL1 antibody C5H9v2, and the triangle represents anti-PDL1 activatable antibody PL15-0003-C5H9v2.

[000280] Figures 21A and 21B are a series of graphs depicting that the presence of tumor-derived proteases capable of cleaving anti-PDL1 activatable antibody PL15-0003-C5H9v2 (depicted by squares) did not lead to high levels of activated activatable antibody in the blood compared to anti-PDL1 antibody C5H9v2 (depicted by circles) or an isotype antibody (depicted by triangles) (Figure 21A) despite the plasma concentrations of the activatable antibody being higher than those of the antibody (Figure 21B).

[000281] Figure 22 is a graph depicting the ability of anti-PDL1 activatable antibody PL07-2001-C5H9v2 to increase CMV-stimulated IFN-gamma secretion as compared to control hIgG4, but with decreased potency relative to anti-PDL1 parental antibody C5H9v2.

[000282] Figures 23A, 23B, 23C, and 23D are a series of images showing the ability of anti-PDL1 activatable antibody PL15-0003-C5H9v2 to be activated and to bind frozen MC38 mouse cancer tissues using an *in situ* imaging method.

[000283] Figure 24 depicts that anti-PDL1 activatable antibody PL15-0003-C5H9v2 exhibited a higher EC_{50} for PDL1 binding and PD1 blocking than did the anti-PDL1 antibody as measured by ELISA. The figure further demonstrates that activation of anti-PDL1 activatable antibody PL15-0003-C5H9v2 by matriptase fully restored PDL1 binding and PD1 blocking activities to levels comparable to those of anti-PDL1 antibody C5H9v2.

[000284] Figures 25A and 25B are a series of graphs depicting the ability of anti-PDL1 activatable antibodies referred to herein as PL07-2001-C5H9v2 and PF07-3001-C5H9v2 and of the anti-PDL1 antibody referred to herein as C5H9 to bind to human or murine PDL1.

[000285] Figures 26A, 26B, and 26C are a series of graphs depicting the abilities of anti-PDL1 activatable antibody PL07-2001-C5H9v2, uPA-activated anti-PDL1 activatable antibody PL07-2001-C5H9v2, MMP14-activated anti-PDL1 activatable antibody PL07-2001-C5H9v2, and anti-PDL1 antibody C5H9v2 to block human PD1 (PD-1) to human PDL1 (Figure 26A), cynomolgus PD1 (PD-1) binding to cynomolgus PDL1 (Figure 26B), or rat PD1 (PD-1) binding to rat PDL1 (Figure 26C).

[000286] Figures 27A and 27B are a series of graphs depicting the ability of anti-PDL1 activatable antibody PL07-2001-C5H9v2, uPA-activated anti-PDL1 activatable antibody PL07-2001-C5H9v2, MMP14-activated anti-PDL1 activatable antibody PL07-2001-C5H9v2, or anti-PDL1 antibody C5H9v2 to human B7-1 binding to human PDL1 (Figure 27A) or cynomolgus B7-1 binding to cynomolgus PDL1 (Figure 27B).

[000287] Figure 28 is a graph depicting the ability of the anti-PDL1 activatable antibodies PL15-0003-C5H9v2, PL15-2001-C5H9v2, and PL15-3001-C5H9v2 to inhibit the growth of MC38 syngeneic tumors similar to positive control anti-PDL1 antibody C5H9v2.

[000288] Figures 29A, 29B, and 29C are a series of graphs depicting that the presence of tumor-derived proteases capable of cleaving the activatable antibodies anti-PDL1 antibody C5H9v2 or anti-PDL1 activatable antibody PL15-0003-C5H9v2, PL15-2001-C5H9v2, or PL15-3001-C5H9v2 did not lead to high levels of activated activatable antibodies in the blood.

[000289] Figure 30 is an illustration depicting the ability of proteases in a tumor implanted into a mouse to activate the anti-PDL1 activatable antibody PL07-2001-C5H9v2.

Detailed Description of the Invention

[000290] The present invention provides monoclonal antibodies (mAbs) and activatable monoclonal antibodies that specifically bind human programmed death ligand 1 (PDL1). PDL1 is a 40kDa type I transmembrane protein that forms a complex with its receptor programmed cell death protein 1 (PD1), also known as CD279. Engagement of PDL1 with its receptor PD1 on T cells delivers a signal that inhibits TCR-mediated activation of IL-2 production and T cell proliferation. Aberrant expression and/or activity of PDL1 and PDL1-related signaling has been implicated in the pathogenesis of many diseases and disorders, such as cancer, inflammation, and autoimmunity.

[000291] The activatable anti-PDL1 antibodies are used in methods of treating, preventing, delaying the progression of, ameliorating and/or alleviating a symptom of a disease or disorder associated with aberrant PDL1 expression and/or activity. For example, the activatable anti-PDL1 antibodies are used in methods of treating, preventing, delaying the progression of, ameliorating and/or alleviating a symptom of a cancer or other neoplastic condition.

[000292] The activatable anti-PDL1 antibodies include an antibody or antigen-binding fragment thereof that specifically binds PDL1 coupled to a masking moiety (MM), such that

coupling of the MM reduces the ability of the antibody or antigen-binding fragment thereof to bind PDL1. In some embodiments, the MM is coupled via a sequence that includes a substrate for a protease, for example, a protease that is co-localized with PDL1 at a treatment site in a subject.

[000293] Exemplary activatable anti-PDL1 antibodies of the invention include, for example, activatable antibodies that include a heavy chain and a light chain that are, or are derived from, antibodies described in the Examples, for example in Example 3 and Example 4.

[000294] In some embodiments, the activatable anti-PDL1 antibody includes a heavy chain that comprises or is derived from an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56, and a light chain that comprises or is derived from the amino acid sequence of SEQ ID NO: 12 or SEQ ID NO: 58.

[000295] In some embodiments, the activatable anti-PDL1 antibody comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence shown in Table 15; a VH CDR2 sequence shown in Table 15; a VH CDR3 sequence shown in Table 15; a VL CDR1 sequence shown in Table 15; a VL CDR2 sequence shown in Table 15; and a VL CDR3 sequence shown in Table 15.

[000296] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 15.

[000297] In some embodiments, the antibody comprises a heavy chain that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein the combination is a combination of the three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 15.

[000298] In some embodiments, the antibody comprises a light chain that comprise a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the three light chain CDR sequences (VL CDR1, VL CDR2, VL CDR3) shown in a single row in Table 15.

[000299] In some embodiments, the activatable anti-PDL1 antibody includes a heavy chain that comprises or is derived from a heavy chain amino acid sequence shown in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes a light chain that comprises or is derived from a light chain amino acid sequence shown in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes a heavy chain that comprises or is derived from a heavy chain amino acid sequence shown in Table 15, and a light chain that comprises or is derived from a light chain amino acid sequence shown in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain variable region and light chain variable region sequences from the combinations shown in Group A in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group B in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group C in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group D in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group E in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group F in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group G in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group H in Table 15. In some embodiments, the activatable anti-PDL1

antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group I in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain and light chain sequences shown in Group J in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group K in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain and light chain sequences shown in Group K in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group L in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group M in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group N in Table 15. In some embodiments, the activatable anti-PDL1 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group O in Table 15.

Table 15. Variable Heavy Chain Region (VH) and Variable Light Chain Region (VL) Sequences for Activatable Antibodies that Bind PDL1 (CDR sequences are underlined; CDR sequences disclosed herein were identified in accordance with the definitions of Kabat, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)))

Group A	
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYGFSWVRQAPGQGLEWMGWITAYNGNTNYAQKLQGRVTM TTDTSTSTVYMELRSLRSDDTAVYYCARDYFYGMDVWGQGTTVTVSS (SEQ ID NO: 248)
VH	QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSTYAISWVRQAPGQGLEWMGGIIPIFGKAHYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVTVSS (SEQ ID NO: 249)
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDVHWVRQAPGQRLEWMGWLHADTGITKFSQKFQGRVTI TRDTSASTAYMELSSLRSEDTAVYYCARERIQLWFDYWGQGT (SEQ ID NO: 250)
VH	QVQLVQSGAEVKKPGSSVKVSCKVSGGIFSTYAINWVRQAPGQGLEWMGGIIPIFGTANHAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARDQGIAAALFDYWGQGTLVTVSS (SEQ ID NO: 251)
VH	EVQLVESGGGLVQPGRSLRLSCAVSGFTFDDYVVHWVRQAPGKGLEWVSGNSGNIGYADSVKGRFTISRD NAKNSLYLQMNSLRAEDTALYYCAVPFDYWGQGTLVTVSS (SEQ ID NO: 252)

VH	QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSSYAISWVRQAPGQGLEWMGGIIPIFGRAHYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVTVSS (SEQ ID NO: 253)
VH	QVQLVQSGAEVKKPGSSVKVSCKTSGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGKAHYAQKFQGRVTI TADESTTTAYMELSSLRSEDTAVYYCARKYDYVSGSPFGMDVWGQGTTVTVSS (SEQ ID NO: 254)
VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAINWVRQAPGQGLEWMGGIIPIFGSANYAQKFQDRVTI TADESTSAAYMELSSLRSEDTAVYYCARDSSGWSRYYMDVWGQGTTVTVSS (SEQ ID NO: 255)
VH	QVQLVQSGAEVKEPGSSVKVSCKASGGTFNSYAISWVRQAPGQGLEWMGGIIPLFGIAHYAQKFQGRVTI TADESTNTAYMDLSSLRSEDTAVYYCARKYSYVSGSPFGMDVWGQGTTVTVSS (SEQ ID NO: 256)
VH	EVQLVESGGGLVQPGRSLRLSCAASGITFDDYGMHWVRQAPGKGLEWVSGISWNRGRIEYADSVKGRFTI SRDNAKNSLYLQMNSLRAEDTALYYCAKGRFRYFDWFLDYWGQGTLVTVSS (SEQ ID NO: 257)
VH	QMQLVQSGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYYVDSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCARDYFWSGFSAFDIWGKGTLVTVS (SEQ ID NO: 449)
VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLVWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTD FTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK (SEQ ID NO: 258)
VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTD FTLTISSLEPEDFAVYYCQQRSNWPTFGQGTKVEIK (SEQ ID NO: 259)
VL	DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK (SEQ ID NO: 260)
VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK (SEQ ID NO: 261)
VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQQYGSSPFGGGTKVEIK (SEQ ID NO: 262)
VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTD FTLTISSLEPEDFAVYYCQQRSNWPTFGQGTRLEIK (SEQ ID NO: 263)
VL	AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQFNSYPFTFGPGTKVDIK (SEQ ID NO: 264)
	DIVMTQSPSTLSASVGDRVTITCRASQGISSWLAWYQQKPGRAPKVLIYKASTLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSYSTPWTFGQGTKLEIK (SEQ ID NO: 450)
Group B	
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVANIKQDGSEKYYVDSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCAREGGWFGELAFDYWGQGTLVTVSS (SEQ ID NO: 265)
VL	EIVLTQSPGTLSLSPGERATLSCRASQRVSSSYLAWYQQKPGQAPRLLIYDASSRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQQYGSLPWTFGQGTKVEIK (SEQ ID NO: 266)
Group C	
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSA (SEQ ID NO: 267)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSGSWIHWVRQAPGKGLEWVAWILPYGGSSYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSA (SEQ ID NO: 451)

VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO: 268)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYYNVPWTFGQGTKVEIKR (SEQ ID NO: 452)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYYAPPWTFGQGTKVEIKR (SEQ ID NO: 453)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYYTVPWTFGQGTKVEIKR (SEQ ID NO: 454)
VL	DIQMTQSPSSLSASVGDRVTITCRASQVINTFLAWYQQKPGKAPKLLIYSASTLASGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYYTVPRTFGQGTKVEIKR (SEQ ID NO: 455)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQGYGVPRTFGQGTKVEIKR (SEQ ID NO: 456)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYLFTP?TFGQGTKVEIKR (SEQ ID NO: 457)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYFITPTTFGQGTKVEIKR (SEQ ID NO: 458)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYYYTP?TFGQGTKVEIKR (SEQ ID NO: 459)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQFFYTP?TFGQGTKVEIKR (SEQ ID NO: 460)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSLFTP?TFGQGTKVEIKR (SEQ ID NO: 461)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSLYTP?TFGQGTKVEIKR (SEQ ID NO: 462)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSWYHP?TFGQGTKVEIKR (SEQ ID NO: 463)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYFYIP?TFGQGTKVEIKR (SEQ ID NO: 464)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYWYTPTTFGQGTKVEIKR (SEQ ID NO: 465)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSYFIP?TFGQGTKVEIKR (SEQ ID NO: 466)
Group D	
VH	METGLRWLLLVAVLKGVQCLSVEESGGRLVTPGTPLTLTCTASGFTITNYHMFWVRQAPGKGLEWIGVIT SSGIGSSSTTYYATWAKGRFTISKTSTTVNLRITSPTTEDTATYFCARDYFTNTYYALDIWGPGTLVTVS S (SEQ ID NO: 467)
VH	QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSTYAISWVRQAPGQGLEWMGGIIPIFGKAHYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVTVSS (SEQ ID NO: 249)

VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDVHWVRQAPGQRLEWMGWLHADTGITKFSQKFQGRVTI TRDTSASTAYMELSSLRSEDTAVYYCARERIQLWFDYWGQGTLVTVSS (SEQ ID NO: 468)
VH	QVQLVQSGAEVKKPGSSVKVSCKVSGGIFSTYAINWVRQAPGQGLEWMGGIIPIFGTANHAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARDQGIAAALFDYWGQGTLVTVSS (SEQ ID NO: 251)
VH	EVQLVESGGGLVQPGRSLRLSCAVSGFTFDDYVVHWVRQAPGKGLEWVSGISGNSGNIGYADSVKGRFTI SRDNAKNSLYLQMNSLRAEDTALYYCAVPFDYWGQGTLVTVSS (SEQ ID NO: 469)
VH	QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSSYAISWVRQAPGQGLEWMGGIIPIFGRAHYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVTVSS (SEQ ID NO: 253)
VH	QVQLVQSGAEVKKPGSSVKVSCKTSGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGKAHYAQKFQGRVTI TADESTTTAYMELSSLRSEDTAVYYCARKYDYVSGSPFGMDVWGQGTTVTVSS (SEQ ID NO: 254)
VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAINWVRQAPGQGLEWMGGIIPIFGSANYAQKFQDRVTI TADESTSAAYMELSSLRSEDTAVYYCARDSSGWSRYYMDVWGQGTTVTVSS (SEQ ID NO: 255)
VH	QVQLVQSGAEVKEPGSSVKVSCKASGGTFNSYAISWVRQAPGQGLEWMGGIIPLFGIAHYAQKFQGRVTI TADESTNTAYMDLSSLRSEDTAVYYCARKYSYVSGSPFGMDVWGQGTTVTVSS (SEQ ID NO: 256)
VH	EVQLVESGGGLVQPGRSLRLSCAASGITFDDYGMHWVRQAPGKGLEWVSGISWNRGRIEYADSVKGRFTI SRDNAKNSLYLQMNSLRAEDTALYYCAKGRFRYFDWFLDYWGQGTLVTVSS (SEQ ID NO: 257)
VL	MDTRAPTQLLGLLLLWLPGARCALVMTQTPSSTSTAVGGTVTIKCQASQSISVYLAWYQQKPGQPPKLLI YSASTLASGVPSRFKGSRSGTEYTLTISGVQREDAATYYCLGSAGS (SEQ ID NO: 470)
VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLVWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTD FTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK (SEQ ID NO: 258)
VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTD FTLTISSLEPEDFAVYYCQQRSNWPTFGQGTKVEIK (SEQ ID NO: 259)
VL	DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK (SEQ ID NO: 260)
VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK (SEQ ID NO: 261)
VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQQYGSSPFGGGTKVEIK (SEQ ID NO: 262)
VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTD FTLTISSLEPEDFAVYYCQQRSNWPTFGQGTRLEIK (SEQ ID NO: 263)
VL	AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQFNSYPFTFGPGTKVDIK (SEQ ID NO: 264)
Group E	
VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLEWVSSIYPSGGITFYADTVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARIKLGTVTTVDYWGQGTLVTVSS (SEQ ID NO: 471)
VL	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVSNRFSGSKSG NTASLTISGLQAEDEADYYCSSYTSSSTRVFGTGTKVTVL (SEQ ID NO: 472)

Group F	
VH	EVKLQESGPSLVKPSQTLSLTCSVTGYSITSDYWNWIRKFPGNKLEYVGYISYTGSTYYNPSLKSRISIT RDTSKNQYYLQLNSVTSEDTATYYCARYGGWLSPFDYWGQGTTLTVSS (SEQ ID NO: 473)
VH	EVQLQESGPGLVAPSQSLSITCTVSGFSLTTYSINWIRQPPGKGLEWLGVMWAGGGTNSNSVLKSRLIIS KDNSKSQVFLKMNSLQTDDTARYYCARYYGNSPYYAIDYWGQGTSVTVSS (SEQ ID NO: 474)
VH	EVKLQESGPSLVKPSQTLSLTCSVTGYSIISDYWNWIRKFPGNKLEYLGYISYTGSTYYNPSLKSRISIT RDTSKNQYYLQLNSVTTEDTATYYCARRGGWLLPFDYWGQGTTLTVSS (SEQ ID NO: 475)
VH	EVKLQESGPSLVKPGASVKLSCKASGYTFTSYDINWVKQRPGQGLEWIGWIFPRDNNTKYNENFKGKATL TVDTSSTTAYMELHSLTSEDSAVYFCTKENWVGDFDYWGQGTTLTLSS (SEQ ID NO: 476)
VH	EVQLQQSGPDLVTPGASVRISCQASGYTFPDYYMNWVKQSHGKSLEWIGDIDPNYGGTTYNQKFKGKAIL TVDRSSSTAYMELRSLTSEDSAVYYCARGALTDWGQGTSLTVSS (SEQ ID NO: 477)
VH	EIVLTQSPATLSLSPGERATLSCRASSSVSYIYWFQQKPGQSPRPLIYAAFNRATGIPARFSGSGSGTDY TLTISSLEPEDFAVYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 478)
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFPDYYMNWVRQAPGQGLEWMGDIDPNYGGTNYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARGALTDWGQGTMVTVSS (SEQ ID NO: 479)
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFPDYYMNWVRQAPGQSLEWMGDIDPNYGGTNYNQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARGALTDWGQGTMVTVSS (SEQ ID NO: 480)
VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFPDYYMNWVRQAPGQSLEWMGDIDPNYGGTNYNQKFQGRVTM TVDRSSSTAYMELSRLRSDDTAVYYCARGALTDWGQGTMVTVSS (SEQ ID NO: 481)
VH	EVQLVESGGGLVQPGRSLRLSCTASGYTFPDYYMNWVRQAPGKGLEWVGDIDPNYGGTTYAASVKGRFTI SVDRSKSIAYLQMSSLKTEDTAVYYCTRGALTDWGQGTMVTVSS (SEQ ID NO: 482)
VH	EVQLVESGGGLVQPGRSLRLSCTASGYTFPDYYMNWVRQAPGKGLEWVGDIDPNYGGTTYNASVKGRFTI SVDRSKSIAYLQMSSLKTEDTAVYYCARGALTDWGQGTMVTVSS (SEQ ID NO: 483)
VL	DIVMTQSHKLMSTSVGDRVSITCKASQDVGTAVAWYQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTD FTLTISNVQSEDLADYFCQQDSSYPLTFGAGTKVELK (SEQ ID NO: 484)
VL	DIVTTQSHKLMSTSVGDRVSITCKASQDVGTAVAWYQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTD FTLTISNVQSEDLADYFCQQDSSYPLTFGAGTKVELK (SEQ ID NO: 485)
VL	DIVMTQSPSSLAVSVGEKVSMGCKSSQSLLYSSNQKNSLAWYQQKPGQSPKLLIDWASTRESGVPDRFTG SGSGTDFTLTISSVKAEDLAVYYCQQYYGYPLTFGAGTKLELK (SEQ ID NO: 486)
VL	DIVMTQSPAIMSASPGEKVTMTCSASSSIRYMHWYQQKPGTSPKRWISDTSKLTSGVPARFSGSGSGTSY ALTISSMEAEDAATYYCHQRSSYPWTFGGGTKLEIK (SEQ ID NO: 487)
VL	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIYWFQQKPGSSPKPWIYATFNLASGVPARFSGSGSGTSY SLTISRVETEDAATYYCQQWSNNPLTFGAGTKLELK (SEQ ID NO: 488)
VL	EIVLTQSPATLSLSPGERATLSCRASSSVSYIYWFQQKPGQAPRLLIYAAFNRATGIPARFSGSGSGTDY TLTISSLEPEDFAVYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 489)
VL	QIVLTQSPATLSLSPGERATLSCRASSSVSYIYWFQQKPGQSPRPLIYATFNLASGIPARFSGSGSGTSY TLTISRLEPEDFAVYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 490)
VL	DIQLTQSPSSLSASVGDRVTITCRASSGVSYIYWFQQKPGKAPKLLIYAAFNLASGVPSRFSGSGSGTEY TLTISSLQPEDFATYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 491)

VL	DIQLTQSPSSLSASVGDRVTITCRASSGVSYIYWFQQKPGKAPKPLIYAAFNLASGVPSRFSGSGSGTEY TLTISSLQPEDFATYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 492)
VL	DIQLTQSPSILSASVGDRVTITCRASSSVSYIYWFQQKPGKAPKPLIYATFNLASGVPSRFSGSGSGTSY TLTISSLQPEDFATYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 493)

Group G	
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTM TTDTSTSTAYMELRSLRSDDTAVYYCARALPSGTILVGGWFDPWGQGTLVTVSS (SEQ ID NO: 494)
VH	EVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYALSWVRQAPGKGLEWVSAISGGGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCAKDVFPETFSMNYGMDVWGQGTLVTVSS (SEQ ID NO: 495)
VH	QVQLVQSGGGVVQPGGSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSLISGDGGSTYYADSVKGRFTI SRDNSKNSLYLQMNSLRTEDTALYYCAKVLLPCSSTSCYGSVGAFDIWGQGTTVTVSS (SEQ ID NO: 496)
VH	QVQLVQSGGSVVRPGESLRLSCVASGFIFDNYDMSWVRQVPGKGLEWVSRVNWNGGSTTYADAVKGRFTI SRDNTKNSLYLQMNNLRAEDTAVYYCVREFVGAYDLWGQGTTVTVSS (SEQ ID NO: 497)
VH	QVQLVQSGAEVKKPGATVKVSCKVFGDTFRGLYIHWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTI TTDESTSTAYMELSSLRSEDTAVYYCASGLRWGIWGWFDPWGQGTLVTVSS (SEQ ID NO: 498)
VH	EVQLVQSGAELKKPGSSVKVSCKAFGGTFSDNAISWVRQAPGQGPEWMGGIIPIFGKPNYAQKFQGRVTI TADESTSTAYMVLSSLRSEDTAVYYCARTMVRGFLGVMDVWGQGTTVTVSS (SEQ ID NO: 499)
VH	QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCAKDQFVTIFGVPRYGMDVWGQGTTVTVSS (SEQ ID NO: 500)
VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTI TADKSTSTAYMELSSLRSEDTAVYYCARGRQMFGAGIDFWGPGTLVTVSS (SEQ ID NO: 501)
VH	EVQLVESGAEVKKPGSSVKVSCKVSGGTFGTYALNWVRQAPGQGLEWMGRIVPLIGLVNYAHNFEGRISI TADKSTGTAYMELSNLRSDDTAVYYCAREVYGGNSDYWGQGTLVTVSS (SEQ ID NO: 502)
VH	QVQLVQSGGEVKKPGASVKVSCKASGYTLSSHGITWVRQAPGQGLEWMGWISAHNGHASNAQKVEDRVTM TTDTSTNTAYMELRSLTADDTAVYYCARVHAALYYGMDVWGQGTLVTVSS (SEQ ID NO: 503)
VH	QVQLQESGGGVVQPGRSLRLSCSASGFTFSRHGMHWVRQAPGKGLEWVAVISHDGSVKYYADSMKGRFSI SRDNSNNTLYLQMDSLRADDTAVYYCARGLSYQVSGWFDPWGQGTLVTVSS (SEQ ID NO: 504)
VH	NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSSPTTVIYEDNQRPSGVPDRFSGSIDTS SNSASLTISGLKTKDEADYYCQSYDGITVIFGGGTKLTVL (SEQ ID NO: 505)
VH	NFMLTQPHSVSGSPGKTVTLPCTRSSGSIASHYVQWYQQRPGSAPTTVIYEDNKRPSGVPDRFSGSIDSS SNSASLSISGLKTEDEADYYCQSYDSSNRWVFGGGTKLTVL (SEQ ID NO: 506)
VH	LPVLTQPASLSASPGASASLTCTLRSGLNVGSYRIYWYQQKPGSRPQYLLNYKSDSNKQQASGVPSRFSG SKDASANAGILLISGLQSEDEADYYCMIWYSSAVVFGGGTKLTVL (SEQ ID NO: 507)
VL	NFMLTQPHSVSESPGKTVTISCTRSSGNIASNYVQWYQQRPGSAPTTVIYEDNQRPSGVPDRFSGSIDSS SNSASLTISGLKTEDEADYYCQSYDSSNLWVFGGGTKLTVL (SEQ ID NO: 508)

VL	SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTA SLTITGAQAEDEADYYCNSRDSSGNHYVFGTGTKVTVL (SEQ ID NO: 509)
VL	LPVLTQAPSVSVAPGKTARITCGGSDIGRKSVHWYQQKPGQAPALVIYSDRDRPSGISERFSGSNSGNTA TLTISRVEAGDEADYYCQVWDNNSDHYVFGAGTELIVL (SEQ ID NO: 510)
VL	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVSNRFSGSKSG NTASLTISGLQAEDEADYYCSSYTSSTLPFGGGTKLTVL (SEQ ID NO: 511)
VL	EIVLTQSPATLSLSPGERATLSCRASQSIGNSLAWYQQKPGQAPRLLMYGASSRATGIPDRFSGSGAGTD FTLTISSLEPEDFATYYCQQHTIPTFSFGPGTKVEVK (SEQ ID NO: 512)
VL	DIVMTQTPSFLSASIGDRVTITCRASQGIGSYLAWYQQRPGEAPKLLIYAASTLQSGVPSRFSGSGSGTD FTLTISNLQPEDFATYYCQQLNNYPITFGQGTRLEIK (SEQ ID NO: 513)
VL	QSALTQPPSVSVSPGQTANIPCSGDKLGNKYAYWYQQKPGQSPVLLIYQDIKRPSRIPERFSGSNSADTA TLTISGTQAMDEADYYCQTWDNSVVFGGGTKLTVL (SEQ ID NO: 514)
VL	NFMLTQPHSVSESPGKTVTISCTRSSGSIDSNYVQWYQQRPGSAPTTVIYEDNQRPSGVPDRFSGSIDSS SNSASLTISGLKTEDEADYYCQSYDSNNRHVIFGGGTKLTVL (SEQ ID NO: 515)
VL	NFMLTQPHSVSESPGKTVTISCTRSSGNIGTNYVQWYQQRPGSAPVALIYEDYRRPSGVPDRFSGSIDSS SNSASLIISGLKPEDEADYYCQSYHSSGWEFGGGTKLTVL (SEQ ID NO: 516)
VL	QSVLTQPPSVSVAPGQTARITCGGNNIGSKGVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCQVWDSSSDHWVFGGGTKLTVL (SEQ ID NO: 517)
VL	NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTTVIYEDNQRPSGVPDRFSGSIDSS SNSASLTISGLKTEDEADYYCQSYDSTTPSVFGGGTKLTVL (SEQ ID NO: 518)
VL	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWTSPHNGLTAFAQILEGRVTM TTDTSTNTAYMELRNLTFDDTAVYFCAKVHPVFSYALDVWGQGTLVTVSS (SEQ ID NO: 519)
VL	EVQLVESGAEVMNPGSSVRVSCRGSGGDFSTYAFSWVRQAPGQGLEWMGRIIPILGIANYAQKFQGRVTI TADKSTSTAYMELSSLRSDDTAVYYCARDGYGSDPVLWGQGTLVTVSS (SEQ ID NO: 520)
VL	EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKVQGRVTM TTDTSTSTGYMELRSLRSDDTAVYYCARGDFRKPFDYWGQGTLVTVSS (SEQ ID NO: 521)
Group H	
VH	EVQLVQSGPELKKPGASVKMSCKASGYTFTSYVMHWVKQAPGQRLEWIGYVNPFNDGTKYNEMFKGRATL TSDKSTSTAYMELSSLRSEDSAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 522)
VH	EVQLVQSGAEVKKPGASVKMSCKASGYTFTSYVMHWVKQAPGQRLEWIGYVNPFNDGTKYNEMFKGRATL TSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 523)
VH	EVQLVQSGAEVKKPGASVKMSCKASGYTFTSYVMHWVRQAPGQRLEWIGYVNPFNDGTKYNEMFKGRATL TSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 524)
VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQRLEWIGYVNPFNDGTKYNEMFKGRATL TSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 525)
VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQRLEWIGYVNPFNDGTKYNEMFKGRATI TSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 526)
VL	DIVLTQSPASLALSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDSGVPSRFSGSG SGTDFTLTINSLEEEDAAMYFCQQSRRVPYTFGQGTKLEIK (SEQ ID NO: 527)

VL	DIVLTQSPATLSLSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDSGVPSRFSGSG SGTDFTLTINSLEAEDAAMYFCQQSRRVPYTFGQGTKLEIK (SEQ ID NO: 528)
VL	EIVLTQSPATLSLSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDSGVPSRFSGSG SGTDFTLTINSLEAEDAAMYFCQQSRRVPYTFGQGTKLEIK (SEQ ID NO: 529)
VL	DIVLTQSPATLSLSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDSGVPSRFSGSG SGTDFTLTINSLEAEDAATYFCQQSRRVPYTFGQGTKLEIK (SEQ ID NO: 530)
Group I	
VH	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTI TADKSTSTAYMELSSLRSEDTAVYYCAREGTIYDSSGYSFDYWGQGTLVTVSS (SEQ ID NO: 531)
VH	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVSM TRDTSTSTVYMELSSLTSEDTAVYYCARDLFPHIYGNYYGMDIWGQGTTVTVSS (SEQ ID NO: 532)
VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTI TADKSTSTAYMELSSLRSEDTAVYYCARLAVPGAFDIWGQGTMVTVSS (SEQ ID NO: 533)
VH	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLAVISYDGSNKYYADSVKGRFTISRD NSKNTLYLQMNSLRAEDTAVYYCARGQWLVTELDYWGQGTLVTVSS (SEQ ID NO: 534)
VH	EVQLVESGSEVEKPGSSVKVSCKASGGTFSDSGISWVRQAPGQGLEWMGGIIPMFATPYYAQKFQDRVTI TADESTSTVYMELSGLRSDDTAVFYCARDRGRGHLPWYFDLWGRGTLVTVSS (SEQ ID NO: 535)
VH	EVQLVESGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARAPYYYYYMDVWGQGTTVTVSS (SEQ ID NO: 536)
VH	EVQLLESGAEVKKPGSSVKVSCKASGGTLSRYALSWVRQAPGQGPEWVGAIIPIFGTPHYSKKFQDRVII TVDTSTNTAFMELSSLRFEDTALYFCARGHDEYDISGYHRLDYWGQGTLVTVSS (SEQ ID NO: 537)
VH	QVQLVQSGSELKKPGSSVKVSCKASGYSFSGYYIHWVRQAPGQGLEWMGWIDPNSGVTNYVRRFQGRVTM TRDTSLSTAYMELSGLTADDTAVYYCARDENLWQFGYLDYWGQGTLVTVSS (SEQ ID NO: 538)
VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSRYGVHWVRQAPGQGLEWMGRLIPIVSMTNYAQKFQDRVSI TTDKSTGTAYMELRSLTSEDTALYYCASVGQQLPWVFFAWGQGTLVTVSS (SEQ ID NO: 539)
VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYADSVRGRFTI SRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS (SEQ ID NO: 540)
VH	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYADSVRGRFTI SRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS (SEQ ID NO: 541)
VH	EVQLVQSGGGLVQPGGSLRLSCAASGFTFSDYGMHWVRQPPGKGLEWLAVISYDGSYKIHADSVQGRFTI SRDNAKNSVFLQMNSLKTEDTAVYYCTTDRKWLAWHGMDVWGQGTTVTVSS (SEQ ID NO: 542)
VH	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARDGIVADFQHWGQGTLVTVSS (SEQ ID NO: 543)
VH	EVQLVESGAEVKKPGASVKVSCKASGDTFSRYGITWVRQAPGRGLEWMGNIVPFFGATNYAQKFQGRLTI TADKSSYTSYMDLSSLRSDDTAVYYCARDHFYGSGGYFDYWGQGTLVTVSS (SEQ ID NO: 544)
VH	EVQLLESGAEVKKPGASVKVSCKASGYTFNSYDINWVRQAPGQGLEWMGGIIPVFGTANYAESFQGRVTM TADHSTSTAYMELNNLRSEDTAVYYCARDRWHYESRPMDVWGQGTTVTVSS (SEQ ID NO: 545)

VH	EVQLVESGGGLVRPGGSLRLACAASGFSFSDYYMTWIRQAPGRGLEWIAYISDSGQTVHYADSVKGRFTI SRDNTKNSLFLQVNTLRAEDTAVYYCAREDLLGYYLQSWGQGTLVTVSS (SEQ ID NO: 546)
VH	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSR ITINPDTSKNQFSLQLNSVTPEDTAVYYCARDEPRAVAGSQAYYYYGMDVWGQGTTVTVSS (SEQ ID NO: 547)
VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSDGSTSYAQKFQGRVTM TRDTSTSTVHMELSSLRSEDTAVYYCARDLFPHIYGNYYGMDIWGQGTTVTVSS (SEQ ID NO: 548)
VH	QMQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYADSVRGRFTI SRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS (SEQ ID NO: 549)
VH	QVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYADSVRGRFTI SRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS (SEQ ID NO: 550)
VL	QSVLTQPPSVSAAPGQKVTISCSGNNSNIANNYVSWYQQLPGTAPKLLIYDNNYRPSGIPDRFSGSKSGT SATLDITGLQTGDEADYYCGVWDGSLTTGVFGGGTKLTVL (SEQ ID NO: 551)
VL	AIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPKLLIYAASTLESGVPSRFSGSGSGTD FTLTISSLQPEDLATYYCQQLHTFPLTFGGGTKVEIK (SEQ ID NO: 552)
VL	QPVLTQPPSASGSPGQSVTISCTGTSSDVGAYNFVSWYRQHPGKAPKLMIYEVNKRPSGVPDRFSGSKSG NTASLTVSGLQAEDEADYYCSSYAGTNSLGIFGTGTKLTVL (SEQ ID NO: 553)
VL	QSVVTQPPSVSAAPGQKVTISCSGSSSDIGNHYVSWYQQLPGTAPKLLIYDNNQRPSGIPDRFSGSKSGT SATLAITGLQTGDEADYYCGTWDNSLSPHLLFGGGTKLTVL (SEQ ID NO: 554)
VL	QSVLTQPPSVSAAPGQKVTISCSGSSSNMGNNYVSWYKQVPGTAPKLLIYENDKRPSGIPDRFSGSKSGT SATLGITGLQTGDEADYYCGTWDNSLSGFVFASGTKVTVL (SEQ ID NO: 555)
VL	QSALTQPASVSGSLGQSVTISCTGSSSDVGSYNLVSWYQQHPGKAPNLMIYDVSKRSGVSNRFSGSKSGN TASLTISGLQAEDEADYYCSSYTGISTVVFGGGTKLTVL (SEQ ID NO: 556)
VL	QSVLTQPASVSGSPGQSITISCTGTSSDVGSYNLVSWYQQHPGKAPKLMIYEVSKRPSGVSNRFSGSKSG NTASLTISGLQAEDEADYYCSSYGGFNNLLFGGGTKLTVL (SEQ ID NO: 557)
VL	DIVMTQSPSSLSASIGDRVTITCRASQRISAYVNWYQQKPGKAPKVLIYAASSLRSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQTYSSPWTFGQGTKVEIK (SEQ ID NO: 558)
VL	QSVLTQPPSASGSPGQSVTISCTGTSSDIGGYDSVSWYQQHPGKAPKLMIYDVSKRPSGVSNRFSGSKSG NTASLTISGLQAEDEADYYCSSYTSSSIFFYVFGTGTKVTVL (SEQ ID NO: 559)
VL	LPVLTQPASVSGSPGQSITISCTGTTSDIGGYDYVSWYQQHPGKAPKLMIYDVSKRPSGVSNRFSGSKSG NTASLTISGLQAEDEADYYCSSYTSSSTHVFGTGTKLTVL (SEQ ID NO: 560)
VL	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVSNRFSGSKSG NTASLTISGLQAEDEADYYCSSYRSSTLGPVFGGGTKLTVL (SEQ ID NO: 561)
VL	QAGLTQPPSVSEAPRQRVTISCSGSSSNIGNNAVNWYQQLPGKAPKLLIYYDDLLPSGVSDRFSGSKSGT SASLAISGLQSEDEADYYCAAWDDSLNGYVFGTGTKLTVL (SEQ ID NO: 562)
VL	QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGVPDRFSGSKSG NTASLTISGLQAEDEADYYCSSYTSSTTHVFGTGTKVTVL (SEQ ID NO: 563)
VL	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPDRFSGSKSGT SATLGITGLQTGDEADYYCGTWDSSLSVWVFGGGTQLTVL (SEQ ID NO: 564)

VL	QSVLTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGRAPRLMIYDVSNRPSGVSNRFSGSKSG NTASLTISGLQAEDEGDYYCSSYTSGGTLGPVFGGGTKLTVL (SEQ ID NO: 565)
VL	QAGLTQPPSASGTPGQRVTISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGSKSGT SASLAISGLQSEDEADYYCAAWDDSLNGWVFGGGTKLTVL (SEQ ID NO: 566)
VL	AIRMTQSPSSLSASVGDRVTITCRASQSISNYLNWYQQRPGKAPNLLIYAASSLQSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQTYSTPYTFGQGTKLEIK (SEQ ID NO: 567)
VL	QSVLTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYRQHPGKAPKLMIYDVSYRPSGVSNRFSGSKSG NTASLTISGLQAEDEADYYCSSYTDSSTRYVFGTGTKLTVL (SEQ ID NO: 568)
VL	QPVLTQPPSASGTPGQRVAISCSGSRSNIEINSVNWYQQLPGTAPKLLIYDNNKRPSGIPDRFSGSKSGT SATLGITGLQTGDEADYYCGSWDSSLSADVFGTGTKLTVL (SEQ ID NO: 569)
VL	QSVLTQPPSVSAAPGKKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSKSGT SASLAISGLQSEDEADYYCATWDDSLNGWVFGGGTKLTVL (SEQ ID NO: 570)
VL	QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNNNRHSGVPDRFSGSKSG TSASLAITGLQAEDEAEFFCGTWDSRLTTYVFGSGTKLTVL (SEQ ID NO: 571)
VL	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPDRFSGSKSGT SATLGITGLQTGDEADYYCGTWDSSLSAVVFGGGTKLTVL (SEQ ID NO: 572)
VL	VIWMTQSPSSLSASVGDRVTITCAASSLQSWYQQKPGKAPKLLIYEASTLESGVPSRFSGSGSGTEFTLT ISSLQPEDFATYYCQQSYSTPYTFGQGTKLEIK (SEQ ID NO: 573)
VL	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQVPGTAPKLLIYDNNKRPSGIPDRFSGSNSDT SATLGITGLQTGDEADYYCGTWDSSLSAWVFGGGTKLTVL (SEQ ID NO: 574)
VL	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPDRFSGSKSGT SATLGITGLQTGDEADYYCGTWDSSLSAGSVVFGGGTKLTVL (SEQ ID NO: 575)
VL	SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCLVWDSSSDHRIFGGGTKLTVL (SEQ ID NO: 576)
VL	SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID NO: 577)
VL	SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID NO: 578)
VL	SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID NO: 579)
Group J*	
HC	QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTL TTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSE STAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPS NTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVD GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 580)

HC	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTI SRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKR VESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK (SEQ ID NO: 581)
LC	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESGVPARFSGSG SGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC (SEQ ID NO: 582)
LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTD FTLTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 583)
Group K	
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSSASTK (SEQ ID NO: 735)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSS (SEQ ID NO: 736)
HC	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTI SADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 737)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO: 268)
LC	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 738)
Group L	
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRFWMSWVRQAPGKGLEWVANINQDGTEKYYVDSVKGRFTI SRDNAKNSLYLQMNSLRAGDTAVYYCANTYYDFWSGHFDYWGQGTLVTVSS (SEQ ID NO: 739)
VH	QEHLVESGGGVVQPGRSLRLSCEASGFTFSNFGMHWVRQAPGKGLEWVAALWSDGSNKYYADSVKGRVTI SRDNSKNTLYLQMNSLRAEDTAVYYCARGRGAPGIPIFGYWGQGTLVTVSS (SEQ ID NO: 740)

VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEWVGRIKRKTDGGTTDYAAPVKGRF TISRDDSKNTLHLQMNSLKTEDTAVYYCTTDDIVVVPAVMREYYFGMDVWGQGTTVTVSS (SEQ ID NO: 741)
VH	QVQLVQSGAEVKKPGASVQVSCKASGYSFTGYYIHWVRQAPGQGLEWMGWINPNSGTKKYAHKFQGRVTM TRDTSIDTAYMILSSLISDDTAVYYCARDEDWNFGSWFDSWGQGTLVTVSS (SEQ ID NO: 742)
VH	QVHLVQSGAEVKKPGASVKVSCKASGYTFTGYYIHWVRQAPGHGLEWMGWLNPNTGTTKYIQNFQGRVTM TRDTSSSTAYMELTRLRSDDTAVYYCARDEDWNYGSWFDTWGQGTLVTVSS (SEQ ID NO: 743)
VH	EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMTWVRQAPGRGLEWVSGIHWHGKRTGYADSVKGRFTI SRDNAKKSLYLQMNSLKGEDTALYHCVRGGMSTGDWFDPWGQGTLVIVSS (SEQ ID NO: 744)
VH	EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMTWVRQVPGKGLEWVSGIHWSGRSTGYADSVKGRFTI SRDNAKNSLYLQMNSLRAEDTALYYCARGGMSTGDWFDPWGQGTLVTVSS (SEQ ID NO: 745)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVGSNYMNWVRQAPGKGLEWVSVIYSGGSTYYADSVKGRFTIS RLTSKNTLYLQMSSLRPEDTAVYYCARGIRGLDVWGQGTTVTVSS (SEQ ID NO: 746)
VH	EERLVESGGDLVQPGGSLRLSCAASGITVGTNYMNWVRQAPGKGLEWVSVISSGGNTHYADSVKGRFIMS RQTSKNTLYLQMNSLETEDTAVYYCARGIRGLDVWGQGTMVTVSS (SEQ ID NO: 747)
VH	QVQLVQSGAEVKMPGSSVRVSCKASGGIFSSSTISWVRQAPGQGLEWMGEIIPVFGTVNYAQKFQDRVIF TADESTTTAYMELSSLKSGDTAVYFCARNWGLGSFYIWGQGTMVTVSS (SEQ ID NO: 748)
VH	EVQLVESGGDLVHPGRSLRLSCAASGFPFDEYAMHWVRQVPGKGLEWVSGISWSNNNIGYADSVKGRFTI SRDNAKNSLYLQMNSLRPEDTAFYYCAKSGIFDSWGQGTLVTVSS (SEQ ID NO: 749)
VH	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVTLISYEGRNKYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCAKDRTLYGMDVWGQGTTVTVSS (SEQ ID NO: 750)
VH	QVTLRESGPALVKTTQTLTLTCTFSGFSLSTNRMCVTWIRQPPGKALEWLARIDWDGVKYYNTSLKTRLT ISKDTSKNQVVLTMTNMDPVDTATFYCARSTSLTFYYFDYWGQGTLVTVSS (SEQ ID NO: 751)
VH	EVQLVESGGGLVQPGGSLRLSCAASEFTVGTNHMNWVRQAPGKGLEWVSVIYSGGNTFYADSVKGRFTIS RHTSKNTLYLQMNSLTAEDTAVYYCARGLGGMDVWGQGTTVTVSS (SEQ ID NO: 752)
VH	EVQLVESGGGLVQRGESLRLYCAASGFTFSKYWMNWVRQAPGKGLEWVANIKGDGSEKYYVDSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCARDYWGSGYYFDFWGQGTLVTVSS (SEQ ID NO: 753)
VH	EVQLVESGGGLVQSGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYYVDSVKGRFTI SRDNAKNSLYLQMNSLRADDTAVYYCARDDIVVVPAPMGYYYYYFGMDVWGQGTTVTVSS (SEQ ID NO: 754)
VH	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDFAMHWVRQAPGKGLEWVSGISWTGGNMDYANSVKGRFTI SREDAKNSLYLQMNSLRAADTALYYCVKDIRGIVATGGAFDIWGRGTMVTVSS (SEQ ID NO: 755)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVGTNYMNWVRQAPGKGLEWISVIYSGGSTFYADSVKGRFTIS RQTSQNTLYLQMNSLRPEDTAVYYCARGIRGFDIWGQGTMVTVSS (SEQ ID NO: 756)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTISTNYMNWVRQAPGKGLEWVAVIYSSGSTYYIDSVKGRFTIS RLTSKNTVYLQMSSLNSEDTAVYYCARGIRGFDIWGQGTMVTVSS (SEQ ID NO: 757)
VH	EVQLVESGGGLVQPGRSLRLSCAASGFTIDDSAMHWVRQTPGKGLEWVSGISWKSGSIGYADSVRGRFTI SRDNAKNSLYLQMNSLRVEDTALYYCVKDIRGNWNYGGNWFDPWGQGTLVTVSS (SEQ ID NO: 758)

VH	EVQLVESGGGLVQPGGSLRLSCEASGFTVGVNHMNWVRQAPGKGLEWVSVIFSSGRTFYGDYVKGRLTIF RQTSQNTVYLQMNSLRSEDTAIYYCARGIGGLDIWGRGTMVTVSS (SEQ ID NO: 759)
VH	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYALHWVRQAPGKGLEWVSGISWTGGTIDYADSVKGRFTI SRDNAKNSLYLQMSSLRTEDTAIYYCTRDIRGNWKYGGWFDPWGQGTLVTVSS (SEQ ID NO: 760)
VH	QVQLVQSGTEVKKPGASVKVSCKASGYTFTAYYMHWVRQAPGQGLDWMGWISPNSGFTNYAQKFQGRVTM TRDTSINTFYMELSGLRSDDTAVYYCAREGSTHHNSFDPWGQGTLVTVSS (SEQ ID NO: 761)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVGTNFMNWVRQAPGKGLEWVSAIYSGGTANYADSVKGRFTIS RDTSRNTLYLQMNSLRTEDTAVYYCARGGGMDVWGQGTTVTVSS (SEQ ID NO: 762)
VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFNTYVLSWVRQAPGQGLEWMGEIIPILGAANYAQNFQGRVTF TTDESTNTAYMDLSSLRSEDTAVYYCARDRTSGGFDPWGQGTLVTVSS (SEQ ID NO: 763)
VH	QVQLVQSGAEVEKPGASVKVSCKASGYIFTHYGISWVRQAPGQGLEWVGWISPYNGYTDYAQKLQGRVTL TTDTSTTTAYMELRNLRSDDTAMYYCSRGRGPYWSFDLWGRGTLVTVSS (SEQ ID NO: 764)
VL	DIQMTQSPSTLSASVGDRVTITCRASQSISNWLAWYQQKPGKAPKLLIYKASSLESGVPSRFSGSGSGTE FTLTISSLQPDDFATYYCQQYHSYSYTFGQGTKEIK (SEQ ID NO: 765)
VL	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYTASSLQSGVPSRFSGSGSGTE FTLTISSLQPEDFATYYCLQHNSYPLTFGGGTKVAIK (SEQ ID NO: 766)
VL	DIQMTQSPSSLSASVGDRVTITCRTSQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTE FTLTISSLQPEDFATYYCLQHNNYPYTFGQGTKLEIK (SEQ ID NO: 767)
VL	DIVMTQTPLSSPVTLGQPASISCRSSQTLVHGDGNTYLSWIQQRPGQPPRLLIYKVSNQFSGVPDRFSGS GAGTDFTLKISRVEAEDVGLYFCMQATHFPITFGQGTRLEIK (SEQ ID NO: 768)
VL	DIVMTQTPLSSPVTLGQPASISCRSSPSLVHSDGNTYLSWLQQRPGQPPRLLIYKISNRFSGVPDRFSGS GAGTDFTLKISRVEAEDVGVYYCMQATHFPITFGQGTRLEIR (SEQ ID NO: 769)
VL	DIQMTQSPSSLSASLGDRVTITCRASQSINSYLNWYQQKPGKAPKLLIYVASSLQSGVPSRFSGSGSGTE FTLTISNLQPEDFATYYCQQSYSTP?ITFGQGTRLEIK (SEQ ID NO: 770)
VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYVASSLQSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSYSTP?ITFGQGTRLEIK (SEQ ID NO: 771)
VL	DIQMTQSPSSLSASVGDRVTITCRASQTINIYLNWYQQKPGRAPRLLIYAASSLQSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCHQSYSTP?ITFGQGTRLEIK (SEQ ID NO: 772)
VL	DIQMTQSPSSLSASVGDRVTITCRASQSMSSYLNWYQQKPGRAPKLLIFAASSLQSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSYSTP?ITFGQGTRLEIK (SEQ ID NO: 773)
VL	EIVLTQSPGTLSLSPGERATLSCRASQSFNFNYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGT DFTLTINRLEPEDFGVFYCQQYESAPWTFGQGTKVEIK (SEQ ID NO: 774)
VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKLLIYAASSLQSGVPSRFSGGGSGTDFTL TISSLRPEDFATYYCQQSYCTPPITFGQGTRLEIK (SEQ ID NO: 775)
VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSYSTP?ITFGQGTRLEIK (SEQ ID NO: 776)
VL	DRVTITCRASQVISNYLAWYQQKPGKVPRLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATY YCQKYNSAPRTFGQGTKVEIK (SEQ ID NO: 777)
VL	DIQMTQSPSSLSASVGDRVTITCRASQNINNYLNWYQQKPGKAPKLLIYAASSFQNAVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSYNTPLTFGGGTKVEIK (SEQ ID NO: 778)

VL	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTE FTLTISSLQPEDFATYYCLQHNSYPYTFGQGTKLEIK (SEQ ID NO: 779)
VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSYSTP?ITFGQGTRLEIK (SEQ ID NO: 780)
Group M	
VH	QSLEESGGRLVKPDETLTITCTVSGIDLSSNGLTWVRQAPGEGLEWIGTINKDASAYYASWAKGRLTISK PSSTKVDLKITSPTTEDTATYFCGRIAFKTGTSIWGPGTLVTVSS (SEQ ID NO: 1108)
VL	AIVMTQTPSPVSAAVGGTVTINCQASESVYSNNYLSWFQQKPGQPPKLLIYLASTLASGVPSRFKGSGSG TQFTLTISGVQCDDAATYYCIGGKSSSTDGNAFGGGTEVVVR (SEQ ID NO: 1109)
Group N	
VH	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARGNIVATITPLDYWGQGTLVTVSS (SEQ ID NO: 1110)
VH	QPVLTQPPSVSAAPGQKVTISCSGSSSNIANNYVSWYQQLPGTAPKLLIFANNKRPSGIPDRFSGSKSGT SAALDITGLQTGDEADYYCGTWDSDLRAGVFGGGTKLTVL (SEQ ID NO: 1111)
VH	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTI TADKSTSTAYMELSSLRSEDTAVYYCAREGTIYDSSGYSFDYWGQGTLVTVSS (SEQ ID NO: 1112)
VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYADSVRGRFTI SRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS (SEQ ID NO: 1113)
VH	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYADSVRGRFTI SRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS (SEQ ID NO: 1114)
VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYADSVRGRFTI SRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS (SEQ ID NO: 1115)
VH	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYADSVRGRFTI SRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS (SEQ ID NO: 1116)
VH	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYADSVRGRFTI SRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS (SEQ ID NO: 1117)
VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYADSVRGRFTI SRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS (SEQ ID NO: 1118)
VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSRYGVHWVRQAPGQGLEWMGRLIPIVSMTNYAQKFQDRVSI TTDKSTGTAYMELRSLTSEDTALYYCASVGQQLPWVFFAWGQGTLVTVSS (SEQ ID NO: 1119)
VH	QMQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYADSVRGRFTI SRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS (SEQ ID NO: 1120)
VH	QVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYADSVRGRFTI SRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS (SEQ ID NO: 1121)
VH	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAYSWVRQAPGQGLEWMGGIIPSFGTANYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARGPIVATITPLDYWGQGTLVTVSS (SEQ ID NO: 1122)
VH	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAYSWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARGPIVATITPLDYWGQGTLVTVSS (SEQ ID NO: 1123)

VH	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAYSWVRQAPGQGLEWMGGIIPSFGTANYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARGPIVATITPLDYWGQGTLVTVSS (SEQ ID NO: 1124)
VH	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPAFGTANYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARGPIVATITPLDYWGQGTLVTVSS (SEQ ID NO: 1125)
VL	SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID NO: 1126)
VL	AIRMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYTTSSLKSGVPSRFSGSGSGTD FTLTISRLQPEDFATYYCQQSYSSTWTFGRGTKVEIK (SEQ ID NO: 1127)
VL	QSVLTQPPSVSAAPGQKVTISCSGNNSNIANNYVSWYQQLPGTAPKLLIYDNNYRPSGIPDRFSGSKSGT SATLDITGLQTGDEADYYCGVWDGSLTTGVFGGGTKLTVL (SEQ ID NO: 1128)
VL	LPVLTQPASVSGSPGQSITISCTGTTSDIGGYDYVSWYQQHPGKAPKLMIYDVSKRPSGVSNRFSGSKSG NTASLTISGLQAEDEADYYCSSYTSSSTHVFGTGTKLTVL (SEQ ID NO: 1129)
VL	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVSNRFSGSKSG NTASLTISGLQAEDEADYYCSSYRSSTLGPVFGGGTKLTVL (SEQ ID NO: 1130)
VL	QAGLTQPPSVSEAPRQRVTISCSGSSSNIGNNAVNWYQQLPGKAPKLLIYYDDLLPSGVSDRFSGSKSGT SASLAISGLQSEDEADYYCAAWDDSLNGYVFGTGTKLTVL (SEQ ID NO: 1131)
VL	QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGVPDRFSGSKSG NTASLTISGLQAEDEADYYCSSYTSSTTHVFGTGTKVTVL (SEQ ID NO: 1132)
VL	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPDRFSGSKSGT SATLGITGLQTGDEADYYCGTWDSSLSVWVFGGGTQLTVL (SEQ ID NO: 1133)
VL	QSVLTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGRAPRLMIYDVSNRPSGVSNRFSGSKSG NTASLTISGLQAEDEGDYYCSSYTSGGTLGPVFGGGTKLTVL (SEQ ID NO: 1134)
VL	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPDRFSGSKSGT SATLGITGLQTGDEADYYCGTWDSSLSAVVFGGGTKLTVL (SEQ ID NO: 1135)
VL	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQVPGTAPKLLIYDNNKRPSGIPDRFSGSNSDT SATLGITGLQTGDEADYYCGTWDSSLSAWVFGGGTKLTVL (SEQ ID NO: 1136)
VL	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPDRFSGSKSGT SATLGITGLQTGDEADYYCGTWDSSLSAGSVVFGGGTKLTVL (SEQ ID NO: 1137)
VL	SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCLVWDSSSDHRIFGGGTKLTVL (SEQ ID NO: 1138)
VL	SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID NO: 1139)
VL	SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID NO: 1140)
VL	SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID NO: 1141)
Group O	
VH	QVQLVQSGSEVKKSGSSVKVSCKTSGGTFSITNYAINWVRQAPGQGLEWMGGILPIFGAAKYAQKFQDRV TITADESTNTAYLELSSLTSEDTAMYYCARGKRWLQSDLQYWGQGTLVTVSS (SEQ ID NO: 1142)

VL	QPVLTQPASVSGSPGQSITISCTGSSSDVGSYDLVSWYQQSPGKVPKLLIYEGVKRPSGVSNRFSGSKSG NTASLTISGLQAEDEADYYCSSYAGTRNFVFGGGTQLTVL (SEQ ID NO: 1143)

Note that the sequences provided for Group J are heavy chain and light chain amino acid sequences; the sequences provided for Group K include variable heavy chain, variable light chain, heavy chain, and light chain amino acid sequences; all other sequences presented in Table 15 are variable heavy chain and variable light chain sequences

[000300] In some embodiments, the activatable anti-PDL1 antibody includes a heavy chain that comprises or is derived from a heavy chain amino acid sequence shown in US Patent Nos. 7,943,743; 8,779,108; 8,217,149; 8,460,927; 7,794,710; 8,741,295; and 8,981,063; in US Patent Application Publication Nos. 2014356353; 20130323249; 2014341917; 20130309250; 20110280877; and 20090317368 (now issued as US Patent No. 8,981,063 listed above); and in PCT Publication Nos. WO2014/100483; WO2012/145493; WO2014/100439; WO2014/195852; WO2014/100079; WO2013/173223; and WO2014/55897, the contents of each of which are hereby incorporated by reference in their entirety. In some embodiments, the activatable anti-PDL1 antibody includes a light chain that comprises or is derived from a light chain amino acid sequence shown in US Patent Nos. 7,943,743; 8,779,108; 8,217,149; 8,460,927; 7,794,710; 8,741,295; and 8,981,063; in US Patent Application Publication Nos. 2014356353; 20130323249; 2014341917; 20130309250; 20110280877; and 20090317368 (now issued as US Patent No. 8,981,063); and in PCT Publication Nos. WO2014/100483; WO2012/145493; WO2014/100439; WO2014/195852; WO2014/100079; WO2013/173223; and WO2014/55897, the contents of each of which are hereby incorporated by reference in their entirety. In some embodiments, the activatable anti-PDL1 antibody includes a heavy chain and a light chain that comprise or are derived from a heavy chain amino acid sequence and a light chain amino acid sequence shown in US Patent Nos. 7,943,743; 8,779,108; 8,217,149; 8,460,927; 7,794,710; 8,741,295; and 8,981,063; in US Patent Application Publication Nos. 2014356353; 20130323249; 2014341917; 20130309250; 20110280877; and 20090317368 (now issued as US Patent No. 8,981,063); and in PCT Publication Nos. WO2014/100483; WO2012/145493; WO2014/100439; WO2014/195852; WO2014/100079; WO2013/173223; and WO2014/55897, the contents of each of which are hereby incorporated by reference in their entirety.

[000301] In some embodiments, the activatable antibody includes an anti-PDL1 antibody that includes at least one CDR sequence selected from the CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, VL CDR3) from the CDR sequences recited in US Patent No. 7,943,743. In some embodiments, the activatable antibody includes an anti-PDL1 antibody that includes at least one CDR, at least two, at least three, at least four, at least five, and/or six CDR sequences selected from the following sequences in US Patent No. 7,943,743: SEQ ID NO: 21, SEQ ID NO: 31, SEQ ID NO: 41, SEQ ID NO: 51, SEQ ID NO: 61, and SEQ ID NO: 71. In some embodiments, the activatable antibody includes an anti-PDL1 antibody that includes at least one CDR, at least two, at least three, at least four, at least five, and/or six CDR sequences selected from SEQ ID NO: 22, SEQ ID NO: 32, SEQ ID NO: 42, SEQ ID NO: 52, SEQ ID NO: 62, and SEQ ID NO: 72. In some embodiments, the activatable antibody includes an anti-PDL1 antibody that includes at least one CDR, at least two, at least three, at least four, at least five, and/or six CDR sequences selected from SEQ ID NO: 23, SEQ ID NO: 33, SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 63, and SEQ ID NO: 73.

[000302] In some embodiments, the activatable antibody includes an anti-PDL1 antibody that includes at least one CDR, at least two, at least three, at least four, at least five, and/or six CDR sequences selected from the following sequences in US Patent No. 7,943,743: a VH CDR1 sequence comprising SEQ ID NO: 21, SEQ ID NO: 22, or SEQ ID NO: 23; a VH CDR2 sequence comprising SEQ ID NO: 31, SEQ ID NO: 32, or SEQ ID NO: 33; a VH CDR3 sequence comprising SEQ ID NO: 41, SEQ ID NO: 42, or SEQ ID NO: 43; a VL CDR1 sequence comprising SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53; a VL CDR2 sequence comprising SEQ ID NO: 61, SEQ ID NO: 62, or SEQ ID NO: 63; and a VL CDR3 sequence comprising SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73.

[000303] In some embodiments, the activatable antibody includes an anti-PDL1 antibody that binds a polypeptide that comprises or is derived from SEQ ID NO: 1 or SEQ ID NO: 3 of US Patent No. 8,460,927.

[000304] In some embodiments, the activatable antibody includes an anti-PDL1 antibody that binds a human PDL1 polypeptide that comprises or is derived from SEQ ID NO: 1 of US Patent No. 8,460,927:

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDL
KVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPVT

SEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTA
ELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET (SEQ
ID NO: 585)

[000305] In some embodiments, the activatable antibody includes an anti-PDL1 antibody that binds a murine PDL1 polypeptide that comprises or is derived from SEQ ID NO: 3 of US Patent No. 8,460,927:

MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKEDEQVIQFVAGEEDL
KPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCCIISYGGADYKRITLKVNAPYRKINQRISVDPATS
EHELICQAEGYPEAEVIWTNSDHQPVSGKRSVTTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAE
LIIPELPATHPPQNRTHWVLLGSILLFLIVVSTVLLFLRKQVRMLDVEKCGVEDTSSKNRNDTQFEET (SEQ
ID NO: 586)

[000306] In some embodiments, the activatable antibody includes an anti-PDL1 antibody that binds a polypeptide that comprises or is derived from SEQ ID NO: 36 or SEQ ID NO: 38 of PCT Publication No. WO 2014/10079.

[000307] In some embodiments, the activatable antibody includes an anti-PDL1 antibody that binds a human PDL1 polypeptide that comprises or is derived from SEQ ID NO: 36 of PCT Publication No. WO 2014/10079.

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDL
KVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPVT
SEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTA
ELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKCGIQDTNSKQSDTHLEET (SEQ ID
NO: 587)

[000308] In some embodiments, the activatable antibody includes an anti-PDL1 antibody that binds a human PDL1 extracellular domain polypeptide that comprises or is derived from SEQ ID NO: 38 of PCT Publication No. WO 2014/10079.

FTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQL
SLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVI
WTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERT
(SEQ ID NO: 588)

[000309] In some embodiments, the activatable antibody includes an anti-PDL1 antibody that is or is derived from BMS-936559 (MDX-1 105). In some embodiments, the activatable antibody includes an anti-PDL1 antibody that is or is derived from MPDL3280A

(RG7446). In some embodiments, the activatable antibody includes an anti-PDL1 antibody that is or is derived from MEDI4736. In some embodiments, the activatable antibody includes an anti-PDL1 antibody that is or is derived from AMP224. In some embodiments, the activatable antibody includes an anti-PDL1 antibody that is or is derived from MSB0010718C.

[000310] In some embodiments, the activatable anti-PDL1 antibody comprises or is derived from an antibody that is manufactured, secreted or otherwise produced by a hybridoma, such as, for example, the hybridoma(s) disclosed in US Patent No. 8,779,108 and deposited at the National Collections of Industrial and Marine Bacteria (NCIMB) under deposit number 41598, as well as the hybridoma(s) disclosed in US Patent No. 8,741,295 and deposited at the Collection Nationale De Cultures De Microorganismes (CNCM) under deposit number I-4080, I-4081, and/or I-4122.

[000311] In some embodiments, the activatable anti-PDL1 antibody includes a CDR sequence shown in Table 16, a combination of VL CDR sequences (VL CDR1, VL CDR2, VL CDR3) selected from the group consisting of those combinations shown in a single row Table 16, a combination of VH CDR sequences (VH CDR1, VH CDR2, VH CDR3) selected from the group consisting of those combinations shown in Table 16, or a combination of VL CDR and VH CDR sequences (VL CDR1, VL CDR2, VL CDR3, VH CDR1, VH CDR2, VH CDR3) selected the group consisting of those combinations shown in Table 16.

Table 16. CDR Sequences for Antibodies and Activatable Antibodies that Bind PDL1

	VL			VH		
AB Name	CDR1 (SEQ ID NO)	CDR2 (SEQ ID NO)	CDR3 (SEQ ID NO)	CDR1 (SEQ ID NO)	CDR2 (SEQ ID NO)	CDR3 (SEQ ID NO)
C8	RASQSISSYLN (209)	KASRLQS (210)	RALKPVT (211)	SYAMS (212)	DITASGQRTTYADS (213)	SKAIFDY (214)
C12	RASQSISSYLN (209)	AASSLQS (215)	SYSTPNT (216)	SYAMS (212)	SINKDGHYTSYADS (217)	NLDEFDY (218)
C16	RASQSISSYLN (209)	SASQLQS (219)	ANSRPST (220)	SYAMS (212)	SIMATGAGTLYADS (221)	DGAGFDY (222)
C20	RASQSISSYLN (209)	NASSLQS (223)	YPYGPG (224)	SYAMS (212)	TITSSGAATYYADS (225)	NYTGFDY (226)

C60	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SIYSTGGATAYADS (229)	SSAGFDY (230)
C1	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIYSTGGATAYADS (231)	SSAGQSRP GFDY (232)
D1	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIYSTGGATAYADS (231)	SSAGQSWP GFDY (233)
G7	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIYSTGGATAYADS (231)	SSAGQSFP GFDY (234)
H9	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIYSTGGATAYADS (231)	WSAAFDY (235)
B10	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIYSTGGATAYADS (231)	WSAGYDY (236)
E10	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIYSTGGATAYADS (231)	WSKGFDY (237)
A05	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIWKQGIVTVYDS (238)	SSAGFDY (230)
C05	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIWRNGIVTVYDS (239)	SSAGFDY (230)
C10	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SDIWKQGMVTVYDS (240)	SSAGFDY (230)
D08	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIWRQGLATAYDS (241)	SSAGFDY (230)
G09	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SEIVATGILTSYDS (242)	SSAGFDY (230)
G10	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIGRQGLITVYDS (243)	SSAGFDY (230)
G12	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIWYQGLVTVYDS (244)	SSAGFDY (230)
E11	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SDIWKQGFATADS (245)	SSAGFDY (230)
D01	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIWKQGIVTVYDS (238)	SSAGFDY (230)
H06	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIWRQGLATAYDS (241)	SSAGFDY (230)
C5H9	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIWRNGIVTVYADS (246)	WSAAFDY (235)

C5B10	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIWRNGIVTVYADS (246)	WSAGYDY (236)
C5E10	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIWRNGIVTVYADS (246)	WSKGFDY (237)
G12H9	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIWYQGLVTVYADS (247)	WSAAFDY (235)
G12B10	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIWYQGLVTVYADS (247)	WSAGYDY (236)
G12E10	RASQSISSYLN (209)	YASTLQS (227)	DNGYPST (228)	SYAMS (212)	SSIWYQGLVTVYADS (247)	WSKGFDY (237)
C5H9 v2	RASQSISSYLN (209)	AASSLQS (215)	DNGYPST (228)	SYAMS (212)	SSIWRNGIVTVYADS (246)	WSAAFDY (235)

[000312] In some embodiments, the activatable anti-PDL1 antibody includes a CDR sequence shown in Table 17, a combination of VL CDR sequences selected from the group consisting of those combinations shown in Table 17, and/or a combination of VH CDR sequences selected from the group consisting of those combinations shown in Table 17.

[000313] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group A in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group A in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group A in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group A in Table 17.

[000314] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group B in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group B in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group B in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group B in Table 17.

[000315] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group C in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group C in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group C in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group C in Table 17.

[000316] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group D in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group D in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group D in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group D in Table 17.

[000317] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group E in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group E in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group E in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group E in Table 17.

[000318] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group F in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group F in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences

selected from the group consisting of the combinations shown in Group F in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group F in Table 17.

[000319] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group G in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group G in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group G in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group G in Table 17.

[000320] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group H in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group H in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group H in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group H in Table 17.

[000321] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group I in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group I in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group I in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group I in Table 17.

[000322] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group J in Table 17. In some embodiments, the activatable anti-

PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group J in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group J in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group J in Table 17.

[000323] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group K in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group K in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group K in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group K in Table 17.

[000324] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group L in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group L in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group L in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group L in Table 17.

[000325] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group M in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group M in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group M in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group M in Table 17.

[000326] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group N in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group N in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group N in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group N in Table 17.

[000327] In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group O in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group O in Table 17. In some embodiments, the activatable anti-PDL1 antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group O in Table 17, and a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group O in Table 17.

[000328] In some embodiments, the activatable anti-PDL1 antibody includes a heavy chain CDR1 sequence selected from the group consisting of the sequences shown in Group P in Table 17.

Table 17. Additional CDR Sequences for Antibodies and Activatable Antibodies that Bind PDL1

Group A					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
RASQSVSSYLV (304)	DASNRAT (293)	QQRSNWPRT (278)	DYGFS (296)	WITAYNGNTNYAQKLQG (284)	DYFYGMDV (269)
RASQSVSSYLA (305)	AASSLQS (215)	QQRSNWPT (279)	TYAIS (297)	GIIPIFGKAHYAQKFQG (285)	KFHFVSGSPFG MDV (270)

RASQGISSWLA (306)	GASSRAT (294)	QQYNSYPYT (280)	SYDVH (298)	WLHADTGITKFSQKFQG (286)	ERIQLWFDY (271)
RASQSVSSSYLA (307)	DASSLES (295)	QQYGSSPWT (281)	TYAIN (299)	GIIPIFGTANHAQKFQG (287)	DQGIAAALFDY (272)
RASQGISSALA (308)	KASTLES (447)	QQYGSSP (282)	DYVVH (300)	GISGNSGNIGYADSVKG (288)	PFDY (273)
		QQFNSYPFT (283)	SYAIS (301)	GIIPIFGRAHYAQKFQG (289)	KYDYVSGSPFG MDV (274)
		QQSYSTPWT (448)	SYAIN (302)	GIIPIFGSANYAQKFQD (290)	DSSGWSRYYMD V (275)
			DYGMH (303)	GIIPLFGIAHYAQKFQG (291)	KYSYVSGSPFG MDV (276)
				GISWNRGRIEYADSVKG (292)	GRFRYFDWFLD Y (277)
					DYFWSGFSAFD I (446)

Group B					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
SGDALPQKYVF (312)	EDSKRPS (313)	YSTDRSGNHRV (314)	TYSMN (309)	SISSSGDYIYYADSVK (310)	DLVTSMVAFDY (311)
RASQRVSSSYLA (318)	DASSRAT (319)	QQYGSLPWT (320)	RYWMS (315)	NIKQDGSEKYYVDSVKG (316)	EGGWFGELAFD Y (317)
RASQSVSSNYLA (324)	GTSSRAT (325)	QQYGSSIFT (326)	SYWMS (321)	NIKQDGGEQYYVDSVK (322)	DWNYGYYDMDV (323)

Group C					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)

RASQ(D/V)(V/I)(S/N)T(A/F)(V/L)A (327)	SAS(F/T)L(Y/A)S (328)	QQ(Y/G/F/S)(L/Y/F/W)(Y/N/A/T/G/F/I)(H/V/P/T/I)P(A/W/R/P/T)T (329)	GFTFS(D/G)SWIH (330)	AWI(S/L)PYGGS(T/S)YYADSVKG (331)	RHWPGGFDY (332)
RASQDVSTAVA (333)	SASFLYS (334)	QQYLYHPAT (335)	GFTFSDSWIH (336)	AWISPYGGSTYYADSVKG (337)	

Group D					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
KSSQSLL(H/N)(S/T)(R/S)TRKNYLA (588)	WASTRES (589)	(Q/K)QSYDVVT (590)	SYW(I/M)H (591)	YINPSS(D/G)Y(H/N)EY(S/N)(E/Q)KF(I/M)D (592)	SGWL(I/V)HGDYYFD(F/Y) (593)
KSSQSLLNSRTRKNYLA (594)		QQSYDVVT (595)	SYWMH (596)	YINPSSDYNEYSEKFMD (597)	SGWLVHGDYYFDY (598)
KSSQSLLHTSTRKNYLA (599)		KQSYDVVT (600)	GYIFTSYWMH (601)	YINPSSGYHEYNQKFID (602)	SGWLIHGDYYFDF (603)
			SYWIH (604)		
			GTTFTSYWIH (605)		

Group E					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)

TGT(N/S)(T/R/S)DVG(A/G)YNYVS (606)	(E/D)V(I/N/D)(D/H/N)RPS (607)	SS(F/Y)T(N/S)(R/T/S)(G/S)(I/T)RV (608)	(K/R/T/Q/G/A/W/M/I/S)Y(V/R/K/L/M/I)M(H/T/N/Q/A/V/Y/W/F/M) (609)	SIYPSGG(F/I)TFYAD(S/T)VKG (610)	IKLGTVTTV(E/D)Y (611)
TGTSSDVGGYNYVS (612)	DVSNRPS (613)	SSYTSSSTRV (614)	SYIMM (615)	SIYPSGGITFYADTVKG (617)	IKLGTVTTVDY (618)

Group F					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
			MYMMM (619)	SIYPSGGITFYADSVKG (620)	TGTSSDVGAYNYVS (621)

Group G					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
(R/S/K)(A/S)S(R/N/D/Q/E/H/K/M/S)(SLLYSS or Absent)(N/D or Absent)(A/Q/E/L/K/M/P/S/T/Y/V)X(R/N/S/T)(A/R/N/C/Q/E/H/I/L/K/M/F/S/T/Y/V)(I/L/M/V)(A/R/N	X(A/C/G/S/T/V)(A/C/H/I/L/M/F/S/T/Y/V)(R/N/D/Q/E/K/S/T)(A/R/N/D/Q/E/G/H/K/M/F/P/S/T/Y)(A/N/S/T) (623)	(R/N/Q/E/H)QX(A/R/N/C/Q/E/H/I/L/K/M/F/S/T/Y/V)(N/G/S)(A/R/N/D/C/Q/E/G/H/I/L/K/M/F/R/S/T/W/Y/V)P(A/R/N/D/Q/E/G/H/K/M/P/S/T/Y) T (624)	G(Y/F)(T/S)(I/L/M/F)(A/R/N/D/C/Q/E/H/I/L/K/M/P/S/T/W/Y/V)(N/D/Q/E/K/P/S/T)(A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/Y/V)(A/R/N/D/C/Q/E/G/H/I/L/K/M/	X(I/L/M/F/V)X(P or Absent)(A/R/N/Q/E/H/K/M/S/T/Y/V)(A/R/N/D/C/Q/E/G/H/K/M/F/P/S/T/Y/V)(N/G/S)(N/G/S)T(A/R/N/Q/E/H/K/M/S/T/Y)(A/R/N/C/Q/E/H/I/L/K/M/F/S/T/W/Y/V)N(A/D/Q/E/K/P/S/T)(A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/Y/V)(I/L/M/F)K(A/G/S (626)	(A/R/N/Q/E/H/K/M/S/T/Y)(A/R/N/D/C/Q/E/G/H/K/M/F/P/S/T/Y/V)(Q/G/H/T/W)XXX(F/Y)(AI or Absent) (627)

/Q/E/H/I/K/M/F/S/T/Y) (622)			F/P/S/T/Y/V)(A/R/C/Q/E/H/I/L/K/M/F/S/T/W/Y/V)N (625)		
RASSSVSYIY (628)	ATFNLAS (629)	HQRSSYPWT (630)	GYTFPDYYMN (631)	DIDPNYGGTTYNQKFKG (632)	GAL (633)
SASSSIRYMH (634)	DTSKLTS (635)	QQDSSYPLT (636)	GYTFTSYDIN (637)	WIFPRDNNTKYNENFKG (638)	ENWVGDF (639)
KASQDVGTAVA (640)	WASTRHT (641)	QQYYGYPLT (642)	GYSITSDYWN (643)	YISYTGSTYYNPS_KS (644)	YGGWLSPF (645)
KSSQSLLYSSNQKNSL (646)	WASTRES (589)		GYSIISDYWN (647)		RGGWLLPF (648)
			GFSLTTYSIN (649)	VMWAGGGTNSNSV_KS (650)	YYGNSPYYAI (651)

Group H					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
TRSSGSIGSNYVQ (652)	EDNQRPS (653)	QSYDSSTWV (654)	SYAIS (301)	WISPIGGSTNYAQKVQG (655)	GLXXXXXXXXXXXXXXDV (656)
TRSSGNIASNYVQ (657)	GKNNRPS (658)	QSYDSSNLWV (659)	SYGIS (660)	WISAYNGNTNYAQKLED (661)	ALPSGTILVGGWFDP (662)
QGDSLRSYYAS (663)	SDRDRPS (664)	NSRDSSGNHYV (665)	SYALS (666)	AISGGGGSTYYADSVKD (667)	DVFPETFSMNYGMDV (668)
GGSDIGRKSVH (669)	DVSNRPS (613)	QVWDNNSDHYV (670)	DYAMH (671)	LISGDGGSTYYADSVKD (672)	VLLPCSSTSCYGSVGAFDI (673)
TGTSSDVGGYNYVS (612)	GASSRAT (294)	SSYTSSTLP (674)	NYDMS (675)	RVNWNGGSTTYADAVKD (676)	EFVGAYDL (677)
RASQSIGNSLA (678)	AASTLQS (679)	QQHTIPTFS (680)	GLYIH (681)	WIIPIFGTANYAQKFED (682)	GLRWGIWGWFDP (683)
RASQGIGSYLA (684)	QDIKRPS (685)	QQLNNYPIT (686)	DNAIS (687)	WIIPIFGKPNYAQKFED (688)	TMVRGFLGVMDV (689)

SGDKLGNKYAY (690)	EDYRRPS (691)	QTWDNSVV (692)	SYAMS (212)	AISGSGGSTYYADS VKD (693)	DQFVTIFGVPR YGMDV (694)
TRSSGSIDSNY VQ (695)	DDSDRPS (696)	QSYDSNNRHVI (697)	TYALN (698)	RIVPLIGLVNYAHN FED (699)	GRQMFGAGIDF (700)
TRSSGNIGTNY VQ (701)	EDNKRPS (702)	QSYHSSGWE (703)	SHGIT (704)	WISAHNGHASNAQK VED (705)	EVYGGNSDY (706)
GGNNIGSKGVH (707)	YKSDSNKQ QAS (708)	QVWDSSSDHWV (709)	RHGMH (710)	VISHDGSVKYYADS MKD (711)	VHAALYYGMDV (712)
TRSSGSIASNY VQ (713)		QSYDSTTPSV (714)		WTSPHNGLTAFAQI LED (715)	GLSYQVSGWFD P (716)
TRSSGSIASHY VQ (717)		QSYDGITVI (718)		RIIPILGIANYAQK FED (719)	VHPVFSYALDV (720)
TLRSGLNVGSY RIY (721)		QSYDSSNRWV (722)	TYAFS (723)	WISAYNGNTNYAQK VED (724)	DGYGSDPVL (725)
		MIWYSSAVV (726)	NYGIS (727)		GDFRKPFDY (728)

Group I					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
RATESVEYYGT SLVQ (729)	AASSVDS (730)	QQSRRVPYT (731)	SYVMH (732)	YVNPFNDGTKYNEMFKG (733)	QAWGYP (734)

Group J					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
RASQDVSTAVA (333)	SASFLYS (334)	QQYLYHPAT (335)	GFTFSDSWIH (336)	AWISPYGGSTYYADSVKG (337)	RHWPGGF DY (332)

Group K					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
QSISNW (781)	KAS (782)	QQYHSYSYT (783)	GFTFSRFW (784)	INQDGTEK (785)	ANTYYDFWSGH FDY (786)
QGIRND (787)	TAS (788)	LQHNSYPLT (789)	GFTFSNFG (790)	LWSDGSNK (791)	ARGRGAPGIPI FGY (792)
QGIRND (787)	AAS (793)	LQHNNYPYT (794)	GFTFSNAW (795)	IKRKTDGGTT (796)	TTDDIVVVPAV MREYYFGMDV (797)
QTLVHGDGNTY (798)	KVS (799)	MQATHFPIT (800)	GYSFTGYY (801)	INPNSGTK (802)	ARDEDWNFGSW FDS (803)
PSLVHSDGNTY (804)	KIS (805)	MQATHFPIT (800)	GYTFTGYY (806)	LNPNTGTT (807)	ARDEDWNYGSW FDT (808)
QSINSY (809)	VAS (810)	QQSYSTPPIT (811)	GFTFDDYG (812)	IHWHGKRT (813)	VRGGMSTGDWF DP (814)
QSISSY (815)	VAS (810)	QQSYSTPPIT (811)	GFTFDDYG (812)	IHWSGRST (816)	ARGGMSTGDWF DP (817)
QTINIY (818)	AAS (793)	HQSYSTPPIT (819)	GFTVGSNY (820)	IYSGGST (821)	ARGIRGLDV (822)
QSMSSY (1011)	AAS (793)	QQSYSTPPIT (811)	GITVGTNY (1012)	ISSGGNT (1013)	ARGIRGLDV (822)
QSFNFNY (823)	GAS (824)	QQYESAPWT (825)	GGIFSSST (826)	IIPVFGTV (827)	ARNWGLGSFYI (828)
QSISSY (815)	AAS (793)	QQSYCTPPIT (829)	GFPFDEYA (830)	ISWSNNNI (831)	AKSGIFDS (832)
QSISSY (815)	AAS (793)	QQSYSTPPIT (811)	GFTFSSYG (833)	ISYEGRNK (834)	AKDRTLYGMDV (835)
QVISNY (836)	AAS (793)	QKYNSAPRT (837)	GFSLSTNRMC (838)	IDWDGVK (839)	ARSTSLTFYYF DY (840)
QNINNY (841)	AAS (793)	QQSYNTPLT (842)	EFTVGTNH (843)	IYSGGNT (844)	ARGLGGMDV (845)
QTISTY (846)	AAS (793)	LQHNSYPYT (847)	GFTFSKYW (848)	IKGDGSEK (849)	ARDYWGSGYYF DF (850)

QSISSY (815)	VVS (851)	QQSYSTPFT (852)	GFTFSSYW (853)	IKQDGSEK (854)	ARDDIVVVPAP MGYYYYYFGMD V (855)
	AAS (793)	QQSYSTPPIT (811)	GFTFDDFA (856)	ISWTGGNM (857)	VKDIRGIVATG GAFDI (858)
			GFTVGTNY (859)	IYSGGST (821)	ARGIRGFDI (860)
			GFTISTNY (861)	IYSSGST (862)	ARGIRGFDI (860)
			GFTIDDSA (863)	ISWKSGSI (864)	VKDIRGNWNYG GNWFDP (865)
			GFTVGVNH (866)	IFSSGRT (867)	ARGIGGLDI (868)
			GFTFDDYA (869)	ISWTGGTI (870)	TRDIRGNWKYG GWFDP (871)
			GYTFTAYY (872)	ISPNSGFT (873)	AREGSTHHNSF DP (874)
			GFTVGTNF (875)	IIPILGAA (876)	ARGGGMDV (877)
			GGTFNTYV (878)	ISPYNGYT (879)	ARDRTSGGFDP (880)
			GYIFTHYG (881)		SRGRGPYWSFD L (882)

Group L					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
QASESVYSNNY LS (1014)	LASTLAS (1015)	IGGKSSSTDG NA (1016)	SNGLT (1017)	TINKDASAYYASWAK G (1018)	IAFKTGTSI (1019)

Group M					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)

RSSKSLLHSNG ITYLY (1020)	QMSNLAS (1021)	AQNLEPPLT (1022)	DYYTH (1023)	WIDPENGKTAYAPKF QG (1024)	GGYDVYFLDY (1025)

Group N					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
KASQDVGIVVA (1026)	WASIRHT (1027)	QQYSNYPLYT (1028)	GFSLTSYGVH (1029)	VIWAGGSTNYNSALM S (1030)	AKPYGNSAMDY (1031)
				VIWAGGSTNYVDSVK G (1032)	AKPYGTSAMDY (1033)
				VIWAGGSTNYADSVK G (1034)	

Group O					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
ASQSVSTSSSS FMH (1035)	YASNLES (1036)	QHSWEIPYT (1037)	SYGMS (1038)	SISSGGSTYYPDSVK G (1039)	GYDSGFAY (1040)
RASQSVSTSSS SYMH (1041)	YASNLES (1036)	QHSWEIPYT (1037)	SYGMS (1038)	SISSGGTTYYPDSVK G (1042)	GYDSGFAY (1040)
KASQSVSNDVA (1043)	YAANRYT (1044)	QQDYTSPYT (1045)	TYGVH (1046)	VIWRGVTTDYNAAFM AS (1047)	LGFYAMDY (1048)
KASQSVSNDVG (1049)	YASNRYS (1050)	QQDYTSPYT (1045)	SYGVH (1051)	VIWSGGVTDYNAAFI S (1052)	LGFYAMDY (1048)
RSSQIIVHSNA NTYLE (1053)	KVSNRFS (1054)	FQGSHVPYT (1055)	TYWMH (1056)	QINPDSTTINTAPSL KD (1057)	PGDYGYDFDC (1058)
SASSSVSSSYL Y (1059)	NTSNLAS (1060)	HQWRSYPPT (1061)	SGYWN (1062)	YISYSGSTYYNPSLK S (1063)	SLLWFSTGFAY (1064)
SANSSVSYMH (1065)	DTSKLAS (1066)	QQWSSNPWT (1067)	SYGVH (1051)	YIWSGGITDYNAAFK S (1068)	LGFYAMDY (1048)
RASQSVSTSSY SYMH (1069)	YASNLES (1036)	QNSWEIPYT (1070)	STGMS (1071)	SISSGGTTYYLGSVQ G (1072)	GYDAGFAY (1073)

KSSQSLLYSSNQKNSLA (1074)	WASNRES (1075)	QQYYSYPLT (1076)	SGYWT (1077)	YIYTGSTYYNPSLKS (1078)	QRDWLGFAY (1079)
RASQSVSTSSYSYVH (1080)	YASNLES (1036)	QHSWEIPYT (1037)	SYGMS (1038)	SISSGGSIYYPDSVKG (1081)	GYDAGFAF (1082)

Group P					
VL			VH		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
			GFTFSMYMMM (1083)		
			GFTFSAYAMA (1084)		
			GFTFSAYRMF (1085)		
			GFTFSAYLMV (1086)		
			GFTFSAYVMF (1087)		
			GFTFSAYVMS (1088)		
			GFTFSGYLMV (1089)		
			GFTFSGYQML (1090)		
			GFTFSGYSMF (1091)		
			GFTFSGYWMA (1092)		
			GFTFSQYLMY (1093)		
			GFTFSQYVMF (1094)		
			GFTFSQYYMY (1095)		
			GFTFSSYLMS (1096)		

			GFTFSSYLMT (1097)		
			GFTFSSYQMV (1098)		
			GFTFSSYSMA (1099)		
			GFTFSSYVMF (1100)		
			GFTFSSYVMS (1101)		
			GFTFSSYVMY (1102)		
			GFTFSSYYMF (1103)		
			GFTFSSYYMV (1104)		
			GFTFSYYSMV (1105)		
			GFTFSWYLMA (1106)		
			GFTFSWYQMS (1107)		

[000329] The ABs in the activatable antibodies of the disclosure specifically bind a PDL1 target, such as, for example, mammalian PDL1, and/or human PDL1. Also included in the disclosure are ABs that bind to the same PDL1 epitope as an antibody of the disclosure and/or an activated activatable antibody described herein. Also included in the disclosure are ABs that compete with an anti-PDL1 antibody and/or an activated anti-PDL1 activatable antibody described herein for binding to a PDL1 target, e.g., human PDL1. Also included in the disclosure are ABs that cross-compete with an anti-PDL1 antibody and/or an activated anti-PDL1 activatable antibody described herein for binding to a PDL1 target, e.g., human PDL1.

[000330] The activatable anti-PDL1 antibodies provided herein include a masking moiety. In some embodiments, the masking moiety is an amino acid sequence that is coupled or otherwise attached to the anti-PDL1 antibody and is positioned within the activatable anti-PDL1 antibody construct such that the masking moiety reduces the ability of the anti-PDL1 antibody to specifically bind PDL1. Suitable masking moieties are

identified using any of a variety of known techniques. For example, peptide masking moieties are identified using the methods described in PCT Publication No. WO 2009/025846 by Daugherty et al., the contents of which are hereby incorporated by reference in their entirety.

[000331] The activatable anti-PDL1 antibodies provided herein include a cleavable moiety. In some embodiments, the cleavable moiety includes an amino acid sequence that is a substrate for a protease, usually an extracellular protease. Suitable substrates are identified using any of a variety of known techniques. For example, peptide substrates are identified using the methods described in U.S. Patent No. 7,666,817 by Daugherty et al.; in U.S. Patent No. 8,563,269 by Stagliano et al.; and in PCT Publication No. WO 2014/026136 by La Porte et al., the contents of each of which are hereby incorporated by reference in their entirety. (*See also* Boulware et al. "Evolutionary optimization of peptide substrates for proteases that exhibit rapid hydrolysis kinetics." Biotechnol Bioeng. 106.3 (2010): 339-46).

[000332] Exemplary substrates include but are not limited to substrates cleavable by one or more of the following enzymes or proteases listed in Table 12.

Table 12: Exemplary Proteases and/or Enzymes

ADAMS, ADAMTS, *e.g.*	Cysteine proteinases, *e.g.*,	Serine proteases, *e.g.*,
ADAM8	Cruzipain	activated protein C
ADAM9	Legumain	Cathepsin A
ADAM10	Otubain-2	Cathepsin G
ADAM12		Chymase
ADAM15	KLKs, *e.g.*,	coagulation factor proteases
ADAM17/TACE	KLK4	(*e.g.*, FVIIa, FIXa, FXa,
		FXIa, FXIIa)
ADAMDEC1	KLK5	
ADAMTS1	KLK6	Elastase
ADAMTS4	KLK7	Granzyme B
ADAMTS5	KLK8	Guanidinobenzoatase
	KLK10	HtrA1
Aspartate proteases, *e.g.*,	KLK11	Human Neutrophil Elastase
BACE	KLK13	Lactoferrin
Renin	KLK14	Marapsin
		NS3/4A
Aspartic cathepsins, *e.g.*,	Metallo proteinases, *e.g.*,	PACE4
Cathepsin D	Meprin	Plasmin
Cathepsin E	Neprilysin	PSA
	PSMA	tPA
Caspases, *e.g.*,	BMP-1	Thrombin
Caspase 1		Tryptase
Caspase 2	MMPs, *e.g.*,	uPA

Caspase 3	MMP1	
Caspase 4	MMP2	Type II Transmembrane Serine Proteases (TTSPs), *e.g.*,
Caspase 5	MMP3	
Caspase 6	MMP7	DESC1
Caspase 7	MMP8	DPP-4
Caspase 8	MMP9	FAP
Caspase 9	MMP10	Hepsin
Caspase 10	MMP11	Matriptase-2
Caspase 14	MMP12	MT-SP1/Matriptase
	MMP13	TMPRSS2
Cysteine cathepsins, *e.g.*,	MMP14	TMPRSS3
Cathepsin B	MMP15	TMPRSS4
Cathepsin C	MMP16	
Cathepsin K	MMP17	
Cathepsin L	MMP19	
Cathepsin S	MMP20	
Cathepsin V/L2	MMP23	
Cathepsin X/Z/P	MMP24	
	MMP26	
	MMP27	

[000333] The activatable anti-PDL1 antibodies described herein overcome a limitation of antibody therapeutics, particularly antibody therapeutics that are known to be toxic to at least some degree *in vivo*. Target-mediated toxicity constitutes a major limitation for the development of therapeutic antibodies. The activatable anti-PDL1 antibodies provided herein are designed to address the toxicity associated with the inhibition of the target in normal tissues by traditional therapeutic antibodies. These activatable anti-PDL1 antibodies remain masked until proteolytically activated at the site of disease. Starting with an anti-PDL1 antibody as a parental therapeutic antibody, the activatable anti-PDL1 antibodies of the invention were engineered by coupling the antibody to an inhibitory mask through a linker that incorporates a protease substrate.

[000334] When the AB is modified with a MM and is in the presence of the target, specific binding of the AB to its target is reduced or inhibited, as compared to the specific binding of the AB not modified with an MM or the specific binding of the parental AB to the target.

[000335] The K_d of the AB modified with a MM towards the target is at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater, or between 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000,

100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times greater than the K_d of the AB not modified with an MM or of the parental AB towards the target. Conversely, the binding affinity of the AB modified with a MM towards the target is at least 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater, or between 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times lower than the binding affinity of the AB not modified with an MM or of the parental AB towards the target.

[000336] In some embodiments, the dissociation constant (K_d) of the MM towards the AB is generally greater than the K_d of the AB towards the target. The K_d of the MM towards the AB can be at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 100,000, 1,000,000 or even 10,000,000 times greater than the K_d of the AB towards the target. Conversely, the binding affinity of the MM towards the AB is generally lower than the binding affinity of the AB towards the target. The binding affinity of MM towards the AB can be at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 100,000, 1,000,000 or even 10,000,000 times lower than the binding affinity of the AB towards the target.

[000337] In some embodiments, the dissociation constant (K_d) of the MM towards the AB is approximately equal to the K_d of the AB towards the target. In some embodiments, the dissociation constant (K_d) of the MM towards the AB is no more than the dissociation constant of the AB towards the target.

[000338] In some embodiments, the dissociation constant (K_d) of the MM towards the AB is less than the dissociation constant of the AB towards the target.

[000339] In some embodiments, the dissociation constant (K_d) of the MM towards the AB is greater than the dissociation constant of the AB towards the target.

[000340] In some embodiments, the MM has a K_d for binding to the AB that is no more than the K_d for binding of the AB to the target.

[000341] In some embodiments, the MM has a K_d for binding to the AB that is no less than the K_d for binding of the AB to the target.

[000342] In some embodiments, the MM has a K_d for binding to the AB that is approximately equal to the K_d for binding of the AB to the target.

[000343] In some embodiments, the MM has a K_d for binding to the AB that is less than the K_d for binding of the AB to the target.

[000344] In some embodiments, the MM has a K_d for binding to the AB that is greater than the K_d for binding of the AB to the target.

[000345] In some embodiments, the MM has a K_d for binding to the AB that is no more than 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or 1,000 fold greater than the K_d for binding of the AB to the target. In some embodiments, the MM has a K_d for binding to the AB that is between 1-5, 2-5, 2-10, 5-10, 5-20, 5-50, 5-100, 10-100, 10-1,000, 20-100, 20-1000, or 100-1,000 fold greater than the K_d for binding of the AB to the target.

[000346] In some embodiments, the MM has an affinity for binding to the AB that is less than the affinity of binding of the AB to the target.

[000347] In some embodiments, the MM has an affinity for binding to the AB that is no more than the affinity of binding of the AB to the target.

[000348] In some embodiments, the MM has an affinity for binding to the AB that is approximately equal of the affinity of binding of the AB to the target.

[000349] In some embodiments, the MM has an affinity for binding to the AB that is no less than the affinity of binding of the AB to the target.

[000350] In some embodiments, the MM has an affinity for binding to the AB that is greater than the affinity of binding of the AB to the target.

[000351] In some embodiments, the MM has an affinity for binding to the AB that is 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or 1,000 less than the affinity of binding of the AB to the target. I In some embodiments, the MM has an affinity for binding to the AB that is between 1-5, 2-5, 2-10, 5-10, 5-20, 5-50, 5-100, 10-100, 10-1,000, 20-100, 20-1000, or 100-1,000 fold less than the affinity of binding of the AB to the target. In some embodiments, the MM has an affinity for binding to the AB that is 2 to 20 fold less than the affinity of binding of the AB to the target. In some embodiments, a MM not covalently linked to the AB and at equimolar concentration to the AB does not inhibit the binding of the AB to the target.

[000352] When the AB is modified with a MM and is in the presence of the target specific binding of the AB to its target is reduced or inhibited, as compared to the specific binding of the AB not modified with an MM or the specific binding of the parental AB to the target. When compared to the binding of the AB not modified with an MM or the binding of the parental AB to the target the AB's ability to bind the target when modified

with an MM can be reduced by at least 50%, 60%, 70%, 80%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and even 100% for at least 2, 4, 6, 8, 12, 28, 24, 30, 36, 48, 60, 72, 84, or 96 hours, or 5, 10, 15, 30, 45, 60, 90, 120, 150, or 180 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or more when measured *in vivo* or in an *in vitro* assay.

[000353] The MM inhibits the binding of the AB to the target. The MM binds the antigen binding domain of the AB and inhibits binding of the AB to the target. The MM can sterically inhibit the binding of the AB to the target. The MM can allosterically inhibit the binding of the AB to its target. In these embodiments when the AB is modified or coupled to a MM and in the presence of target there is no binding or substantially no binding of the AB to the target, or no more than 0.001%, 0.01%, 0.1%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, or 50% binding of the AB to the target, as compared to the binding of the AB not modified with an MM, the parental AB, or the AB not coupled to an MM to the target, for at least 2, 4, 6, 8, 12, 28, 24, 30, 36, 48, 60, 72, 84, or 96 hours, or 5, 10, 15, 30, 45, 60, 90, 120, 150, or 180 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or longer when measured *in vivo* or in an *in vitro* assay.

[000354] When an AB is coupled to or modified by a MM, the MM 'masks' or reduces or otherwise inhibits the specific binding of the AB to the target. When an AB is coupled to or modified by a MM, such coupling or modification can effect a structural change that reduces or inhibits the ability of the AB to specifically bind its target.

[000355] An AB coupled to or modified with an MM can be represented by the following formulae (in order from an amino (N) terminal region to carboxyl (C) terminal region:

(MM)-(AB)

(AB)-(MM)

(MM)-L-(AB)

(AB)-L-(MM)

where MM is a masking moiety, the AB is an antibody or antibody fragment thereof, and the L is a linker. In many embodiments, it may be desirable to insert one or more linkers, *e.g.*, flexible linkers, into the composition so as to provide for flexibility.

[000356] In certain embodiments, the MM is not a natural binding partner of the AB. In some embodiments, the MM contains no or substantially no homology to any natural binding partner of the AB. In some embodiments, the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% similar to any

natural binding partner of the AB. In some embodiments, the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 25% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 50% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 20% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 10% identical to any natural binding partner of the AB.

[000357] In some embodiments, the activatable antibodies include an AB that is modified by an MM and also includes one or more cleavable moieties (CM). Such activatable antibodies exhibit activatable/switchable binding, to the AB's target. Activatable antibodies generally include an antibody or antibody fragment (AB), modified by or coupled to a masking moiety (MM) and a modifiable or cleavable moiety (CM). In some embodiments, the CM contains an amino acid sequence that serves as a substrate for at least one protease.

[000358] The elements of the activatable antibodies are arranged so that the MM and CM are positioned such that in a cleaved (or relatively active) state and in the presence of a target, the AB binds a target while the activatable antibody is in an uncleaved (or relatively inactive) state in the presence of the target, specific binding of the AB to its target is reduced or inhibited. The specific binding of the AB to its target can be reduced due to the inhibition or masking of the AB's ability to specifically bind its target by the MM.

[000359] The K_d of the AB modified with a MM and a CM towards the target is at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater, or between 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times greater than the K_d of the AB not modified with an MM and a CM or of the parental AB towards the target. Conversely, the binding affinity of the AB modified with a MM and a CM towards the target is at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater, or between 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-

1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times lower than the binding affinity of the AB not modified with an MM and a CM or of the parental AB towards the target.

[000360] When the AB is modified with a MM and a CM and is in the presence of the target but not in the presence of a modifying agent (for example at least one protease), specific binding of the AB to its target is reduced or inhibited, as compared to the specific binding of the AB not modified with an MM and a CM or of the parental AB to the target. When compared to the binding of the parental AB or the binding of an AB not modified with an MM and a CM to its target, the AB's ability to bind the target when modified with an MM and a CM can be reduced by at least 50%, 60%, 70%, 80%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and even 100% for at least 2, 4, 6, 8, 12, 28, 24, 30, 36, 48, 60, 72, 84, or 96 hours or 5, 10, 15, 30, 45, 60, 90, 120, 150, or 180 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or longer when measured *in vivo* or in an *in vitro* assay.

[000361] As used herein, the term cleaved state refers to the condition of the activatable antibodies following modification of the CM by at least one protease. The term uncleaved state, as used herein, refers to the condition of the activatable antibodies in the absence of cleavage of the CM by a protease. As discussed above, the term "activatable antibodies" is used herein to refer to an activatable antibody in both its uncleaved (native) state, as well as in its cleaved state. It will be apparent to the ordinarily skilled artisan that in some embodiments a cleaved activatable antibody may lack an MM due to cleavage of the CM by protease, resulting in release of at least the MM (*e.g.*, where the MM is not joined to the activatable antibodies by a covalent bond (*e.g.*, a disulfide bond between cysteine residues).

[000362] By activatable or switchable is meant that the activatable antibody exhibits a first level of binding to a target when the activatable antibody is in a inhibited, masked or uncleaved state (*i.e.*, a first conformation), and a second level of binding to the target in the uninhibited, unmasked and/or cleaved state (*i.e.*, a second conformation), where the second level of target binding is greater than the first level of binding. In general, the access of target to the AB of the activatable antibody is greater in the presence of a cleaving agent capable of cleaving the CM, i.e., a protease, than in the absence of such a cleaving agent. Thus, when the activatable antibody is in the uncleaved state, the AB is inhibited from target binding and can be masked from target binding (*i.e.*, the first conformation is such the

AB cannot bind the target), and in the cleaved state the AB is not inhibited or is unmasked to target binding.

[000363] The CM and AB of the activatable antibodies are selected so that the AB represents a binding moiety for a given target, and the CM represents a substrate for a protease. In some embodiments, the protease is co-localized with the target at a treatment site or diagnostic site in a subject. As used herein, co-localized refers to being at the same site or relatively close nearby. In some embodiments, a protease cleaves a CM yielding an activated antibody that binds to a target located nearby the cleavage site. The activatable antibodies disclosed herein find particular use where, for example, a protease capable of cleaving a site in the CM, i.e., a protease, is present at relatively higher levels in or in close proximity to target-containing tissue of a treatment site or diagnostic site than in tissue of non-treatment sites (for example in healthy tissue). In some embodiments, a CM of the disclosure is also cleaved by one or more other proteases. In some embodiments, it is the one or more other proteases that is co-localized with the target and that is responsible for cleavage of the CM *in vivo*.

[000364] In some embodiments activatable antibodies provide for reduced toxicity and/or adverse side effects that could otherwise result from binding of the AB at non-treatment sites if the AB were not masked or otherwise inhibited from binding to the target.

[000365] In general, an activatable antibody can be designed by selecting an AB of interest and constructing the remainder of the activatable antibody so that, when conformationally constrained, the MM provides for masking of the AB or reduction of binding of the AB to its target. Structural design criteria can be to be taken into account to provide for this functional feature.

[000366] Activatable antibodies exhibiting a switchable phenotype of a desired dynamic range for target binding in an inhibited versus an uninhibited conformation are provided. Dynamic range generally refers to a ratio of (a) a maximum detected level of a parameter under a first set of conditions to (b) a minimum detected value of that parameter under a second set of conditions. For example, in the context of an activatable antibody, the dynamic range refers to the ratio of (a) a maximum detected level of target protein binding to an activatable antibody in the presence of at least one protease capable of cleaving the CM of the activatable antibodies to (b) a minimum detected level of target protein binding to an activatable antibody in the absence of the protease. The dynamic range of an activatable antibody can be calculated as the ratio of the dissociation constant of an

activatable antibody cleaving agent (*e.g.*, enzyme) treatment to the dissociation constant of the activatable antibodies cleaving agent treatment. The greater the dynamic range of an activatable antibody, the better the switchable phenotype of the activatable antibody. Activatable antibodies having relatively higher dynamic range values (*e.g.*, greater than 1) exhibit more desirable switching phenotypes such that target protein binding by the activatable antibodies occurs to a greater extent (*e.g.*, predominantly occurs) in the presence of a cleaving agent (*e.g.*, enzyme) capable of cleaving the CM of the activatable antibodies than in the absence of a cleaving agent.

[000367] Activatable antibodies can be provided in a variety of structural configurations. Exemplary formulae for activatable antibodies are provided below. It is specifically contemplated that the N- to C-terminal order of the AB, MM and CM may be reversed within an activatable antibody. It is also specifically contemplated that the CM and MM may overlap in amino acid sequence, *e.g.*, such that the CM is contained within the MM.

[000368] For example, activatable antibodies can be represented by the following formula (in order from an amino (N) terminal region to carboxyl (C) terminal region:

(MM)-(CM)-(AB)

(AB)-(CM)-(MM)

where MM is a masking moiety, CM is a cleavable moiety, and AB is an antibody or fragment thereof. It should be noted that although MM and CM are indicated as distinct components in the formulae above, in all exemplary embodiments (including formulae) disclosed herein it is contemplated that the amino acid sequences of the MM and the CM could overlap, *e.g.*, such that the CM is completely or partially contained within the MM. In addition, the formulae above provide for additional amino acid sequences that may be positioned N-terminal or C-terminal to the activatable antibodies elements.

[000369] In certain embodiments, the MM is not a natural binding partner of the AB. In some embodiments, the MM contains no or substantially no homology to any natural binding partner of the AB. In some embodiments, the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% similar to any natural binding partner of the AB. In some embodiments, the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 50% identical to any natural binding partner of the AB. In some embodiments,

the MM is no more than 25% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 20% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 10% identical to any natural binding partner of the AB.

[000370] In many embodiments it may be desirable to insert one or more linkers, *e.g.*, flexible linkers, into the activatable antibody construct so as to provide for flexibility at one or more of the MM-CM junction, the CM-AB junction, or both. For example, the AB, MM, and/or CM may not contain a sufficient number of residues (*e.g.*, Gly, Ser, Asp, Asn, especially Gly and Ser, particularly Gly) to provide the desired flexibility. As such, the switchable phenotype of such activatable antibody constructs may benefit from introduction of one or more amino acids to provide for a flexible linker. In addition, as described below, where the activatable antibody is provided as a conformationally constrained construct, a flexible linker can be operably inserted to facilitate formation and maintenance of a cyclic structure in the uncleaved activatable antibody.

[000371] For example, in certain embodiments an activatable antibody comprises one of the following formulae (where the formula below represent an amino acid sequence in either N- to C-terminal direction or C- to N-terminal direction):

(MM)-L1-(CM)-(AB)

(MM)-(CM)-L2-(AB)

(MM)-L1-(CM)-L2-(AB)

wherein MM, CM, and AB are as defined above; wherein L1 and L2 are each independently and optionally present or absent, are the same or different flexible linkers that include at least 1 flexible amino acid (*e.g.*, Gly). In addition, the formulae above provide for additional amino acid sequences that may be positioned N-terminal or C-terminal to the activatable antibodies elements. Examples include, but are not limited to, targeting moieties (*e.g.*, a ligand for a receptor of a cell present in a target tissue) and serum half-life extending moieties (*e.g.*, polypeptides that bind serum proteins, such as immunoglobulin (*e.g.*, IgG) or serum albumin (*e.g.*, human serum albumin (HAS)).

[000372] The CM is specifically cleaved by at least one protease at a rate of about 0.001-1500 x 10^4 $M^{-1}S^{-1}$ or at least 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 2.5, 5, 7.5, 10, 15, 20, 25, 50, 75, 100, 125, 150, 200, 250, 500, 750, 1000, 1250, or 1500 x 10^4 $M^{-1}S^{-1}$. In some embodiments, the CM is specifically cleaved at a rate of about 100,000 $M^{-1}S^{-1}$. In some

embodiments, the CM is specifically cleaved at a rate from about 1x10E2 to about 1x10E6 $M^{-1}S^{-1}$ (i.e., from about $1x10^2$ to about $1x10^6$ $M^{-1}S^{-1}$).

[000373] For specific cleavage by an enzyme, contact between the enzyme and CM is made. When the activatable antibody comprising an AB coupled to a MM and a CM is in the presence of target and sufficient enzyme activity, the CM can be cleaved. Sufficient enzyme activity can refer to the ability of the enzyme to make contact with the CM and effect cleavage. It can readily be envisioned that an enzyme may be in the vicinity of the CM but unable to cleave because of other cellular factors or protein modification of the enzyme.

[000374] Linkers suitable for use in compositions described herein are generally ones that provide flexibility of the modified AB or the activatable antibodies to facilitate the inhibition of the binding of the AB to the target. Such linkers are generally referred to as flexible linkers. Suitable linkers can be readily selected and can be of any of a suitable of different lengths, such as from 1 amino acid (*e.g.*, Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length.

[000375] Exemplary flexible linkers include glycine polymers (G)n, glycine-serine polymers (including, for example, (GS)n, (GSGGS)n (SEQ ID NO: 191) and (GGGS)n (SEQ ID NO: 192), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers are relatively unstructured, and therefore may be able to serve as a neutral tether between components. Glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, Rev. Computational Chem. 11173-142 (1992)). Exemplary flexible linkers include, but are not limited to Gly-Gly-Ser-Gly (SEQ ID NO: 193), Gly-Gly-Ser-Gly-Gly (SEQ ID NO: 194), Gly-Ser-Gly-Ser-Gly (SEQ ID NO: 195), Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 196), Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 197), Gly-Ser-Ser-Ser-Gly (SEQ ID NO: 198), and the like. The ordinarily skilled artisan will recognize that design of an activatable antibodies can include linkers that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure to provide for a desired activatable antibodies structure.

[000376] The disclosure also provides compositions and methods that include an activatable anti-PDL1 antibody that includes an antibody or antibody fragment (AB) that specifically binds PDL1, where the AB is coupled to a masking moiety (MM) that decreases the ability of the AB to bind its target. In some embodiments, the activatable anti-PDL1 antibody further includes a cleavable moiety (CM) that is a substrate for a protease. The compositions and methods provided herein enable the attachment of one or more agents to one or more cysteine residues in the AB without compromising the activity (e.g., the masking, activating or binding activity) of the activatable anti-PDL1 antibody. In some embodiments, the compositions and methods provided herein enable the attachment of one or more agents to one or more cysteine residues in the AB without reducing or otherwise disturbing one or more disulfide bonds within the MM. The compositions and methods provided herein produce an activatable anti-PDL1 antibody that is conjugated to one or more agents, *e.g.*, any of a variety of therapeutic, diagnostic and/or prophylactic agents, for example, in some embodiments, without any of the agent(s) being conjugated to the MM of the activatable anti-PDL1 antibody. The compositions and methods provided herein produce conjugated activatable anti-PDL1 antibodies in which the MM retains the ability to effectively and efficiently mask the AB of the activatable antibody in an uncleaved state. The compositions and methods provided herein produce conjugated activatable anti-PDL1 antibodies in which the activatable antibody is still activated, *i.e.*, cleaved, in the presence of a protease that can cleave the CM.

[000377] The activatable anti-PDL1 antibodies have at least one point of conjugation for an agent, but in the methods and compositions provided herein less than all possible points of conjugation are available for conjugation to an agent. In some embodiments, the one or more points of conjugation are sulfur atoms involved in disulfide bonds. In some embodiments, the one or more points of conjugation are sulfur atoms involved in interchain disulfide bonds. In some embodiments, the one or more points of conjugation are sulfur atoms involved in interchain sulfide bonds, but not sulfur atoms involved in intrachain disulfide bonds. In some embodiments, the one or more points of conjugation are sulfur atoms of cysteine or other amino acid residues containing a sulfur atom. Such residues may occur naturally in the antibody structure or may be incorporated into the antibody by site-directed mutagenesis, chemical conversion, or mis-incorporation of non-natural amino acids.

[000378] Also provided are methods of preparing a conjugate of an activatable anti-PDL1 antibody having one or more interchain disulfide bonds in the AB and one or more intrachain disulfide bonds in the MM, and a drug reactive with free thiols is provided. The method generally includes partially reducing interchain disulfide bonds in the activatable antibody with a reducing agent, such as, for example, TCEP; and conjugating the drug reactive with free thiols to the partially reduced activatable antibody. As used herein, the term partial reduction refers to situations where an activatable anti-PDL1 antibody is contacted with a reducing agent and less than all disulfide bonds, *e.g.*, less than all possible sites of conjugation are reduced. In some embodiments, less than 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10% or less than 5% of all possible sites of conjugation are reduced.

[000379] In yet other embodiments, a method of reducing and conjugating an agent, e.g., a drug, to an activatable anti-PDL1 antibody resulting in selectivity in the placement of the agent is provided. The method generally includes partially reducing the activatable anti-PDL1 antibody with a reducing agent such that any conjugation sites in the masking moiety or other non-AB portion of the activatable antibody are not reduced, and conjugating the agent to interchain thiols in the AB. The conjugation site(s) are selected so as to allow desired placement of an agent to allow conjugation to occur at a desired site. The reducing agent is, for example, TCEP. The reduction reaction conditions such as, for example, the ratio of reducing agent to activatable antibody, the length of incubation, the temperature during the incubation, the pH of the reducing reaction solution, etc., are determined by identifying the conditions that produce a conjugated activatable antibody in which the MM retains the ability to effectively and efficiently mask the AB of the activatable antibody in an uncleaved state. The ratio of reduction agent to activatable anti-PDL1 antibody will vary depending on the activatable antibody. In some embodiments, the ratio of reducing agent to activatable anti-PDL1 antibody will be in a range from about 20:1 to 1:1, from about 10:1 to 1:1, from about 9:1 to 1:1, from about 8:1 to 1:1, from about 7:1 to 1:1, from about 6:1 to 1:1, from about 5:1 to 1:1, from about 4:1 to 1:1, from about 3:1 to 1:1, from about 2:1 to 1:1, from about 20:1 to 1:1.5, from about 10:1 to 1:1.5, from about 9:1 to 1:1.5, from about 8:1 to 1:1.5, from about 7:1 to 1:1.5, from about 6:1 to 1:1.5, from about 5:1 to 1:1.5, from about 4:1 to 1:1.5, from about 3:1 to 1:1.5, from about 2:1 to 1:1.5, from about 1.5:1 to 1:1.5, or from about 1:1 to 1:1.5. In some embodiments, the ratio is in a range of from about 5:1 to 1:1. In some embodiments, the ratio is in a range of from about 5:1 to 1.5:1. In some

embodiments, the ratio is in a range of from about 4:1 to 1:1. In some embodiments, the ratio is in a range from about 4:1 to 1.5:1. In some embodiments, the ratio is in a range from about 8:1 to about 1:1. In some embodiments, the ratio is in a range of from about 2.5:1 to 1:1.

[000380] In some embodiments, a method of reducing interchain disulfide bonds in the AB of an activatable anti-PDL1 antibody and conjugating an agent, e.g., a thiol-containing agent such as a drug, to the resulting interchain thiols to selectively locate agent(s) on the AB is provided. The method generally includes partially reducing the AB with a reducing agent to form at least two interchain thiols without forming all possible interchain thiols in the activatable antibody; and conjugating the agent to the interchain thiols of the partially reduced AB. For example, the AB of the activatable antibody is partially reduced for about 1 hour at about 37°C at a desired ratio of reducing agent:activatable antibody. In some embodiments, the ratio of reducing agent to activatable antibody will be in a range from about 20:1 to 1:1, from about 10:1 to 1:1, from about 9:1 to 1:1, from about 8:1 to 1:1, from about 7:1 to 1:1, from about 6:1 to 1:1, from about 5:1 to 1:1, from about 4:1 to 1:1, from about 3:1 to 1:1, from about 2:1 to 1:1, from about 20:1 to 1:1.5, from about 10:1 to 1:1.5, from about 9:1 to 1:1.5, from about 8:1 to 1:1.5, from about 7:1 to 1:1.5, from about 6:1 to 1:1.5, from about 5:1 to 1:1.5, from about 4:1 to 1:1.5, from about 3:1 to 1:1.5, from about 2:1 to 1:1.5, from about 1.5:1 to 1:1.5, or from about 1:1 to 1:1.5. In some embodiments, the ratio is in a range of from about 5:1 to 1:1. In some embodiments, the ratio is in a range of from about 5:1 to 1.5:1. In some embodiments, the ratio is in a range of from about 4:1 to 1:1. In some embodiments, the ratio is in a range from about 4:1 to 1.5:1. In some embodiments, the ratio is in a range from about 8:1 to about 1:1. In some embodiments, the ratio is in a range of from about 2.5:1 to 1:1.

[000381] The thiol-containing reagent can be, for example, cysteine or N-acetyl cysteine. The reducing agent can be, for example, TCEP. In some embodiments, the reduced activatable antibody can be purified prior to conjugation, using for example, column chromatography, dialysis, or diafiltration. Alternatively, the reduced antibody is not purified after partial reduction and prior to conjugation.

[000382] The invention also provides partially reduced activatable anti-PDL1 antibodies in which at least one interchain disulfide bond in the activatable antibody has been reduced with a reducing agent without disturbing any intrachain disulfide bonds in the activatable antibody, wherein the activatable antibody includes an antibody or an antigen

binding fragment thereof (AB) that specifically binds to PDL1, a masking moiety (MM) that inhibits the binding of the AB of the activatable antibody in an uncleaved state to the PDL1 target, and a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease. In some embodiments the MM is coupled to the AB via the CM. In some embodiments, one or more intrachain disulfide bond(s) of the activatable antibody is not disturbed by the reducing agent. In some embodiments, one or more intrachain disulfide bond(s) of the MM within the activatable antibody is not disturbed by the reducing agent. In some embodiments, the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM. In some embodiments, reducing agent is TCEP.

[000383] The disclosure also provides partially reduced activatable antibodies in which at least one interchain disulfide bond in the activatable antibody has been reduced with a reducing agent without disturbing any intrachain disulfide bonds in the activatable antibody, wherein the activatable antibody includes an antibody or an antigen binding fragment thereof (AB) that specifically binds to the target, e.g., PDL1, a masking moiety (MM) that inhibits the binding of the AB of the activatable antibody in an uncleaved state to the target, and a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for at least one protease. In some embodiments, the MM is coupled to the AB via the CM. In some embodiments, one or more intrachain disulfide bond(s) of the activatable antibody is not disturbed by the reducing agent. In some embodiments, one or more intrachain disulfide bond(s) of the MM within the activatable antibody is not disturbed by the reducing agent. In some embodiments, the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM. In some embodiments, reducing agent is TCEP.

[000384] In some embodiments, the activatable antibodies described herein also include an agent conjugated to the activatable antibody. In some embodiments, the conjugated agent is a therapeutic agent, such as an anti-inflammatory and/or an antineoplastic agent. In such embodiments, the agent is conjugated to a carbohydrate moiety of the activatable antibody, for example, in some embodiments, where the carbohydrate moiety is located outside the antigen-binding region of the antibody or antigen-binding

fragment in the activatable antibody. In some embodiments, the agent is conjugated to a sulfhydryl group of the antibody or antigen-binding fragment in the activatable antibody.

[000385] In some embodiments, the agent is a cytotoxic agent such as a toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

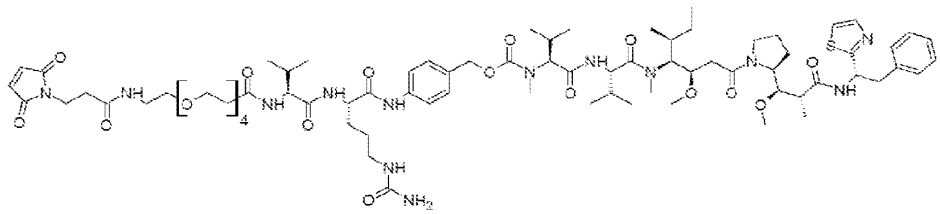
[000386] In some embodiments, the agent is a detectable moiety such as, for example, a label or other marker. For example, the agent is or includes a radiolabeled amino acid, one or more biotinyl moieties that can be detected by marked avidin (*e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods), one or more radioisotopes or radionuclides, one or more fluorescent labels, one or more enzymatic labels, and/or one or more chemiluminescent agents. In some embodiments, detectable moieties are attached by spacer molecules.

[000387] The disclosure also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate). Suitable cytotoxic agents include, for example, dolastatins and derivatives thereof (*e.g.* auristatin E, AFP, MMAF, MMAE, MMAD, DMAF, DMAE). For example, the agent is monomethyl auristatin E (MMAE) or monomethyl auristatin D (MMAD). In some embodiments, the agent is an agent selected from the group listed in Table 11. In some embodiments, the agent is a dolastatin. In some embodiments, the agent is an auristatin or derivative thereof. In some embodiments, the agent is auristatin E or a derivative thereof. In some embodiments, the agent is monomethyl auristatin E (MMAE). In some embodiments, the agent is monomethyl auristatin D (MMAD). In some embodiments, the agent is a maytansinoid or maytansinoid derivative. In some embodiments, the agent is DM1 or DM4. In some embodiments, the agent is a duocarmycin or derivative thereof. In some embodiments, the agent is a calicheamicin or derivative thereof. In some embodiments, the agent is a pyrrolobenzodiazepine. In some embodiments, the agent is a pyrrolobenzodiazepine dimer.

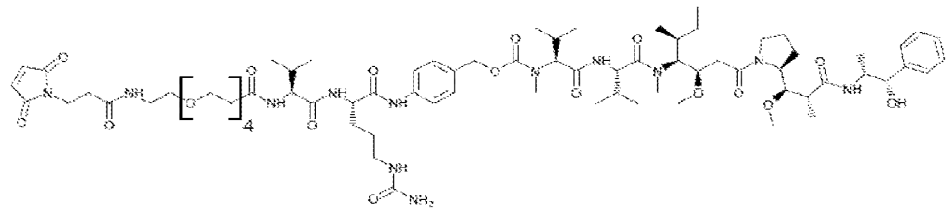
[000388] In some embodiments, the agent is linked to the AB using a maleimide caproyl-valine-citrulline linker or a maleimide PEG-valine-citrulline linker. In some embodiments, the agent is linked to the AB using a maleimide caproyl-valine-citrulline linker. In some embodiments, the agent is linked to the AB using a maleimide PEG-valine-citrulline linker In some embodiments, the agent is monomethyl auristatin D (MMAD)

linked to the AB using a maleimide PEG-valine-citrulline-para-aminobenzyloxycarbonyl linker, and this linker payload construct is referred to herein as "vc-MMAD." In some embodiments, the agent is monomethyl auristatin E (MMAE) linked to the AB using a maleimide PEG-valine-citrulline-para-aminobenzyloxycarbonyl linker, and this linker payload construct is referred to herein as "vc-MMAE." The structures of vc-MMAD and vc-MMAE are shown below:

vc-MMAD:



vc-MMAE:



[000389] The disclosure also provides conjugated activatable antibodies that include an activatable antibody linked to monomethyl auristatin D (MMAD) payload, wherein the activatable antibody includes an antibody or an antigen binding fragment thereof (AB) that specifically binds to a target, a masking moiety (MM) that inhibits the binding of the AB of the activatable antibody in an uncleaved state to the target, and cleavable moiety (CM) coupled to the AB, and the CM is a polypeptide that functions as a substrate for at least one MMP protease.

[000390] In some embodiments, the MMAD-conjugated activatable antibody can be conjugated using any of several methods for attaching agents to ABs: (a) attachment to the carbohydrate moieties of the AB, or (b) attachment to sulfhydryl groups of the AB, or (c) attachment to amino groups of the AB, or (d) attachment to carboxylate groups of the AB.

[000391] In some embodiments, the MMAD payload is conjugated to the AB via a linker. In some embodiments, the MMAD payload is conjugated to a cysteine in the AB via

a linker. In some embodiments, the MMAD payload is conjugated to a lysine in the AB via a linker. In some embodiments, the MMAD payload is conjugated to another residue of the AB via a linker, such as those residues disclosed herein. In some embodiments, the linker is a thiol-containing linker. In some embodiments, the linker is a cleavable linker. In some embodiments, the linker is a non-cleavable linker. In some embodiments, the linker is selected from the group consisting of the linkers shown in Tables 13 and 14. In some embodiments, the activatable antibody and the MMAD payload are linked via a maleimide caproyl-valine-citrulline linker. In some embodiments, the activatable antibody and the MMAD payload are linked via a maleimide PEG-valine-citrulline linker. In some embodiments, the activatable antibody and the MMAD payload are linked via a maleimide caproyl-valine-citrulline-para-aminobenzyloxycarbonyl linker. In some embodiments, the activatable antibody and the MMAD payload are linked via a maleimide PEG-valine-citrulline-para-aminobenzyloxycarbonyl linker. In some embodiments, the MMAD payload is conjugated to the AB using the partial reduction and conjugation technology disclosed herein.

[000392] In some embodiments, the polyethylene glycol (PEG) component of a linker of the present disclosure is formed from 2 ethylene glycol monomers, 3 ethylene glycol monomers, 4 ethylene glycol monomers, 5 ethylene glycol monomers, 6 ethylene glycol monomers, 7 ethylene glycol monomers 8 ethylene glycol monomers, 9 ethylene glycol monomers, or at least 10 ethylene glycol monomers. In some embodiments of the present disclosure, the PEG component is a branched polymer. In some embodiments of the present disclosure, the PEG component is an unbranched polymer. In some embodiments, the PEG polymer component is functionalized with an amino group or derivative thereof, a carboxyl group or derivative thereof, or both an amino group or derivative thereof and a carboxyl group or derivative thereof.

[000393] In some embodiments, the PEG component of a linker of the present disclosure is an amino-tetra-ethylene glycol-carboxyl group or derivative thereof. In some embodiments, the PEG component of a linker of the present disclosure is an amino-tri-ethylene glycol-carboxyl group or derivative thereof. In some embodiments, the PEG component of a linker of the present disclosure is an amino-di-ethylene glycol-carboxyl group or derivative thereof. In some embodiments, an amino derivative is the formation of an amide bond between the amino group and a carboxyl group to which it is conjugated. In some embodiments, a carboxyl derivative is the formation of an amide bond between the

carboxyl group and an amino group to which it is conjugated. In some embodiments, a carboxyl derivative is the formation of an ester bond between the carboxyl group and an hydroxyl group to which it is conjugated.

[000394] Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

[000395] Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. (*See* WO94/11026).

[000396] Table 11 lists some of the exemplary pharmaceutical agents that may be employed in the herein described disclosure but in no way is meant to be an exhaustive list.

Table 11: Exemplary Pharmaceutical Agents for Conjugation

CYTOTOXIC AGENTS	
Auristatins	Turbostatin
Auristatin E	Phenstatins
Monomethyl auristatin D (MMAD)	Hydroxyphenstatin
Monomethyl auristatin E (MMAE)	Spongistatin 5
Desmethyl auristatin E (DMAE)	Spongistatin 7
Auristatin F	Halistatin 1
Monomethyl auristatin F (MMAF)	Halistatin 2
Desmethyl auristatin F (DMAF)	Halistatin 3
Auristatin derivatives, *e.g.*, amides thereof	Modified Bryostatins
Auristatin tyramine	Halocomstatins

Auristatin quinoline
Dolastatins
Dolastatin derivatives
Dolastatin 16 DmJ
Dolastatin 16 Dpv
Maytansinoids, *e.g.* DM-1; DM-4
Maytansinoid derivatives
Duocarmycin
Duocarmycin derivatives
Alpha-amanitin
Anthracyclines
Doxorubicin

Daunorubicin
Bryostatins
Camptothecin
Camptothecin derivatives
7-substituted Camptothecin
10, 11-
Difluoromethylenedioxycamptothecin
Combretastatins
Debromoaplysiatoxin

Kahalalide-F
Discodermolide
Ecteinascidins

ANTIVIRALS

Acyclovir
Vira A
Symmetrel

ANTIFUNGALS
Nystatin

ADDITIONAL ANTI-NEOPLASTICS
Adriamycin
Cerubidine
Bleomycin
Alkeran
Velban
Oncovin
Fluorouracil
Methotrexate
Thiotepa
Bisantrene
Novantrone
Thioguanine
Procarabizine

Pyrrolobenzimidazoles (PBI)
Cibrostatin6
Doxaliform
Anthracyclins analogues

Cemadotin analogue (CemCH2-SH)
Pseudomonas toxin A (PE38) variant
Pseudomonas toxin A (ZZ-PE38) variant
ZJ-101
OSW-1
4-Nitrobenzyloxycarbonyl Derivatives of
O6-Benzylguanine
Topoisomerase inhibitors
Hemiasterlin
Cephalotaxine
Homoharringtonine
Pyrrolobenzodiazepine dimers (PBDs)
Pyrrolobenzodiazepenes

Functionalized pyrrolobenzodiazepenes
Functionalized pyrrolobenzodiazepene
dimers
Calicheamicins
Podophyllotoxins
Taxanes
Vinca alkaloids

CONJUGATABLE DETECTION REAGENTS
Fluorescein and derivatives thereof
Fluorescein isothiocyanate (FITC)

RADIOPHARMACEUTICALS
^{125}I
^{131}I
^{89}Zr
^{111}In
^{123}I
^{131}I
^{99}mTc
^{201}Tl
^{133}Xe
^{11}C
^{62}Cu
^{18}F
^{68}Ga
^{13}N
^{15}O
^{38}K

Cytarabine

ANTI-BACTERIALS
Aminoglycosides
Streptomycin
Neomycin
Kanamycin
Amikacin
Gentamicin
Tobramycin
Streptomycin B
Spectinomycin
Ampicillin
Sulfanilamide
Polymyxin
Chloramphenicol

^{82}Rb
^{99}mTc (Technetium)

HEAVY METALS
Barium
Gold
Platinum

ANTI-MYCOPLASMALS
Tylosine
Spectinomycin

[000397] Those of ordinary skill in the art will recognize that a large variety of possible moieties can be coupled to the resultant antibodies of the disclosure. (*See, for example*, "Conjugate Vaccines", Contributions to Microbiology and Immunology, J. M. Cruse and R. E. Lewis, Jr (eds), Carger Press, New York, (1989), the entire contents of which are incorporated herein by reference).

[000398] Coupling may be accomplished by any chemical reaction that will bind the two molecules so long as the antibody and the other moiety retain their respective activities. This linkage can include many chemical mechanisms, for instance covalent binding, affinity binding, intercalation, coordinate binding and complexation. In some embodiments, the binding is, however, covalent binding. Covalent binding can be achieved either by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent linking agents are useful in coupling protein molecules, such as the antibodies of the present disclosure, to other molecules. For example, representative coupling agents can include organic compounds such as thioesters, carbodiimides, succinimide esters, diisocyanates, glutaraldehyde, diazobenzenes and hexamethylene diamines. This listing is not intended to be exhaustive of the various classes of coupling agents known in the art but, rather, is exemplary of the more common coupling agents. (*See* Killen and Lindstrom, Jour. Immun. 133:1335-2549 (1984); Jansen et al., Immunological Reviews 62:185-216 (1982); and Vitetta et al., Science 238:1098 (1987).

[000399] In some embodiments, in addition to the compositions and methods provided herein, the conjugated activatable antibody can also be modified for site-specific conjugation through modified amino acid sequences inserted or otherwise included in the activatable antibody sequence. These modified amino acid sequences are designed to allow for controlled placement and/or dosage of the conjugated agent within a conjugated activatable antibody. For example, the activatable antibody can be engineered to include cysteine substitutions at positions on light and heavy chains that provide reactive thiol groups and do not negatively impact protein folding and assembly, nor alter antigen binding. In some embodiments, the activatable antibody can be engineered to include or otherwise introduce one or more non-natural amino acid residues within the activatable antibody to provide suitable sites for conjugation. In some embodiments, the activatable antibody can be engineered to include or otherwise introduce enzymatically activatable peptide sequences within the activatable antibody sequence.

[000400] Suitable linkers are described in the literature. (*See, for example*, Ramakrishnan, S. et al., Cancer Res. 44:201-208 (1984) describing use of MBS (M-maleimidobenzoyl-N-hydroxysuccinimide ester). *See also*, U.S. Patent No. 5,030,719, describing use of halogenated acetyl hydrazide derivative coupled to an antibody by way of an oligopeptide linker. In some embodiments, suitable linkers include: (i) EDC (1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride; (ii) SMPT (4-succinimidyloxycarbonyl-alpha-methyl-alpha-(2-pridyl-dithio)-toluene (Pierce Chem. Co., Cat. (21558G); (iii) SPDP (succinimidyl-6 [3-(2-pyridyldithio) propionamido]hexanoate (Pierce Chem. Co., Cat #21651G); (iv) Sulfo-LC-SPDP (sulfosuccinimidyl 6 [3-(2-pyridyldithio)-propianamide] hexanoate (Pierce Chem. Co. Cat. #2165-G); and (v) sulfo-NHS (N-hydroxysulfo-succinimide: Pierce Chem. Co., Cat. #24510) conjugated to EDC. Additional linkers include, but are not limited to, SMCC, sulfo-SMCC, SPDB, or sulfo-SPDB.

[000401] The linkers described above contain components that have different attributes, thus leading to conjugates with differing physio-chemical properties. For example, sulfo-NHS esters of alkyl carboxylates are more stable than sulfo-NHS esters of aromatic carboxylates. NHS-ester containing linkers are less soluble than sulfo-NHS esters. Further, the linker SMPT contains a sterically hindered disulfide bond, and can form conjugates with increased stability. Disulfide linkages, are in general, less stable than other linkages because the disulfide linkage is cleaved *in vitro*, resulting in less conjugate

available. Sulfo-NHS, in particular, can enhance the stability of carbodimide couplings. Carbodimide couplings (such as EDC) when used in conjunction with sulfo-NHS, forms esters that are more resistant to hydrolysis than the carbodimide coupling reaction alone.

[000402] In some embodiments, the linkers are cleavable. In some embodiments, the linkers are non-cleavable. In some embodiments, two or more linkers are present. The two or more linkers are all the same, *i.e.*, cleavable or non-cleavable, or the two or more linkers are different, *i.e.*, at least one cleavable and at least one non-cleavable.

[000403] The present disclosure utilizes several methods for attaching agents to ABs: (a) attachment to the carbohydrate moieties of the AB, or (b) attachment to sulfhydryl groups of the AB, or (c) attachment to amino groups of the AB, or (d) attachment to carboxylate groups of the AB. According to the disclosure, ABs may be covalently attached to an agent through an intermediate linker having at least two reactive groups, one to react with AB and one to react with the agent. The linker, which may include any compatible organic compound, can be chosen such that the reaction with AB (or agent) does not adversely affect AB reactivity and selectivity. Furthermore, the attachment of linker to agent might not destroy the activity of the agent. Suitable linkers for reaction with oxidized antibodies or oxidized antibody fragments include those containing an amine selected from the group consisting of primary amine, secondary amine, hydrazine, hydrazide, hydroxylamine, phenylhydrazine, semicarbazide and thiosemicarbazide groups. Such reactive functional groups may exist as part of the structure of the linker, or may be introduced by suitable chemical modification of linkers not containing such groups.

[000404] According to the present disclosure, suitable linkers for attachment to reduced ABs include those having certain reactive groups capable of reaction with a sulfhydryl group of a reduced antibody or fragment. Such reactive groups include, but are not limited to: reactive haloalkyl groups (including, for example, haloacetyl groups), p-mercuribenzoate groups and groups capable of Michael-type addition reactions (including, for example, maleimides and groups of the type described by Mitra and Lawton, 1979, J. Amer. Chem. Soc. 101: 3097-3110).

[000405] According to the present disclosure, suitable linkers for attachment to neither oxidized nor reduced Abs include those having certain functional groups capable of reaction with the primary amino groups present in unmodified lysine residues in the Ab. Such reactive groups include, but are not limited to, NHS carboxylic or carbonic esters, sulfo-NHS carboxylic or carbonic esters, 4-nitrophenyl carboxylic or carbonic esters,

pentafluorophenyl carboxylic or carbonic esters, acyl imidazoles, isocyanates, and isothiocyanates.

[000406] According to the present disclosure, suitable linkers for attachment to neither oxidized nor reduced Abs include those having certain functional groups capable of reaction with the carboxylic acid groups present in aspartate or glutamate residues in the Ab, which have been activated with suitable reagents. Suitable activating reagents include EDC, with or without added NHS or sulfo-NHS, and other dehydrating agents utilized for carboxamide formation. In these instances, the functional groups present in the suitable linkers would include primary and secondary amines, hydrazines, hydroxylamines, and hydrazides.

[000407] The agent may be attached to the linker before or after the linker is attached to the AB. In certain applications it may be desirable to first produce an AB-linker intermediate in which the linker is free of an associated agent. Depending upon the particular application, a specific agent may then be covalently attached to the linker. In some embodiments, the AB is first attached to the MM, CM and associated linkers and then attached to the linker for conjugation purposes.

[000408] *Branched Linkers*: In specific embodiments, branched linkers that have multiple sites for attachment of agents are utilized. For multiple site linkers, a single covalent attachment to an AB would result in an AB-linker intermediate capable of binding an agent at a number of sites. The sites may be aldehyde or sulfhydryl groups or any chemical site to which agents can be attached.

[000409] In some embodiments, higher specific activity (or higher ratio of agents to AB) can be achieved by attachment of a single site linker at a plurality of sites on the AB. This plurality of sites may be introduced into the AB by either of two methods. First, one may generate multiple aldehyde groups and/or sulfhydryl groups in the same AB. Second, one may attach to an aldehyde or sulfhydryl of the AB a "branched linker" having multiple functional sites for subsequent attachment to linkers. The functional sites of the branched linker or multiple site linker may be aldehyde or sulfhydryl groups, or may be any chemical site to which linkers may be attached. Still higher specific activities may be obtained by combining these two approaches, that is, attaching multiple site linkers at several sites on the AB.

[000410] *Cleavable Linkers*: Peptide linkers that are susceptible to cleavage by enzymes of the complement system, such as but not limited to u-plasminogen activator, tissue plasminogen activator, trypsin, plasmin, or another enzyme having proteolytic

activity may be used in one embodiment of the present disclosure. According to one method of the present disclosure, an agent is attached via a linker susceptible to cleavage by complement. The antibody is selected from a class that can activate complement. The antibody-agent conjugate, thus, activates the complement cascade and releases the agent at the target site. According to another method of the present disclosure, an agent is attached via a linker susceptible to cleavage by enzymes having a proteolytic activity such as a u-plasminogen activator, a tissue plasminogen activator, plasmin, or trypsin. These cleavable linkers are useful in conjugated activatable antibodies that include an extracellular toxin, *e.g.*, by way of non-limiting example, any of the extracellular toxins shown in Table 11.

[000411] Non-limiting examples of cleavable linker sequences are provided in Table 13.

Table 13: Exemplary Linker Sequences for Conjugation

Types of Cleavable Sequences	Amino Acid Sequence
Plasmin cleavable sequences	
Pro-urokinase	PRFKIIGG (SEQ ID NO: 396) PRFRIIGG (SEQ ID NO: 397)
TGFβ	SSRHRRALD (SEQ ID NO: 398)
Plasminogen	RKSSIIIRMRDVVL (SEQ ID NO: 399)
Staphylokinase	SSSFDKGKYKKGDDA (SEQ ID NO: 400) SSSFDKGKYKRGDDA (SEQ ID NO: 401)
Factor Xa cleavable sequences	IEGR (SEQ ID NO: 402) IDGR (SEQ ID NO: 403) GGSIDGR (SEQ ID NO: 404)
MMP cleavable sequences	
Gelatinase A	PLGLWA (SEQ ID NO: 405)
Collagenase cleavable sequences	
Calf skin collagen (α1(I) chain)	GPQGIAGQ (SEQ ID NO: 406)
Calf skin collagen (α2(I) chain)	GPQGLLGA (SEQ ID NO: 407)
Bovine cartilage collagen (α1(II) chain)	GIAGQ (SEQ ID NO: 408)
Human liver collagen (α1(III) chain)	GPLGIAGI (SEQ ID NO: 409)
Human α_2M	GPEGLRVG (SEQ ID NO: 410)
Human PZP	YGAGLGVV (SEQ ID NO: 411) AGLGVVER (SEQ ID NO: 412) AGLGISST (SEQ ID NO: 413)
Rat α_1M	EPQALAMS (SEQ ID NO: 414) QALAMSAI (SEQ ID NO: 415)
Rat α_2M	AAYHLVSQ (SEQ ID NO: 416) MDAFLESS (SEQ ID NO: 417)
Rat α_1I_3(2J)	ESLPVVAV (SEQ ID NO: 418)

Rat α_1I_3(27J)	SAPAVESE (SEQ ID NO: 419)
Human fibroblast collagenase (autolytic cleavages)	DVAQFVLT (SEQ ID NO: 420)
	VAQFVLTE (SEQ ID NO: 421)
	AQFVLTEG (SEQ ID NO: 422)
	PVQPIGPQ (SEQ ID NO: 423)

[000412] In addition, agents may be attached via disulfide bonds (for example, the disulfide bonds on a cysteine molecule) to the AB. Since many tumors naturally release high levels of glutathione (a reducing agent) this can reduce the disulfide bonds with subsequent release of the agent at the site of delivery. In some embodiments, the reducing agent that would modify a CM would also modify the linker of the conjugated activatable antibody.

[000413] *Spacers and Cleavable Elements*: In some embodiments, it may be necessary to construct the linker in such a way as to optimize the spacing between the agent and the AB of the activatable antibody. This may be accomplished by use of a linker of the general structure:

$$W - (CH_2)n - Q$$

wherein

W is either --NH--CH_2-- or --CH_2--;

Q is an amino acid, peptide; and

n is an integer from 0 to 20.

[000414] In some embodiments, the linker may comprise a spacer element and a cleavable element. The spacer element serves to position the cleavable element away from the core of the AB such that the cleavable element is more accessible to the enzyme responsible for cleavage. Certain of the branched linkers described above may serve as spacer elements.

[000415] Throughout this discussion, it should be understood that the attachment of linker to agent (or of spacer element to cleavable element, or cleavable element to agent) need not be particular mode of attachment or reaction. Any reaction providing a product of suitable stability and biological compatibility is acceptable.

[000416] *Serum Complement and Selection of Linkers*: According to one method of the present disclosure, when release of an agent is desired, an AB that is an antibody of a class that can activate complement is used. The resulting conjugate retains both the ability to bind antigen and activate the complement cascade. Thus, according to this embodiment of the present disclosure, an agent is joined to one end of the cleavable linker or cleavable

element and the other end of the linker group is attached to a specific site on the AB. For example, if the agent has an hydroxy group or an amino group, it may be attached to the carboxy terminus of a peptide, amino acid or other suitably chosen linker via an ester or amide bond, respectively. For example, such agents may be attached to the linker peptide via a carbodimide reaction. If the agent contains functional groups that would interfere with attachment to the linker, these interfering functional groups can be blocked before attachment and deblocked once the product conjugate or intermediate is made. The opposite or amino terminus of the linker is then used either directly or after further modification for binding to an AB that is capable of activating complement.

[000417] Linkers (or spacer elements of linkers) may be of any desired length, one end of which can be covalently attached to specific sites on the AB of the activatable antibody. The other end of the linker or spacer element may be attached to an amino acid or peptide linker.

[000418] Thus when these conjugates bind to antigen in the presence of complement the amide or ester bond that attaches the agent to the linker will be cleaved, resulting in release of the agent in its active form. These conjugates, when administered to a subject, will accomplish delivery and release of the agent at the target site, and are particularly effective for the in vivo delivery of pharmaceutical agents, antibiotics, antimetabolites, antiproliferative agents and the like as presented in but not limited to those in Table 11.

[000419] *Linkers for Release without Complement Activation*: In yet another application of targeted delivery, release of the agent without complement activation is desired since activation of the complement cascade will ultimately lyse the target cell. Hence, this approach is useful when delivery and release of the agent should be accomplished without killing the target cell. Such is the goal when delivery of cell mediators such as hormones, enzymes, corticosteroids, neurotransmitters, genes or enzymes to target cells is desired. These conjugates may be prepared by attaching the agent to an AB that is not capable of activating complement via a linker that is mildly susceptible to cleavage by serum proteases. When this conjugate is administered to an individual, antigen-antibody complexes will form quickly whereas cleavage of the agent will occur slowly, thus resulting in release of the compound at the target site.

[000420] *Biochemical Cross Linkers*: In some embodiments, the activatable antibody may be conjugated to one or more therapeutic agents using certain biochemical cross-linkers. Cross-linking reagents form molecular bridges that tie together functional groups of

two different molecules. To link two different proteins in a step-wise manner, hetero-bifunctional cross-linkers can be used that eliminate unwanted homopolymer formation.

[000421] Peptidyl linkers cleavable by lysosomal proteases are also useful, for example, Val-Cit, Val-Ala or other dipeptides. In addition, acid-labile linkers cleavable in the low-pH environment of the lysosome may be used, for example: bis-sialyl ether. Other suitable linkers include cathepsin-labile substrates, particularly those that show optimal function at an acidic pH.

[000422] Exemplary hetero-bifunctional cross-linkers are referenced in Table 14.

Table 14: Exemplary Hetero-Bifunctional Cross Linkers

HETERO-BIFUNCTIONAL CROSS-LINKERS

Linker	Reactive Toward	Advantages and Applications	Spacer Arm Length after cross-linking (Angstroms)
SMPT	Primary amines Sulfhydryls	Greater stability	11.2 Å
SPDP	Primary amines Sulfhydryls	Thiolation Cleavable cross-linking	6.8 Å
LC-SPDP	Primary amines Sulfhydryls	Extended spacer arm	15.6 Å
Sulfo-LC-SPDP	Primary amines Sulfhydryls	Extender spacer arm Water-soluble	15.6 Å
SMCC	Primary amines Sulfhydryls	Stable maleimide reactive group Enzyme-antibody conjugation Hapten-carrier protein conjugation	11.6 Å
Sulfo-SMCC	Primary amines Sulfhydryls	Stable maleimide reactive group Water-soluble Enzyme-antibody conjugation	11.6 Å
MBS	Primary amines Sulfhydryls	Enzyme-antibody conjugation Hapten-carrier protein conjugation	9.9 Å
Sulfo-MBS	Primary amines Sulfhydryls	Water-soluble	9.9 Å
SIAB	Primary amines Sulfhydryls	Enzyme-antibody conjugation	10.6 Å
Sulfo-SIAB	Primary amines Sulfhydryls	Water-soluble	10.6 Å
SMPB	Primary amines Sulfhydryls	Extended spacer arm Enzyme-antibody conjugation	14.5 Å
Sulfo-SMPB	Primary amines Sulfhydryls	Extended spacer arm Water-soluble	14.5 Å

EDE/Sulfo-NHS	Primary amines Carboxyl groups	Hapten-Carrier conjugation	0
ABH	Carbohydrates Nonselective	Reacts with sugar groups	11.9 Å

[000423] *Non-Cleavable Linkers or Direct Attachment*: In some embodiments of the disclosure, the conjugate may be designed so that the agent is delivered to the target but not released. This may be accomplished by attaching an agent to an AB either directly or via a non-cleavable linker.

[000424] These non-cleavable linkers may include amino acids, peptides, D-amino acids or other organic compounds that may be modified to include functional groups that can subsequently be utilized in attachment to ABs by the methods described herein. A-general formula for such an organic linker could be

$$W - (CH_2)n - Q$$

wherein

W is either --NH--CH_2-- or --CH_2--;

Q is an amino acid, peptide; and

n is an integer from 0 to 20.

[000425] *Non-Cleavable Conjugates*: In some embodiments, a compound may be attached to ABs that do not activate complement. When using ABs that are incapable of complement activation, this attachment may be accomplished using linkers that are susceptible to cleavage by activated complement or using linkers that are not susceptible to cleavage by activated complement.

[000426] The antibodies disclosed herein can also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang et al., Proc. Natl Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

[000427] Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present disclosure can be conjugated to the liposomes as described in Martin et al., J. Biol. Chem., 257: 286-288 (1982) via a disulfide-interchange reaction.

Definitions:

[000428] Unless otherwise defined, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. The term "a" entity or "an" entity refers to one or more of that entity. For example, a compound refers to one or more compounds. As such, the terms "a", "an", "one or more" and "at least one" can be used interchangeably. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well-known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. *See e.g.*, Sambrook *et al.* Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[000429] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[000430] As used herein, the term "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. By "specifically bind" or "immunoreacts with" or "immunospecifically bind" is meant that the antibody reacts with one or more antigenic determinants of the desired antigen and does not react with other polypeptides or binds at much lower affinity ($K_d > 10^{-6}$). Antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, domain antibody, single chain, Fab, and $F(ab')_2$ fragments, scFvs, and an Fab expression library.

[000431] The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. In general, antibody molecules obtained from humans relate to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG_1, IgG_2, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain.

[000432] The term "monoclonal antibody" (mAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

[000433] The term "antigen-binding site" or "binding portion" refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains, referred to as "hypervariable regions," are interposed between more conserved flanking stretches known as "framework regions," or "FRs". Thus, the term "FR" refers to amino acid sequences that are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as "complementarity-determining regions," or "CDRs." The assignment of amino acids to each domain is in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and

1991)), or Chothia & Lesk J. Mol. Biol. 196:901-917 (1987), Chothia *et al.* Nature 342:878-883 (1989).

[000434] As used herein, the term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin, an scFv, or a T-cell receptor. The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. For example, antibodies may be raised against N-terminal or C-terminal peptides of a polypeptide. An antibody is said to specifically bind an antigen when the dissociation constant is ≤ 1 μM; in some embodiments, ≤ 100 nM and in some embodiments, ≤ 10 nM.

[000435] As used herein, the terms "specific binding," "immunological binding," and "immunological binding properties" refer to the non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and geometric parameters that equally influence the rate in both directions. Thus, both the "on rate constant" (K_{on}) and the "off rate constant" (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. (*See* Nature 361:186-87 (1993)). The ratio of K_{off} /K_{on} enables the cancellation of all parameters not related to affinity, and is equal to the dissociation constant K_d. (*See, generally*, Davies et al. (1990) Annual Rev Biochem 59:439-473). An antibody of the present disclosure is said to specifically bind to the target, when the binding constant (K_d) is ≤ 1 μM, in some embodiments ≤ 100 nM, in some embodiments ≤ 10 nM, and in some embodiments ≤ 100 pM to about 1 pM, as measured by assays such as radioligand binding assays or similar assays known to those skilled in the art.

[000436] The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated polynucleotide" (1) is not associated with all or a portion

of a polynucleotide in which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence. Polynucleotides in accordance with the disclosure include the nucleic acid molecules encoding the heavy chain immunoglobulin molecules shown herein, and nucleic acid molecules encoding the light chain immunoglobulin molecules shown herein.

[000437] The term "isolated protein" referred to herein means a protein of cDNA, recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the "isolated protein" (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, *e.g.,* free of murine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

[000438] The term "polypeptide" is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein fragments, and analogs are species of the polypeptide genus. Polypeptides in accordance with the disclosure comprise the heavy chain immunoglobulin molecules shown herein, and the light chain immunoglobulin molecules shown herein, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules with light chain immunoglobulin molecules, such as kappa light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs thereof.

[000439] The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and that has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

[000440] The term "operably linked" as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

[000441] The term "control sequence" as used herein refers to polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism in prokaryotes, such control sequences generally include promoter, ribosomal

binding site, and transcription termination sequence in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. The term "polynucleotide" as referred to herein means nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[000442] The term oligonucleotide referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. In some embodiments, oligonucleotides are 10 to 60 bases in length and in some embodiments, 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, *e.g.*, for probes, although oligonucleotides may be double stranded, *e.g.*, for use in the construction of a gene mutant. Oligonucleotides of the disclosure are either sense or antisense oligonucleotides.

[000443] The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotide linkages such as phosphorothioate, phosphorodithioate, phosphoroselerloate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate, phosphoronmidate, and the like. *See e.g.*, LaPlanche *et al.* Nucl. Acids Res. 14:9081 (1986); Stec *et al.* J. Am. Chem. Soc. 106:6077 (1984), Stein *et al.* Nucl. Acids Res. 16:3209 (1988), Zon *et al.* Anti Cancer Drug Design 6:539 (1991); Zon *et al.* Oligonucleotides and Analogues: A Practical Approach, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec *et al.* U.S. Patent No. 5,151,510; Uhlmann and Peyman Chemical Reviews 90:543 (1990). An oligonucleotide can include a label for detection, if desired.

[000444] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. *See* Immunology - A Synthesis (2nd Edition, E.S. Golub and D.R. Green, Eds., Sinauer Associates, Sunderland, Mass. (1991)). Stereoisomers (*e.g.*, D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α-, α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino

acids may also be suitable components for polypeptides of the present disclosure. Examples of unconventional amino acids include: 4 hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε -N-acetyllysine, O-phosphoserine, N- acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ-N-methylarginine, and other similar amino acids and imino acids (*e.g.*, 4- hydroxyproline). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

[000445] Similarly, unless specified otherwise, the left-hand end of single- stranded polynucleotide sequences is the 5' end the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction sequence regions on the DNA strand having the same sequence as the RNA and that are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences", sequence regions on the DNA strand having the same sequence as the RNA and that are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

[000446] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, in some embodiments, at least 90 percent sequence identity, in some embodiments, at least 95 percent sequence identity, and in some embodiments, at least 99 percent sequence identity.

[000447] In some embodiments, residue positions that are not identical differ by conservative amino acid substitutions.

[000448] As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present disclosure, providing that the variations in the amino acid sequence maintain at least 75%, in some embodiments, at least 80%, 90%, 95%, and in some embodiments, 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families: (1) acidic amino acids are aspartate, glutamate; (2) basic amino acids are lysine, arginine, histidine; (3) non-polar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and (4) uncharged polar amino acids are glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. The hydrophilic amino acids include

arginine, asparagine, aspartate, glutamine, glutamate, histidine, lysine, serine, and threonine. The hydrophobic amino acids include alanine, cysteine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine and valine. Other families of amino acids include (i) serine and threonine, which are the aliphatic-hydroxy family; (ii) asparagine and glutamine, which are the amide containing family; (iii) alanine, valine, leucine and isoleucine, which are the aliphatic family; and (iv) phenylalanine, tryptophan, and tyrosine, which are the aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. Suitable amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. In some embodiments, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie *et al.* Science 253:164 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the disclosure.

[000449] Suitable amino acid substitutions are those that: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (5) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (for example, conservative amino acid substitutions) may be made in the naturally- occurring sequence (for example, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the

parent sequence (*e.g.*, a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et at. Nature 354:105 (1991).

[000450] The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino terminal and/or carboxy-terminal deletion and/or one or more internal deletion(s), but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence deduced, for example, from a full length cDNA sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, in some embodiments, at least 14 amino acids long, in some embodiments, at least 20 amino acids long, usually at least 50 amino acids long, and in some embodiments, at least 70 amino acids long. The term "analog" as used herein refers to polypeptides that are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of a deduced amino acid sequence and that has specific binding to the target, under suitable binding conditions. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally- occurring sequence. Analogs typically are at least 20 amino acids long, in some embodiments, at least 50 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

[000451] The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

[000452] As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, *e.g.*, by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (*e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.*, ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent labels (*e.g.*, FITC, rhodamine, lanthanide phosphors), enzymatic labels (*e.g.*, horseradish

peroxidase, p-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance. The term "pharmaceutical agent or drug" as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient.

[000453] Other chemistry terms herein are used according to conventional usage in the art, as exemplified by The McGraw-Hill Dictionary of Chemical Terms (Parker, S., Ed., McGraw-Hill, San Francisco (1985)).

[000454] As used herein, "substantially pure" means an object species is the predominant species present (*i.e.,* on a molar basis it is more abundant than any other individual species in the composition), and in some embodiments, a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present.

[000455] Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, in some embodiments, more than about 85%, 90%, 95%, and 99%. In some embodiments, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

[000456] The term patient includes human and veterinary subjects.

[000457] Antibodies and/or activatable antibodies of the disclosure specifically bind a given target, e.g., a human target protein such as human PDL1. Also included in the disclosure are antibodies and/or activatable antibodies that bind to the same epitope as the antibodies and/or activatable antibodies described herein. Also included in the disclosure are antibodies and/or antibodies activatable antibodies that compete with an anti-PDL1 antibody and/or an anti-PDL1 activatable antibody described herein for binding to PDL1, e.g., human PDL1. Also included in the disclosure are antibodies and/or antibodies activatable antibodies that cross-compete with an anti-PDL1 antibody and/or an anti-PDL1 activatable antibody described herein for binding to PDL1, e.g., human PDL1.

[000458] Those skilled in the art will recognize that it is possible to determine, without undue experimentation, if a monoclonal antibody (*e.g.*, a murine monoclonal or humanized

antibody) has the same specificity as a monoclonal antibody used in the methods described herein by ascertaining whether the former prevents the latter from binding to the target. If the monoclonal antibody being tested competes with the monoclonal antibody of the disclosure, as shown by a decrease in binding by the monoclonal antibody of the disclosure, then the two monoclonal antibodies bind to the same, or a closely related, epitope. An alternative method for determining whether a monoclonal antibody has the specificity of a monoclonal antibody of the disclosure is to pre-incubate the monoclonal antibody of the disclosure with the target and then add the monoclonal antibody being tested to determine if the monoclonal antibody being tested is inhibited in its ability to bind the target. If the monoclonal antibody being tested is inhibited then, in all likelihood, it has the same, or functionally equivalent, epitopic specificity as the monoclonal antibody of the disclosure.

Multispecific Activatable Antibodies

[000459] The disclosure also provides multispecific anti-PDL1 activatable antibodies. The multispecific activatable antibodies provided herein are multispecific antibodies that recognize PDL1 and at least one or more different antigens or epitopes and that include at least one masking moiety (MM) linked to at least one antigen- or epitope-binding domain of the multispecific antibody such that coupling of the MM reduces the ability of the antigen- or epitope-binding domain to bind its target. In some embodiments, the MM is coupled to the antigen- or epitope-binding domain of the multispecific antibody via a cleavable moiety (CM) that functions as a substrate for at least one protease. The activatable multispecific antibodies provided herein are stable in circulation, activated at intended sites of therapy and/or diagnosis but not in normal, *i.e.*, healthy tissue, and, when activated, exhibit binding to a target that is at least comparable to the corresponding, unmodified multispecific antibody.

[000460] In some embodiments, the multispecific activatable antibodies are designed to engage immune effector cells, also referred to herein as immune-effector cell engaging multispecific activatable antibodies. In some embodiments, the multispecific activatable antibodies are designed to engage leukocytes, also referred to herein as leukocyte engaging multispecific activatable antibodies. In some embodiments, the multispecific activatable antibodies are designed to engage T cells, also referred to herein as T-cell engaging multispecific activatable antibodies. In some embodiments, the multispecific activatable antibodies engage a surface antigen on a leukocyte, such as on a T cell, on a natural killer (NK) cell, on a myeloid mononuclear cell, on a macrophage, and/or on another immune

effector cell. In some embodiments, the immune effector cell is a leukocyte. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is a NK cell. In some embodiments, the immune effector cell is a mononuclear cell, such as a myeloid mononuclear cell. In some embodiments, the multispecific activatable antibodies are designed to bind or otherwise interact with more than one target and/or more than one epitope, also referred to herein as multi-antigen targeting activatable antibodies. As used herein, the terms "target" and "antigen" are used interchangeably.

[000461] In some embodiments, immune effector cell engaging multispecific activatable antibodies of the disclosure include a targeting antibody or antigen-binding fragment thereof that binds PDL1 and an immune effector cell engaging antibody or antigen-binding portion thereof, where at least one of the targeting antibody or antigen-binding fragment thereof and/or the immune effector cell engaging antibody or antigen-binding portion thereof is masked. In some embodiments, the immune effector cell engaging antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds a first, immune effector cell engaging target, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind the first target. In some embodiments, the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds PDL1, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind PDL1. In some embodiments, the immune effector cell engaging antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds a first, immune effector cell engaging target, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind the first target, and the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds PDL1, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind PDL1. In some embodiments, the non-immune effector cell engaging antibody is a cancer targeting antibody. In some embodiments the non-immune cell effector antibody is an IgG. In some embodiments the immune effector cell engaging antibody is a scFv. In some embodiments the PDL1-targeting antibody (e.g., non-immune cell effector antibody) is an IgG and the immune effector cell engaging

antibody is a scFv. In some embodiments, the immune effector cell is a leukocyte. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is a NK cell. In some embodiments, the immune effector cell is a myeloid mononuclear cell.

[000462] In some embodiments, T-cell engaging multispecific activatable antibodies of the disclosure include a PDL1-targeting antibody or antigen-binding fragment thereof and a T-cell engaging antibody or antigen-binding portion thereof, where at least one of the PDL1-targeting antibody or antigen-binding fragment thereof and/or the T-cell engaging antibody or antigen-binding portion thereof is masked. In some embodiments, the T-cell engaging antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds a first, T-cell engaging target, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind the first target. In some embodiments, the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds PDL1, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind PDL1. In some embodiments, the T-cell engaging antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds a first, T-cell engaging target, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind the first target, and the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds PDL1, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind PDL1.

[000463] In some embodiments of an immune effector cell engaging multispecific activatable antibody, one antigen is PDL1, and another antigen is typically a stimulatory or inhibitory receptor present on the surface of a T-cell, natural killer (NK) cell, myeloid mononuclear cell, macrophage, and/or other immune effector cell, such as, but not limited to, B7-H4, BTLA, CD3, CD4, CD8, CD16a, CD25, CD27, CD28, CD32, CD56, CD137, CTLA-4, GITR, HVEM, ICOS, LAG3, NKG2D, OX40, PD1, TIGIT, TIM3, or VISTA. In some embodiments, the antigen is a stimulatory receptor present on the surface of a T cell or NK cell; examples of such stimulatory receptors include, but are not limited to, CD3, CD27, CD28, CD137 (also referred to as 4-1BB), GITR, HVEM, ICOS, NKG2D, and OX40. In

some embodiments, the antigen is an inhibitory receptor present on the surface of a T-cell; examples of such inhibitory receptors include, but are not limited to, BTLA, CTLA-4, LAG3, PD1, TIGIT, TIM3, and NK-expressed KIRs. The antibody domain conferring specificity to the T-cell surface antigen may also be substituted by a ligand or ligand domain that binds to a T-cell receptor, a NK-cell receptor, a macrophage receptor, and/or other immune effector cell receptor, such as, but not limited to, B7-1, B7-2, B7H3, PDL1, PDL2, or TNFSF9.

[000464] In some embodiments, the T-cell engaging multispecific activatable antibody includes an anti-CD3 epsilon (CD3ε, also referred to herein as CD3e and CD3) scFv and a targeting antibody or antigen-binding fragment thereof, where at least one of the anti-CD3ε scFv and/or the targeting antibody or antigen-binding portion thereof is masked. In some embodiments, the CD3ε scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3ε, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3ε. In some embodiments, the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds PDL1, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind PDL1. In some embodiments, the CD3ε scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3ε, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3ε, and the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds PDL1, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind PDL1.

[000465] In some embodiments, the multi-antigen targeting antibodies and/or multi-antigen targeting activatable antibodies include at least a first antibody or antigen-binding fragment thereof that binds a first target and/or first epitope and a second antibody or antigen-binding fragment thereof that binds a second target and/or a second epitope. In some embodiments, the multi-antigen targeting antibodies and/or multi-antigen targeting activatable antibodies bind two or more different targets. In some embodiments, the multi-antigen targeting antibodies and/or multi-antigen targeting activatable antibodies bind two or more different epitopes on the same target. In some embodiments, the multi-antigen

targeting antibodies and/or multi-antigen targeting activatable antibodies bind a combination of two or more different targets and two or more different epitopes on the same target.

[000466] In some embodiments, a multispecific activatable antibody comprising an IgG has the IgG variable domains masked. In some embodiments, a multispecific activatable antibody comprising a scFv has the scFv domains masked. In some embodiments, a multispecific activatable antibody has both IgG variable domains and scFv domains, where at least one of the IgG variable domains is coupled to a masking moiety. In some embodiments, a multispecific activatable antibody has both IgG variable domains and scFv domains, where at least one of the scFv domains is coupled to a masking moiety. In some embodiments, a multispecific activatable antibody has both IgG variable domains and scFv domains, where at least one of the IgG variable domains is coupled to a masking moiety and at least one of the scFv domains is coupled to a masking moiety. In some embodiments, a multispecific activatable antibody has both IgG variable domains and scFv domains, where each of the IgG variable domains and the scFv domains is coupled to its own masking moiety. In some embodiments, one antibody domain of a multispecific activatable antibody has specificity for a target antigen and another antibody domain has specificity for a T-cell surface antigen. In some embodiments, one antibody domain of a multispecific activatable antibody has specificity for a target antigen and another antibody domain has specificity for another target antigen. In some embodiments, one antibody domain of a multispecific activatable antibody has specificity for an epitope of a target antigen and another antibody domain has specificity for another epitope of the target antigen.

[000467] In a multispecific activatable antibody, a scFv can be fused to the carboxyl terminus of the heavy chain of an IgG activatable antibody, to the carboxyl terminus of the light chain of an IgG activatable antibody, or to the carboxyl termini of both the heavy and light chains of an IgG activatable antibody. In a multispecific activatable antibody, a scFv can be fused to the amino terminus of the heavy chain of an IgG activatable antibody, to the amino terminus of the light chain of an IgG activatable antibody, or to the amino termini of both the heavy and light chains of an IgG activatable antibody. In a multispecific activatable antibody, a scFv can be fused to any combination of one or more carboxyl termini and one or more amino termini of an IgG activatable antibody. In some embodiments, a masking moiety (MM) linked to a cleavable moiety (CM) is attached to and masks an antigen

binding domain of the IgG. In some embodiments, a masking moiety (MM) linked to a cleavable moiety (CM) is attached to and masks an antigen binding domain of at least one scFv. In some embodiments, a masking moiety (MM) linked to a cleavable moiety (CM) is attached to and masks an antigen binding domain of an IgG and a masking moiety (MM) linked to a cleavable moiety (CM) is attached to and masks an antigen binding domain of at least one scFv.

[000468] The disclosure provides examples of multispecific activatable antibody structures which include, but are not limited to, the following: $(VL\text{-}CL)_2$:(VH-CH1-CH2-CH3-L4-VH*-L3-VL*-L2-CM-L1-MM)$_2$; $(VL\text{-}CL)_2$:(VH-CH1-CH2-CH3-L4-VL*-L3-VH*-L2-CM-L1-MM)$_2$; (MM-L1-CM-L2-VL-CL)$_2$:(VH-CH1-CH2-CH3-L4-VH*-L3-VL*)$_2$; (MM-L1-CM-L2-VL-CL)$_2$:(VH-CH1-CH2-CH3-L4-VL*-L3-VH*)$_2$; $(VL\text{-}CL)_2$:(MM-L1-CM-L2-VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; $(VL\text{-}CL)_2$:(MM-L1-CM-L2-VH*-L3-VL*-L4-VH-CH1-CH2-CH3)$_2$; (MM-L1-CM-L2-VL-CL)$_2$:(VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; (MM-L1-CM-L2-VL-CL)$_2$:(VH*-L3-VL*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VH*-L3-VL*-L2-CM-L1-MM)$_2$:(VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VL*-L3-VH*-L2-CM-L1-MM)$_2$:(VH-CH1-CH2-CH3)$_2$; (MM-L1-CM-L2-VL*-L3-VH*-L4-VL-CL)$_2$:(VH-CH1-CH2-CH3)$_2$; (MM-L1-CM-L2-VH*-L3-VL*-L4-VL-CL)$_2$:(VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VH*-L3-VL*-L2-CM-L1-MM)$_2$: (MM-L1-CM-L2-VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VH*-L3-VL*-L2-CM-L1-MM)$_2$: (MM-L1-CM-L2-VH*-L3-VL*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VL*-L3-VH*-L2-CM-L1-MM)$_2$: (MM-L1-CM-L2-VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VL*-L3-VH*-L2-CM-L1-MM)$_2$: (MM-L1-CM-L2-VH*-L3-VL*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VH*-L3-VL*)$_2$: (MM-L1-CM-L2-VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VH*-L3-VL*)$_2$: (MM-L1-CM-L2-VH*-L3-VL*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VL*-L3-VH*)$_2$: (MM-L1-CM-L2-VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VL*-L3-VH*)$_2$: (MM-L1-CM-L2-VH*-L3-VL*-L4-VH-CH1-CH2-CH3)2; (VL-CL-L4-VH*-L3-VL*-L2-CM-L1-MM)$_2$: (VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VH*-L3-VL*-L2-CM-L1-MM)$_2$: (VH*-L3-VL*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VL*-L3-VH*-L2-CM-L1-MM)$_2$: (VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; or (VL-CL-L4-VL*-L3-VH*-L2-CM-L1-MM)$_2$: (VH*-L3-VL*-L4-VH-CH1-CH2-CH3)$_2$, wherein: VL and VH represent the light and heavy variable domains of the first specificity, contained in the IgG; VL* and VH* represent the variable domains of the second specificity, contained in the scFv; L1 is a linker peptide connecting the masking moiety

(MM) and the cleavable moiety (CM); L2 is a linker peptide connecting the cleavable moiety (CM), and the antibody; L3 is a linker peptide connecting the variable domains of the scFv; L4 is a linker peptide connecting the antibody of the first specificity to the antibody of the second specificity; CL is the light-chain constant domain; and CH1, CH2, CH3 are the heavy chain constant domains. The first and second specificities may be toward any antigen or epitope.

[000469] In some embodiments of a T-cell engaging multispecific activatable antibody, one antigen is PDL1, and another antigen is typically a stimulatory (also referred to herein as activating) or inhibitory receptor present on the surface of a T-cell, natural killer (NK) cell, myeloid mononuclear cell, macrophage, and/or other immune effector cell, such as, but not limited to, B7-H4, BTLA, CD3, CD4, CD8, CD16a, CD25, CD27, CD28, CD32, CD56, CD137 (also referred to as TNFRSF9), CTLA-4, GITR, HVEM, ICOS, LAG3, NKG2D, OX40, PD1, TIGIT, TIM3, or VISTA. The antibody domain conferring specificity to the T-cell surface antigen may also be substituted by a ligand or ligand domain that binds to a T-cell receptor, a NK-cell receptor, a macrophage receptor, and/or other immune effector cell receptor, such as, but not limited to, PDL1.

[000470] In some embodiments, the targeting antibody is an anti-PDL1 antibody disclosed herein. In some embodiments, the targeting antibody can be in the form an activatable antibody. In some embodiments, the scFv(s) can be in the form of a Pro-scFv (see, e.g., WO 2009/025846, WO 2010/081173).

[000471] In some embodiments, the scFv is specific for binding CD3ε, and comprises or is derived from an antibody or fragment thereof that binds CD3ε, e.g., CH2527, FN18, H2C, OKT3, 2C11, UCHT1, or V9. In some embodiments, the scFv is specific for binding CTLA-4 (also referred to herein as CTLA and CTLA4).

[000472] In some embodiments, the anti-CTLA-4 scFv includes the amino acid sequence:

GGGSGGGGSGSGGGSGGGGSGGGEIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQA
PRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIKRSG
GSTITSYNVYYTKLSSSGTQVQLVQTGGGVVQPGRSLRLSCAASGSTFSSYAMSWVRQAPGKGLEWV
SAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATNSLYWYFDLWGRGTLVT
VSSAS (SEQ ID NO: 424)

[000473] In some embodiments, the anti-CTLA-4 scFv includes the amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 424.

[000474] In some embodiments, the anti-CD3ε scFv includes the amino acid sequence:

GGGSGGGGSGSGGGSGGGGSGGGQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQG
LEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQ
GTTLTVSSGGGGSGGGGSGGGGSQIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPK
RWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINR
(SEQ ID NO: 425)

[000475] In some embodiments, the anti-CD3ε scFv includes the amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 425.

[000476] In some embodiments, the scFv is specific for binding one or more T-cells, one or more NK-cells and/or one or more macrophages. In some embodiments, the scFv is specific for binding a target selected from the group consisting of B7-H4, BTLA, CD3, CD4, CD8, CD16a, CD25, CD27, CD28, CD32, CD56, CD137, CTLA-4, GITR, HVEM, ICOS, LAG3, NKG2D, OX40, PD1, TIGIT, TIM3, or VISTA.

[000477] In some embodiments, the multispecific activatable antibody also includes an agent conjugated to the AB. In some embodiments, the agent is a therapeutic agent. In some embodiments, the agent is an antineoplastic agent. In some embodiments, the agent is a toxin or fragment thereof. In some embodiments, the agent is conjugated to the multispecific activatable antibody via a linker. In some embodiments, the agent is conjugated to the AB via a cleavable linker. In some embodiments, the linker is a non-cleavable linker. In some embodiments, the agent is a microtubule inhibitor. In some embodiments, the agent is a nucleic acid damaging agent, such as a DNA alkylator or DNA intercalator, or other DNA damaging agent. In some embodiments, the linker is a cleavable linker. In some embodiments, the agent is an agent selected from the group listed in Table 11. In some embodiments, the agent is a dolastatin. In some embodiments, the agent is an auristatin or derivative thereof. In some embodiments, the agent is auristatin E or a derivative thereof. In some embodiments, the agent is monomethyl auristatin E (MMAE). In some embodiments, the agent is monomethyl auristatin D (MMAD). In some embodiments, the agent is a maytansinoid or maytansinoid derivative. In some embodiments, the agent is

DM1 or DM4. In some embodiments, the agent is a duocarmycin or derivative thereof. In some embodiments, the agent is a calicheamicin or derivative thereof. In some embodiments, the agent is a pyrrolobenzodiazepine. In some embodiments, the agent is a pyrrolobenzodiazepine dimer.

[000478] In some embodiments, the multispecific activatable antibody also includes a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent.

[000479] In some embodiments, the multispecific activatable antibody naturally contains one or more disulfide bonds. In some embodiments, the multispecific activatable antibody can be engineered to include one or more disulfide bonds.

[000480] The disclosure also provides an isolated nucleic acid molecule encoding a multispecific activatable antibody described herein, as well as vectors that include these isolated nucleic acid sequences. The disclosure provides methods of producing a multispecific activatable antibody by culturing a cell under conditions that lead to expression of the activatable antibody, wherein the cell comprises such a nucleic acid molecule. In some embodiments, the cell comprises such a vector.

[000481] The disclosure also provides a method of manufacturing multispecific activatable antibodies of the disclosure by (a) culturing a cell comprising a nucleic acid construct that encodes the multispecific activatable antibody under conditions that lead to expression of the multispecific activatable, and (b) recovering the multispecific activatable antibody. Suitable AB, MM, and/or CM include any of the AB, MM, and/or CM disclosed herein.

[000482] The disclosure also provides multispecific activatable antibodies and/or multispecific activatable antibody compositions that include at least a first antibody or antigen-binding fragment thereof (AB1) that specifically binds a first target or first epitope and a second antibody or antigen-biding fragment thereof (AB2) that binds a second target or a second epitope, where at least AB1 is coupled or otherwise attached to a masking moiety (MM1), such that coupling of the MM1 reduces the ability of AB1 to bind its target. In some embodiments, the MM1 is coupled to AB1 via a first cleavable moiety (CM1) sequence that includes a substrate for a protease, for example, a protease that is co-localized with the target of AB1 at a treatment site or a diagnostic site in a subject. The multispecific activatable antibodies provided herein are stable in circulation, activated at intended sites of therapy and/or diagnosis but not in normal, *i.e.*, healthy tissue, and, when activated, exhibit binding to the target of AB1 that is at least comparable to the corresponding, unmodified

multispecific antibody. Suitable AB, MM, and/or CM include any of the AB, MM, and/or CM disclosed herein.

[000483] The disclosure also provides compositions and methods that include a multispecific activatable antibody that includes at least a first antibody or antibody fragment (AB1) that specifically binds a target and a second antibody or antibody fragment (AB2), where at least the first AB in the multispecific activatable antibody is coupled to a masking moiety (MM1) that decreases the ability of AB1 to bind its target. In some embodiments, each AB is coupled to a MM that decreases the ability of its corresponding AB to each target. For example, in bispecific activatable antibody embodiments, AB1 is coupled to a first masking moiety (MM1) that decreases the ability of AB1 to bind its target, and AB2 is coupled to a second masking moiety (MM2) that decreases the ability of AB2 to bind its target. In some embodiments, the multispecific activatable antibody comprises more than two AB regions; in such embodiments, AB1 is coupled to a first masking moiety (MM1) that decreases the ability of AB1 to bind its target, AB2 is coupled to a second masking moiety (MM2) that decreases the ability of AB2 to bind its target, AB3 is coupled to a third masking moiety (MM3) that decreases the ability of AB3 to bind its target, and so on for each AB in the multispecific activatable antibody. Suitable AB, MM, and/or CM include any of the AB, MM, and/or CM disclosed herein.

[000484] In some embodiments, the multispecific activatable antibody further includes at least one cleavable moiety (CM) that is a substrate for a protease, where the CM links a MM to an AB. For example, in some embodiments, the multispecific activatable antibody includes at least a first antibody or antibody fragment (AB1) that specifically binds a target and a second antibody or antibody fragment (AB2), where at least the first AB in the multispecific activatable antibody is coupled via a first cleavable moiety (CM1) to a masking moiety (MM1) that decreases the ability of AB1 to bind its target. In some bispecific activatable antibody embodiments, AB1 is coupled via CM1 to MM1, and AB2 is coupled via a second cleavable moiety (CM2) to a second masking moiety (MM2) that decreases the ability of AB2 to bind its target. In some embodiments, the multispecific activatable antibody comprises more than two AB regions; in some of these embodiments, AB1 is coupled via CM1 to MM1, AB2 is coupled via CM2 to MM2, and AB3 is coupled via a third cleavable moiety (CM3) to a third masking moiety (MM3) that decreases the ability of AB3 to bind its target, and so on for each AB in the multispecific activatable

antibody. Suitable AB, MM, and/or CM include any of the AB, MM, and/or CM disclosed herein.

Activatable antibodies Having Non-Binding Steric Moieties or Binding Partners for Non-Binding Steric Moieties

[000485] The disclosure also provides activatable antibodies that include non-binding steric moieties (NB) or binding partners (BP) for non-binding steric moieties, where the BP recruits or otherwise attracts the NB to the activatable antibody. The activatable antibodies provided herein include, for example, an activatable antibody that includes a non-binding steric moiety (NB), a cleavable linker (CL) and antibody or antibody fragment (AB) that binds a target; an activatable antibody that includes a binding partner for a non-binding steric moiety (BP), a CL and an AB; and an activatable antibody that includes a BP to which an NB has been recruited, a CL and an AB that binds the target. Activatable antibodies in which the NB is covalently linked to the CL and AB of the activatable antibody or is associated by interaction with a BP that is covalently linked to the CL and AB of the activatable antibody are referred to herein as "NB-containing activatable antibodies." By activatable or switchable is meant that the activatable antibody exhibits a first level of binding to a target when the activatable antibody is in an inhibited, masked or uncleaved state (*i.e.*, a first conformation), and a second level of binding to the target when the activatable antibody is in an uninhibited, unmasked and/or cleaved state (*i.e.*, a second conformation, *i.e.*, activated antibody), where the second level of target binding is greater than the first level of target binding. The activatable antibody compositions can exhibit increased bioavailability and more favorable biodistribution compared to conventional antibody therapeutics.

[000486] In some embodiments, activatable antibodies provide for reduced toxicity and/or adverse side effects that could otherwise result from binding of the at non-treatment sites and/or non-diagnostic sites if the AB were not masked or otherwise inhibited from binding to such a site.

[000487] Anti-PDL1 activatable antibodies that include a non-binding steric moiety (NB) can be made using the methods set forth in PCT Publication No. WO 2013/192546, the contents of which are hereby incorporated by reference in their entirety.

Use Of Antibodies, Conjugated Antibodies, Activatable Antibodies, and Conjugated Activatable Antibodies

[000488] It will be appreciated that administration of therapeutic entities in accordance with the disclosure will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present disclosure, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. *See* also Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance." Regul. Toxicol Pharmacol. 32(2):210-8 (2000), Wang W. "Lyophilization and development of solid protein pharmaceuticals." Int. J. Pharm. 203(1-2):1-60 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." J Pharm Sci.89(8):967-78 (2000), Powell *et al.* "Compendium of excipients for parenteral formulations" PDA J Pharm Sci Technol. 52:238-311 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[000489] Therapeutic formulations of the disclosure, which include an anti-PDL1 antibody and/or activatable anti-PDL1 antibody, such as by way of non-limiting example, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody, are used to prevent, treat or otherwise ameliorate a disease or disorder associated with aberrant target expression and/or activity. For example, therapeutic formulations of the disclosure, which include an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody, are used to treat or otherwise ameliorate a cancer or other neoplastic condition, inflammation, an inflammatory disorder, and/or an autoimmune disease. In some embodiments, the cancer is a solid tumor or a hematologic malignancy where the target is expressed. In some embodiments, the cancer is a solid tumor

where the target is expressed. In some embodiments, the cancer is a hematologic malignancy where the target is expressed. In some embodiments, the target is expressed on parenchyma (e.g., in cancer, the portion of an organ or tissue that often carries out function(s) of the organ or tissue). In some embodiments, the target is expressed on a cell, tissue, or organ. In some embodiments, the target is expressed on stroma (i.e., the connective supportive framework of a cell, tissue, or organ). In some embodiments, the target is expressed on an osteoblast. In some embodiments, the target is expressed on the endothelium (vasculature). In some embodiments, the target is expressed on a cancer stem cell. In some embodiments, the agent to which the antibody and/or the activatable antibody is conjugated is a microtubule inhibitor. In some embodiments, the agent to which the antibody and/or the activatable antibody is conjugated is a nucleic acid damaging agent.

[000490] Efficaciousness of prevention, amelioration or treatment is determined in association with any known method for diagnosing or treating the disease or disorder associated with target expression and/or activity, such as, for example, aberrant target expression and/or activity. Prolonging the survival of a subject or otherwise delaying the progression of the disease or disorder associated with target expression and/or activity, e.g., aberrant target expression and/or activity, in a subject indicates that the antibody, conjugated antibody, activatable antibody and/or conjugated activatable antibody confers a clinical benefit.

[000491] An antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody can be administered in the form of pharmaceutical compositions. Principles and considerations involved in preparing such compositions, as well as guidance in the choice of components are provided, for example, in Remington : The Science And Practice Of Pharmacy 19th ed. (Alfonso R. Gennaro, et al., editors) Mack Pub. Co., Easton, Pa.: 1995; Drug Absorption Enhancement: Concepts, Possibilities, Limitations, And Trends, Harwood Academic Publishers, Langhorne, Pa., 1994; and Peptide And Protein Drug Delivery (Advances In Parenteral Sciences, Vol. 4), 1991, M. Dekker, New York.

[000492] In some embodiments where antibody fragments are used, the smallest fragment that specifically binds to the binding domain of the target protein is selected. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. (*See*, *e.g.*,

Marasco et al., Proc. Natl. Acad. Sci. USA, 90: 7889-7893 (1993)). The formulation can also contain more than one active compounds as necessary for the particular indication being treated, for example, in some embodiments, those with complementary activities that do not adversely affect each other. In some embodiments, or in addition, the composition can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[000493] The active ingredients can also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions.

[000494] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[000495] Sustained-release preparations can 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. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT ™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[000496] In some embodiments, the antibody, the conjugated antibody, activatable antibody and/or conjugated activatable antibody contains a detectable label. An intact antibody, or a fragment thereof (*e.g.*, Fab, scFv, or $F(ab)_2$) is used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary

antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. Included within the usage of the term "biological sample", therefore, is blood and a fraction or component of blood including blood serum, blood plasma, or lymph. That is, the detection method of the disclosure can be used to detect an analyte mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of an analyte mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of an analyte protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, immunochemical staining, and immunofluorescence. *In vitro* techniques for detection of an analyte genomic DNA include Southern hybridizations. Procedures for conducting immunoassays are described, for example in "ELISA: Theory and Practice: Methods in Molecular Biology", Vol. 42, J. R. Crowther (Ed.) Human Press, Totowa, NJ, 1995; "Immunoassay", E. Diamandis and T. Christopoulus, Academic Press, Inc., San Diego, CA, 1996; and "Practice and Theory of Enzyme Immunoassays", P. Tijssen, Elsevier Science Publishers, Amsterdam, 1985. Furthermore, *in vivo* techniques for detection of an analyte protein include introducing into a subject a labeled anti-analyte protein antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

[000497] The antibodies, conjugated antibodies, activatable antibodies and/or conjugated activatable antibodies of the disclosure are also useful in a variety of diagnostic and prophylactic formulations. In one embodiment, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody is administered to patients that are at risk of developing one or more of the aforementioned disorders. A patient's or organ's predisposition to one or more of the aforementioned disorders can be determined using genotypic, serological or biochemical markers.

[000498] In some embodiments of the disclosure, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody is administered to human individuals diagnosed with a clinical indication associated with one or more of the aforementioned disorders. Upon diagnosis, an antibody, a conjugated antibody, an

activatable antibody and/or a conjugated activatable antibody is administered to mitigate or reverse the effects of the clinical indication.

[000499] An antibody, a conjugated antibody, an activatable antibody, and/or a conjugated activatable antibody of the disclosure is also useful in the detection of a target in patient samples and accordingly are useful as diagnostics. For example, the antibodies and/or activatable antibodies, and conjugated versions thereof, of the disclosure are used in *in vitro* assays, *e.g.*, ELISA, to detect target levels in a patient sample.

[000500] In one embodiment, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody of the disclosure is immobilized on a solid support (*e.g.*, the well(s) of a microtiter plate). The immobilized antibody, conjugated antibody, activatable antibody and/or conjugated activatable antibody serves as a capture antibody for any target that may be present in a test sample. Prior to contacting the immobilized antibody and/or activatable antibody, and/or conjugated versions thereof, with a patient sample, the solid support is rinsed and treated with a blocking agent such as milk protein or albumin to prevent nonspecific adsorption of the analyte.

[000501] Subsequently the wells are treated with a test sample suspected of containing the antigen, or with a solution containing a standard amount of the antigen. Such a sample is, *e.g.*, a serum sample from a subject suspected of having levels of circulating antigen considered to be diagnostic of a pathology. After rinsing away the test sample or standard, the solid support is treated with a second antibody that is detectably labeled. The labeled second antibody serves as a detecting antibody. The level of detectable label is measured, and the concentration of target antigen in the test sample is determined by comparison with a standard curve developed from the standard samples.

[000502] It will be appreciated that based on the results obtained using the antibodies and activatable antibodies of the disclosure, and conjugated versions thereof, in an *in vitro* diagnostic assay, it is possible to stage a disease in a subject based on expression levels of the target antigen. For a given disease, samples of blood are taken from subjects diagnosed as being at various stages in the progression of the disease, and/or at various points in the therapeutic treatment of the disease. Using a population of samples that provides statistically significant results for each stage of progression or therapy, a range of concentrations of the antigen that may be considered characteristic of each stage is designated.

[000503] An antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody can also be used in diagnostic and/or imaging methods. In some embodiments, such methods are *in vitro* methods. In some embodiments, such methods are *in vivo* methods. In some embodiments, such methods are *in situ* methods. In some embodiments, such methods are *ex vivo* methods. For example, activatable antibodies having an enzymatically cleavable CM can be used to detect the presence or absence of an enzyme that is capable of cleaving the CM. Such activatable antibodies can be used in diagnostics, which can include *in vivo* detection (*e.g.*, qualitative or quantitative) of enzyme activity (or, in some embodiments, an environment of increased reduction potential such as that which can provide for reduction of a disulfide bond) through measured accumulation of activated antibodies (i.e., antibodies resulting from cleavage of an activatable antibody) in a given cell or tissue of a given host organism. Such accumulation of activated antibodies indicates not only that the tissue expresses enzymatic activity (or an increased reduction potential depending on the nature of the CM) but also that the tissue expresses target to which the activated antibody binds.

[000504] For example, the CM can be selected to be substrate for at least one protease found at the site of a tumor, at the site of a viral or bacterial infection at a biologically confined site (*e.g.*, such as in an abscess, in an organ, and the like), and the like. The AB can be one that binds a target antigen. Using methods as disclosed herein, or when appropriate, methods familiar to one skilled in the art, a detectable label (*e.g.*, a fluorescent label or radioactive label or radiotracer) can be conjugated to an AB or other region of an antibody and/or activatable antibody. Suitable detectable labels are discussed in the context of the above screening methods and additional specific examples are provided below. Using an AB specific to a protein or peptide of the disease state, along with at least one protease whose activity is elevated in the disease tissue of interest, activatable antibodies will exhibit an increased rate of binding to disease tissue relative to tissues where the CM specific enzyme is not present at a detectable level or is present at a lower level than in disease tissue or is inactive (e.g., in zymogen form or in complex with an inhibitor). Since small proteins and peptides are rapidly cleared from the blood by the renal filtration system, and because the enzyme specific for the CM is not present at a detectable level (or is present at lower levels in non-disease tissues or is present in inactive conformation), accumulation of activated antibodies in the disease tissue is enhanced relative to non-disease tissues.

[000505] In another example, activatable antibodies can be used to detect the presence or absence of a cleaving agent in a sample. For example, where the activatable antibodies contain a CM susceptible to cleavage by an enzyme, the activatable antibodies can be used to detect (either qualitatively or quantitatively) the presence of an enzyme in the sample. In another example, where the activatable antibodies contain a CM susceptible to cleavage by reducing agent, the activatable antibodies can be used to detect (either qualitatively or quantitatively) the presence of reducing conditions in a sample. To facilitate analysis in these methods, the activatable antibodies can be detectably labeled, and can be bound to a support (*e.g.*, a solid support, such as a slide or bead). The detectable label can be positioned on a portion of the activatable antibody that is not released following cleavage, for example, the detectable label can be a quenched fluorescent label or other label that is not detectable until cleavage has occurred. The assay can be conducted by, for example, contacting the immobilized, detectably labeled activatable antibodies with a sample suspected of containing an enzyme and/or reducing agent for a time sufficient for cleavage to occur, then washing to remove excess sample and contaminants. The presence or absence of the cleaving agent (*e.g.*, enzyme or reducing agent) in the sample is then assessed by a change in detectable signal of the activatable antibodies prior to contacting with the sample *e.g.*, the presence of and/or an increase in detectable signal due to cleavage of the activatable antibody by the cleaving agent in the sample.

[000506] Such detection methods can be adapted to also provide for detection of the presence or absence of a target that is capable of binding the AB of the activatable antibodies when cleaved. Thus, the assays can be adapted to assess the presence or absence of a cleaving agent and the presence or absence of a target of interest. The presence or absence of the cleaving agent can be detected by the presence of and/or an increase in detectable label of the activatable antibodies as described above, and the presence or absence of the target can be detected by detection of a target-AB complex *e.g.*, by use of a detectably labeled anti-target antibody.

[000507] Activatable antibodies are also useful in *in situ* imaging for the validation of activatable antibody activation, *e.g.*, by protease cleavage, and binding to a particular target. *In situ* imaging is a technique that enables localization of proteolytic activity and target in biological samples such as cell cultures or tissue sections. Using this technique, it is possible to confirm both binding to a given target and proteolytic activity based on the presence of a detectable label (e.g., a fluorescent label).

[000508] These techniques are useful with any frozen cells or tissue derived from a disease site (*e.g.* tumor tissue) or healthy tissues. These techniques are also useful with fresh cell or tissue samples.

[000509] In these techniques, an activatable antibody is labeled with a detectable label. The detectable label may be a fluorescent dye, (e.g. a fluorophore, Fluorescein Isothiocyanate (FITC), Rhodamine Isothiocyanate (TRITC), an Alexa Fluor® label), a near infrared (NIR) dye (e.g., Qdot® nanocrystals), a colloidal metal, a hapten, a radioactive marker, biotin and an amplification reagent such as streptavidin, or an enzyme (e.g. horseradish peroxidase or alkaline phosphatase).

[000510] Detection of the label in a sample that has been incubated with the labeled, activatable antibody indicates that the sample contains the target and contains a protease that is specific for the CM of the activatable antibody. In some embodiments, the presence of the protease can be confirmed using broad spectrum protease inhibitors such as those described herein, and/or by using an agent that is specific for the protease, for example, an antibody such as A11, which is specific for the protease matriptase and inhibits the proteolytic activity of matriptase; see e.g., International Publication Number WO 2010/129609, published 11 November 2010. The same approach of using broad spectrum protease inhibitors such as those described herein, and/or by using a more selective inhibitory agent can be used to identify a protease that is specific for the CM of the activatable antibody. In some embodiments, the presence of the target can be confirmed using an agent that is specific for the target, *e.g.*, another antibody, or the detectable label can be competed with unlabeled target. In some embodiments, unlabeled activatable antibody could be used, with detection by a labeled secondary antibody or more complex detection system.

[000511] Similar techniques are also useful for *in vivo* imaging where detection of the fluorescent signal in a subject, *e.g.*, a mammal, including a human, indicates that the disease site contains the target and contains a protease that is specific for the CM of the activatable antibody.

[000512] These techniques are also useful in kits and/or as reagents for the detection, identification or characterization of protease activity in a variety of cells, tissues, and organisms based on the protease-specific CM in the activatable antibody.

[000513] The disclosure provides methods of using the antibodies and/or activatable antibodies in a variety of diagnostic and/or prophylactic indications. For example, the

disclosure provides methods of detecting presence or absence of a cleaving agent and a target of interest in a subject or a sample by (i) contacting a subject or sample with an activatable antibody, wherein the activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, e.g., a protease, and an antigen binding domain or fragment thereof (AB) that specifically binds the target of interest, wherein the activatable antibody in an uncleaved, non-activated state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB and is not a modified form of a natural binding partner of the AB; and (b) wherein, in an uncleaved, non-activated state, the MM interferes with specific binding of the AB to the target, and in a cleaved, activated state the MM does not interfere or compete with specific binding of the AB to the target; and (ii) measuring a level of activated activatable antibody in the subject or sample, wherein a detectable level of activated activatable antibody in the subject or sample indicates that the cleaving agent and the target are present in the subject or sample and wherein no detectable level of activated activatable antibody in the subject or sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent and/or not sufficiently present in the subject or sample. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the activatable antibody comprises a detectable label. In some embodiments, the detectable label is positioned on the AB. In some embodiments, measuring the level of activatable antibody in the subject or sample is accomplished using a secondary reagent that specifically binds to the activated antibody, wherein the reagent comprises a detectable label. In some embodiments, the secondary reagent is an antibody comprising a detectable label.

[000514] The disclosure also provides methods of detecting presence or absence of a cleaving agent in a subject or a sample by (i) contacting a subject or sample with an activatable antibody in the presence of a target of interest, *e.g.*, the target, wherein the activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, e.g., a protease, and an antigen binding domain or fragment thereof (AB) that specifically binds the target of interest, wherein the activatable antibody in an uncleaved, non-activated state comprises a structural arrangement from N-terminus to C-

terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB and is not a modified form of a natural binding partner of the AB; and (b) wherein, in an uncleaved, non-activated state, the MM interferes with specific binding of the AB to the target, and in a cleaved, activated state the MM does not interfere or compete with specific binding of the AB to the target; and (ii) measuring a level of activated activatable antibody in the subject or sample, wherein a detectable level of activated activatable antibody in the subject or sample indicates that the cleaving agent is present in the subject or sample and wherein no detectable level of activated activatable antibody in the subject or sample indicates that the cleaving agent is absent and/or not sufficiently present in the subject or sample. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the activatable antibody comprises a detectable label. In some embodiments, the detectable label is positioned on the AB. In some embodiments, measuring the level of activatable antibody in the subject or sample is accomplished using a secondary reagent that specifically binds to the activated antibody, wherein the reagent comprises a detectable label. In some embodiments, the secondary reagent is an antibody comprising a detectable label.

[000515] The disclosure also provides kits for use in methods of detecting presence or absence of a cleaving agent and the target in a subject or a sample, where the kits include at least an activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, e.g., a protease, and an antigen binding domain or fragment thereof (AB) that specifically binds the target of interest, wherein the activatable antibody in an uncleaved, non-activated state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB and is not a modified form of a natural binding partner of the AB; and (b) wherein, in an uncleaved, non-activated state, the MM interferes with specific binding of the AB to the target, and in a cleaved, activated state the MM does not interfere or compete with specific binding of the AB to the target; and (ii) measuring a level of activated activatable antibody in the subject or sample, wherein a detectable level of activated activatable antibody in the subject or

sample indicates that the cleaving agent is present in the subject or sample and wherein no detectable level of activated activatable antibody in the subject or sample indicates that the cleaving agent is absent and/or not sufficiently present in the subject or sample. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the activatable antibody comprises a detectable label. In some embodiments, the detectable label is positioned on the AB. In some embodiments, measuring the level of activatable antibody in the subject or sample is accomplished using a secondary reagent that specifically binds to the activated antibody, wherein the reagent comprises a detectable label. In some embodiments, the secondary reagent is an antibody comprising a detectable label.

[000516] The disclosure also provides methods of detecting presence or absence of a cleaving agent in a subject or a sample by (i) contacting a subject or sample with an activatable antibody, wherein the activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, e.g., a protease, an antigen binding domain (AB) that specifically binds the target, and a detectable label, wherein the activatable antibody in an uncleaved, non-activated state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB and is not a modified form of a natural binding partner of the AB; wherein, in an uncleaved, non-activated state, the MM interferes with specific binding of the AB to the target, and in a cleaved, activated state the MM does not interfere or compete with specific binding of the AB to the target; and wherein the detectable label is positioned on a portion of the activatable antibody that is released following cleavage of the CM; and (ii) measuring a level of detectable label in the subject or sample, wherein a detectable level of the detectable label in the subject or sample indicates that the cleaving agent is absent and/or not sufficiently present in the subject or sample and wherein no detectable level of the detectable label in the subject or sample indicates that the cleaving agent is present in the subject or sample. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the activatable antibody comprises a detectable label. In some embodiments, the detectable label is positioned on the AB. In

some embodiments, measuring the level of activatable antibody in the subject or sample is accomplished using a secondary reagent that specifically binds to the activated antibody, wherein the reagent comprises a detectable label. In some embodiments, the secondary reagent is an antibody comprising a detectable label.

[000517] The disclosure also provides kits for use in methods of detecting presence or absence of a cleaving agent and the target in a subject or a sample, where the kits include at least an activatable antibody and/or conjugated activatable antibody (e.g., an activatable antibody to which a therapeutic agent is conjugated) described herein for use in contacting a subject or biological sample and means for detecting the level of activated activatable antibody and/or conjugated activatable antibody in the subject or biological sample, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent and the target are present in the subject or biological sample and wherein no detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent and/or not sufficiently present in the subject or biological sample, such that the target binding and/or protease cleavage of the activatable antibody cannot be detected in the subject or biological sample.

[000518] The disclosure also provides methods of detecting presence or absence of a cleaving agent in a subject or a sample by (i) contacting a subject or biological sample with an activatable antibody in the presence of the target, and (ii) measuring a level of activated activatable antibody in the subject or biological sample, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent is present in the subject or biological sample and wherein no detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent is absent and/or not sufficiently present in the subject or biological sample at a detectable level, such that protease cleavage of the activatable antibody cannot be detected in the subject or biological sample. Such an activatable antibody includes a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, e.g., a protease, and an antigen binding domain or fragment thereof (AB) that specifically binds the target, wherein the activatable antibody in an uncleaved (*i.e.*, non-activated) state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding

partner of the AB; and (b) wherein the MM of the activatable antibody in an uncleaved state interferes with specific binding of the AB to the target, and wherein the MM of an activatable antibody in a cleaved (*i.e.*, activated) state does not interfere or compete with specific binding of the AB to the target. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the detectable label is attached to the masking moiety. In some embodiments, the detectable label is attached to the cleavable moiety N-terminal to the protease cleavage site. In some embodiments, a single antigen binding site of the AB is masked. In some embodiments wherein an antibody of the disclosure has at least two antigen binding sites, at least one antigen binding site is masked and at least one antigen binding site is not masked. In some embodiments all antigen binding sites are masked. In some embodiments, the measuring step includes use of a secondary reagent comprising a detectable label.

[000519] The disclosure also provides kits for use in methods of detecting presence or absence of a cleaving agent and the target in a subject or a sample, where the kits include at least an activatable antibody and/or conjugated activatable antibody described herein for use in contacting a subject or biological sample with an activatable antibody in the presence of the target, and measuring a level of activated activatable antibody in the subject or biological sample, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent is present in the subject or biological sample and wherein no detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent is absent and/or not sufficiently present in the subject or biological sample at a detectable level, such that protease cleavage of the activatable antibody cannot be detected in the subject or biological sample. Such an activatable antibody includes a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, e.g., a protease, and an antigen binding domain or fragment thereof (AB) that specifically binds the target, wherein the activatable antibody in an uncleaved (*i.e.*, non-activated) state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB; and (b) wherein the MM of the activatable antibody in an uncleaved state interferes with specific binding of the AB to the target, and wherein the MM of an activatable antibody in a cleaved (*i.e.*, activated) state

does not interfere or compete with specific binding of the AB to the target. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the detectable label is attached to the masking moiety. In some embodiments, the detectable label is attached to the cleavable moiety N-terminal to the protease cleavage site. In some embodiments, a single antigen binding site of the AB is masked. In some embodiments wherein an antibody of the disclosure has at least two antigen binding sites, at least one antigen binding site is masked and at least one antigen binding site is not masked. In some embodiments all antigen binding sites are masked. In some embodiments, the measuring step includes use of a secondary reagent comprising a detectable label.

[000520] The disclosure also provides kits for use in methods of detecting presence or absence of a cleaving agent in a subject or a sample, where the kits include at least an activatable antibody and/or conjugated activatable antibody described herein for use in contacting a subject or biological sample and means for detecting the level of activated activatable antibody and/or conjugated activatable antibody in the subject or biological sample, wherein the activatable antibody includes a detectable label that is positioned on a portion of the activatable antibody that is released following cleavage of the CM, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent is absent and/or not sufficiently present in the subject or biological sample such that the target binding and/or protease cleavage of the activatable antibody cannot be detected in the subject or biological sample, and wherein no detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent is present in the subject or biological sample at a detectable level.

[000521] The disclosure provides methods of detecting presence or absence of a cleaving agent and the target in a subject or a sample by (i) contacting a subject or biological sample with an activatable antibody, wherein the activatable antibody includes a detectable label that is positioned on a portion of the activatable antibody that is released following cleavage of the CM and (ii) measuring a level of activated activatable antibody in the subject or biological sample, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent and/or not sufficiently present in the subject or biological sample, such that the target binding and/or protease cleavage of the activatable

antibody cannot be detected in the subject or biological sample, and wherein a reduced detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent and the target are present in the subject or biological sample. A reduced level of detectable label is, for example, a reduction of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% and/or about 100%. Such an activatable antibody includes a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, and an antigen binding domain or fragment thereof (AB) that specifically binds the target, wherein the activatable antibody in an uncleaved (*i.e.*, non-activated) state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB; and (b) wherein the MM of the activatable antibody in an uncleaved state interferes with specific binding of the AB to the target, and wherein the MM of an activatable antibody in a cleaved (*i.e.*, activated) state does not interfere or compete with specific binding of the AB to the target. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the activatable antibody comprises a detectable label. In some embodiments, the detectable label is positioned on the AB. In some embodiments, measuring the level of activatable antibody in the subject or sample is accomplished using a secondary reagent that specifically binds to the activated antibody, wherein the reagent comprises a detectable label. In some embodiments, the secondary reagent is an antibody comprising a detectable label.

[000522] The disclosure also provides kits for use in methods of detecting presence or absence of a cleaving agent and the target in a subject or a sample, where the kits include at least an activatable antibody and/or conjugated activatable antibody described herein for use in contacting a subject or biological sample and means for detecting the level of activated activatable antibody and/or conjugated activatable antibody in the subject or biological sample, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent and/or not sufficiently present in the subject or biological sample, such that the target binding and/or protease cleavage of the activatable antibody cannot be

detected in the subject or biological sample, and wherein a reduced detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent and the target are present in the subject or biological sample. A reduced level of detectable label is, for example, a reduction of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% and/or about 100%.

[000523] The disclosure also provides methods of detecting presence or absence of a cleaving agent in a subject or a sample by (i) contacting a subject or biological sample with an activatable antibody, wherein the activatable antibody includes a detectable label that is positioned on a portion of the activatable antibody that is released following cleavage of the CM; and (ii) measuring a level of detectable label in the subject or biological sample, wherein a detectable level of the detectable label in the subject or biological sample indicates that the cleaving agent is absent and/or not sufficiently present in the subject or biological sample at a detectable level, such that protease cleavage of the activatable antibody cannot be detected in the subject or biological sample, and wherein a reduced detectable level of the detectable label in the subject or biological sample indicates that the cleaving agent is present in the subject or biological sample. A reduced level of detectable label is, for example, a reduction of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% and/or about 100%. Such an activatable antibody includes a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, and an antigen binding domain or fragment thereof (AB) that specifically binds the target, wherein the activatable antibody in an uncleaved (*i.e.*, non-activated) state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB; and (b) wherein the MM of the activatable antibody in an uncleaved state interferes with specific binding of the AB to the target, and wherein the MM of an activatable antibody in a cleaved (*i.e.*, activated) state does not interfere or compete with specific binding of the AB to the target. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an

agent. In some embodiments, the activatable antibody comprises a detectable label. In some embodiments, the detectable label is positioned on the AB. In some embodiments, measuring the level of activatable antibody in the subject or sample is accomplished using a secondary reagent that specifically binds to the activated antibody, wherein the reagent comprises a detectable label. In some embodiments, the secondary reagent is an antibody comprising a detectable label.

[000524] The disclosure also provides kits for use in methods of detecting presence or absence of a cleaving agent of interest in a subject or a sample, where the kits include at least an activatable antibody and/or conjugated activatable antibody described herein for use in contacting a subject or biological sample and means for detecting the level of activated activatable antibody and/or conjugated activatable antibody in the subject or biological sample, wherein the activatable antibody includes a detectable label that is positioned on a portion of the activatable antibody that is released following cleavage of the CM, wherein a detectable level of the detectable label in the subject or biological sample indicates that the cleaving agent, the target, or both the cleaving agent and the target are absent and/or not sufficiently present in the subject or biological sample, such that the target binding and/or protease cleavage of the activatable antibody cannot be detected in the subject or biological sample, and wherein a reduced detectable level of the detectable label in the subject or biological sample indicates that the cleaving agent and the target are present in the subject or biological sample. A reduced level of detectable label is, for example, a reduction of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% and/or about 100%.

[000525] In some embodiments of these methods and kits, the activatable antibody includes a detectable label. In some embodiments of these methods and kits, the detectable label includes an imaging agent, a contrasting agent, an enzyme, a fluorescent label, a chromophore, a dye, one or more metal ions, or a ligand-based label. In some embodiments of these methods and kits, the imaging agent comprises a radioisotope. In some embodiments of these methods and kits, the radioisotope is indium or technetium. In some embodiments of these methods and kits, the contrasting agent comprises iodine, gadolinium or iron oxide. In some embodiments of these methods and kits, the enzyme comprises horseradish peroxidase, alkaline phosphatase, or β-galactosidase. In some embodiments of these methods and kits, the fluorescent label comprises yellow fluorescent protein (YFP),

cyan fluorescent protein (CFP), green fluorescent protein (GFP), modified red fluorescent protein (mRFP), red fluorescent protein tdimer2 (RFP tdimer2), HCRED, or a europium derivative. In some embodiments of these methods and kits, the luminescent label comprises an N- methylacrydium derivative. In some embodiments of these methods, the label comprises an Alexa Fluor® label, such as Alex Fluor® 680 or Alexa Fluor® 750. In some embodiments of these methods and kits, the ligand-based label comprises biotin, avidin, streptavidin or one or more haptens.

[000526] In some embodiments of these methods and kits, the subject is a mammal. In some embodiments of these methods and kits, the subject is a human. In some embodiments, the subject is a non-human mammal, such as a non-human primate, companion animal (e.g., cat, dog, horse), farm animal, work animal, or zoo animal. In some embodiments, the subject is a rodent.

[000527] In some embodiments of these methods, the method is an *in vivo* method. In some embodiments of these methods, the method is an *in situ* method. In some embodiments of these methods, the method is an *ex vivo* method. In some embodiments of these methods, the method is an *in vitro* method.

[000528] In some embodiments, *in situ* imaging and/or *in vivo* imaging are useful in methods to identify which patients to treat. For example, in *in situ* imaging, the activatable antibodies are used to screen patient samples to identify those patients having the appropriate protease(s) and target(s) at the appropriate location, *e.g.*, at a tumor site.

[000529] In some embodiments *in situ* imaging is used to identify or otherwise refine a patient population suitable for treatment with an activatable antibody of the disclosure. For example, patients that test positive for both the target (e.g., the target) and a protease that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody being tested (e.g., accumulate activated antibodies at the disease site) are identified as suitable candidates for treatment with such an activatable antibody comprising such a CM. Likewise, patients that test negative for either or both of the target (*e.g.*, the target) and the protease that cleaves the substrate in the CM in the activatable antibody being tested using these methods might be identified as suitable candidates for another form of therapy. In some embodiments, such patients that test negative with respect to a first activatable antibody can be tested with other activatable antibodies comprising different CMs until a suitable activatable antibody for treatment is identified (e.g., an activatable antibody comprising a CM that is cleaved by the patient at the site of disease). In some embodiments,

the patient is then administered a therapeutically effective amount of the activatable antibody for which the patient tested positive.

[000530] In some embodiments *in vivo* imaging is used to identify or otherwise refine a patient population suitable for treatment with an activatable antibody of the disclosure. For example, patients that test positive for both the target (e.g., the target) and a protease that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody being tested (e.g., accumulate activated antibodies at the disease site) are identified as suitable candidates for treatment with such an activatable antibody comprising such a CM. Likewise, patients that test negative might be identified as suitable candidates for another form of therapy. In some embodiments, such patients that test negative with respect to a first activatable antibody can be tested with other activatable antibodies comprising different CMs until a suitable activatable antibody for treatment is identified (e.g., an activatable antibody comprising a CM that is cleaved by the patient at the site of disease). In some embodiments, the patient is then administered a therapeutically effective amount of the activatable antibody for which the patient tested positive.

[000531] In some embodiments of the methods and kits, the method or kit is used to identify or otherwise refine a patient population suitable for treatment with an activatable antibody of the disclosure. For example, patients that test positive for both the target (e.g., the target) and a protease that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody being tested in these methods are identified as suitable candidates for treatment with such an activatable antibody comprising such a CM. Likewise, patients that test negative for both of the targets (*e.g.*, the target) and the protease that cleaves the substrate in the CM in the activatable antibody being tested using these methods might be identified as suitable candidates for another form of therapy. In some embodiments, such patients can be tested with other activatable antibodies until a suitable activatable antibody for treatment is identified (e.g., an activatable antibody comprising a CM that is cleaved by the patient at the site of disease). In some embodiments, patients that test negative for either of the target (*e.g.*, the target) are identified as suitable candidates for treatment with such an activatable antibody comprising such a CM. In some embodiments, patients that test negative for either of the target (*e.g.*, the target) are identified as not being suitable candidates for treatment with such an activatable antibody comprising such a CM. In some embodiments, such patients can be tested with other activatable antibodies until a suitable activatable antibody for treatment is identified (e.g., an activatable antibody comprising a

CM that is cleaved by the patient at the site of disease). In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the activatable antibody comprises a detectable label. In some embodiments, the detectable label is positioned on the AB. In some embodiments, measuring the level of activatable antibody in the subject or sample is accomplished using a secondary reagent that specifically binds to the activated antibody, wherein the reagent comprises a detectable label. In some embodiments, the secondary reagent is an antibody comprising a detectable label.

[000532] In some embodiments, a method or kit is used to identify or otherwise refine a patient population suitable for treatment with an anti-the target activatable antibody and/or conjugated activatable antibody (e.g., activatable antibody to which a therapeutic agent is conjugated) of the disclosure, followed by treatment by administering that activatable antibody and/or conjugated activatable antibody to a subject in need thereof. For example, patients that test positive for both the targets (e.g., the target) and a protease that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody and/or conjugated activatable antibody being tested in these methods are identified as suitable candidates for treatment with such antibody and/or such a conjugated activatable antibody comprising such a CM, and the patient is then administered a therapeutically effective amount of the activatable antibody and/or conjugated activatable antibody that was tested. Likewise, patients that test negative for either or both of the target (e.g., the target) and the protease that cleaves the substrate in the CM in the activatable antibody being tested using these methods might be identified as suitable candidates for another form of therapy. In some embodiments, such patients can be tested with other antibody and/or conjugated activatable antibody until a suitable antibody and/or conjugated activatable antibody for treatment is identified (*e.g.*, an activatable antibody and/or conjugated activatable antibody comprising a CM that is cleaved by the patient at the site of disease). In some embodiments, the patient is then administered a therapeutically effective amount of the activatable antibody and/or conjugated activatable antibody for which the patient tested positive.

[000533] In some embodiments of these methods and kits, the MM is a peptide having a length from about 4 to 40 amino acids. In some embodiments of these methods and kits, the activatable antibody comprises a linker peptide, wherein the linker peptide is positioned between the MM and the CM. In some embodiments of these methods and kits, the

activatable antibody comprises a linker peptide, where the linker peptide is positioned between the AB and the CM. In some embodiments of these methods and kits, the activatable antibody comprises a first linker peptide (L1) and a second linker peptide (L2), wherein the first linker peptide is positioned between the MM and the CM and the second linker peptide is positioned between the AB and the CM. In some embodiments of these methods and kits, each of L1 and L2 is a peptide of about 1 to 20 amino acids in length, and wherein each of L1 and L2 need not be the same linker. In some embodiments of these methods and kits, one or both of L1 and L2 comprises a glycine-serine polymer. In some embodiments of these methods and kits, at least one of L1 and L2 comprises an amino acid sequence selected from the group consisting of (GS)n, (GSGGS)n (SEQ ID NO: 191) and (GGGS)n (SEQ ID NO: 192), where n is an integer of at least one. In some embodiments of these methods and kits, at least one of L1 and L2 comprises an amino acid sequence having the formula (GGS)n, where n is an integer of at least one. In some embodiments of these methods and kits, at least one of L1 and L2 comprises an amino acid sequence selected from the group consisting of Gly-Gly-Ser-Gly (SEQ ID NO: 193), Gly-Gly-Ser-Gly-Gly (SEQ ID NO: 194), Gly-Ser-Gly-Ser-Gly (SEQ ID NO: 195), Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 196), Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 197), and Gly-Ser-Ser-Ser-Gly (SEQ ID NO: 198).

[000534] In some embodiments of these methods and kits, the AB comprises an antibody or antibody fragment sequence selected from the cross-reactive antibody sequences presented herein. In some embodiments of these methods and kits, the AB comprises a Fab fragment, a scFv or a single chain antibody (scAb).

[000535] In some embodiments of these methods and kits, the cleaving agent is a protease that is co-localized in the subject or sample with the target and the CM is a polypeptide that functions as a substrate for the protease, wherein the protease cleaves the CM in the activatable antibody when the activatable antibody is exposed to the protease. In some embodiments of these methods and kits, the CM is a polypeptide of up to 15 amino acids in length. In some embodiments of these methods and kits, the CM is coupled to the N-terminus of the AB. In some embodiments of these methods and kits, the CM is coupled to the C-terminus of the AB. In some embodiments of these methods and kits, the CM is coupled to the N-terminus of a VL chain of the AB.

[000536] The antibodies, conjugated antibodies, activatable antibodies and/or conjugated activatable antibodies of the disclosure are used in diagnostic and prophylactic

formulations. In one embodiment, an activatable antibody is administered to patients that are at risk of developing one or more of the aforementioned inflammation, inflammatory disorders, cancer or other disorders.

[000537] A patient's or organ's predisposition to one or more of the aforementioned disorders can be determined using genotypic, serological or biochemical markers.

[000538] In some embodiments of the disclosure, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody is administered to human individuals diagnosed with a clinical indication associated with one or more of the aforementioned disorders. Upon diagnosis, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody is administered to mitigate or reverse the effects of the clinical indication.

[000539] Antibodies, conjugated antibodies, activatable antibodies and/or conjugated activatable antibodies of the disclosure are also useful in the detection of the target in patient samples and accordingly are useful as diagnostics. For example, the antibodies, conjugated antibodies, the activatable antibodies and/or conjugated activatable antibodies of the disclosure are used in *in vitro* assays, *e.g.*, ELISA, to detect target levels in a patient sample.

[000540] In one embodiment, an antibody and/or activatable antibody of the disclosure is immobilized on a solid support (*e.g.*, the well(s) of a microtiter plate). The immobilized antibody and/or activatable antibody serves as a capture antibody for any target that may be present in a test sample. Prior to contacting the immobilized antibody and/or activatable antibody with a patient sample, the solid support is rinsed and treated with a blocking agent such as milk protein or albumin to prevent nonspecific adsorption of the analyte.

[000541] Subsequently the wells are treated with a test sample suspected of containing the antigen, or with a solution containing a standard amount of the antigen. Such a sample is, *e.g.*, a serum sample from a subject suspected of having levels of circulating antigen considered to be diagnostic of a pathology. After rinsing away the test sample or standard, the solid support is treated with a second antibody that is detectably labeled. The labeled second antibody serves as a detecting antibody. The level of detectable label is measured, and the concentration of target antigen in the test sample is determined by comparison with a standard curve developed from the standard samples.

[000542] It will be appreciated that based on the results obtained using the antibodies and/or activatable antibodies of the disclosure in an *in vitro* diagnostic assay, it is possible

to stage a disease in a subject based on expression levels of the Target antigen. For a given disease, samples of blood are taken from subjects diagnosed as being at various stages in the progression of the disease, and/or at various points in the therapeutic treatment of the disease. Using a population of samples that provides statistically significant results for each stage of progression or therapy, a range of concentrations of the antigen that may be considered characteristic of each stage is designated.

[000543] Antibodies, conjugated antibodies, activatable antibodies and/or conjugated activatable antibodies can also be used in diagnostic and/or imaging methods. In some embodiments, such methods are *in vitro* methods. In some embodiments, such methods are *in vivo* methods. In some embodiments, such methods are *in situ* methods. In some embodiments, such methods are *ex vivo* methods. For example, activatable antibodies having an enzymatically cleavable CM can be used to detect the presence or absence of an enzyme that is capable of cleaving the CM. Such activatable antibodies can be used in diagnostics, which can include *in vivo* detection (*e.g.*, qualitative or quantitative) of enzyme activity (or, in some embodiments, an environment of increased reduction potential such as that which can provide for reduction of a disulfide bond) through measured accumulation of activated antibodies (i.e., antibodies resulting from cleavage of an activatable antibody) in a given cell or tissue of a given host organism. Such accumulation of activated antibodies indicates not only that the tissue expresses enzymatic activity (or an increased reduction potential depending on the nature of the CM) but also that the tissue expresses target to which the activated antibody binds.

[000544] For example, the CM can be selected to be a protease substrate for a protease found at the site of a tumor, at the site of a viral or bacterial infection at a biologically confined site (*e.g.*, such as in an abscess, in an organ, and the like), and the like. The AB can be one that binds a target antigen. Using methods familiar to one skilled in the art, a detectable label (*e.g.*, a fluorescent label or radioactive label or radiotracer) can be conjugated to an AB or other region of an activatable antibody. Suitable detectable labels are discussed in the context of the above screening methods and additional specific examples are provided below. Using an AB specific to a protein or peptide of the disease state, along with a protease whose activity is elevated in the disease tissue of interest, activatable antibodies will exhibit an increased rate of binding to disease tissue relative to tissues where the CM specific enzyme is not present at a detectable level or is present at a lower level than in disease tissue or is inactive (e.g., in zymogen form or in complex with an

inhibitor). Since small proteins and peptides are rapidly cleared from the blood by the renal filtration system, and because the enzyme specific for the CM is not present at a detectable level (or is present at lower levels in non-disease tissues or is present in inactive conformation), accumulation of activated antibodies in the disease tissue is enhanced relative to non-disease tissues.

[000545] In another example, activatable antibodies can be used to detect the presence or absence of a cleaving agent in a sample. For example, where the activatable antibodies contain a CM susceptible to cleavage by an enzyme, the activatable antibodies can be used to detect (either qualitatively or quantitatively) the presence of an enzyme in the sample. In another example, where the activatable antibodies contain a CM susceptible to cleavage by reducing agent, the activatable antibodies can be used to detect (either qualitatively or quantitatively) the presence of reducing conditions in a sample. To facilitate analysis in these methods, the activatable antibodies can be detectably labeled, and can be bound to a support (*e.g.*, a solid support, such as a slide or bead). The detectable label can be positioned on a portion of the activatable antibody that is not released following cleavage, for example, the detectable label can be a quenched fluorescent label or other label that is not detectable until cleavage has occurred. The assay can be conducted by, for example, contacting the immobilized, detectably labeled activatable antibodies with a sample suspected of containing an enzyme and/or reducing agent for a time sufficient for cleavage to occur, then washing to remove excess sample and contaminants. The presence or absence of the cleaving agent (*e.g.*, enzyme or reducing agent) in the sample is then assessed by a change in detectable signal of the activatable antibodies prior to contacting with the sample *e.g.*, the presence of and/or an increase in detectable signal due to cleavage of the activatable antibody by the cleaving agent in the sample.

[000546] Such detection methods can be adapted to also provide for detection of the presence or absence of a target that is capable of binding the AB of the activatable antibodies when cleaved. Thus, the assays can be adapted to assess the presence or absence of a cleaving agent and the presence or absence of a target of interest. The presence or absence of the cleaving agent can be detected by the presence of and/or an increase in detectable label of the activatable antibodies as described above, and the presence or absence of the target can be detected by detection of a target-AB complex *e.g.*, by use of a detectably labeled anti-target antibody.

[000547] Activatable antibodies are also useful in *in situ* imaging for the validation of activatable antibody activation, *e.g.*, by protease cleavage, and binding to a particular target. *In situ* imaging is a technique that enables localization of proteolytic activity and target in biological samples such as cell cultures or tissue sections. Using this technique, it is possible to confirm both binding to a given target and proteolytic activity based on the presence of a detectable label (e.g., a fluorescent label).

[000548] These techniques are useful with any frozen cells or tissue derived from a disease site (*e.g.* tumor tissue) or healthy tissues. These techniques are also useful with fresh cell or tissue samples.

[000549] In these techniques, an activatable antibody is labeled with a detectable label. The detectable label may be a fluorescent dye, (e.g. Fluorescein Isothiocyanate (FITC), Rhodamine Isothiocyanate (TRITC), a near infrared (NIR) dye (e.g., Qdot® nanocrystals), a colloidal metal, a hapten, a radioactive marker, biotin and an amplification reagent such as streptavidin, or an enzyme (e.g. horseradish peroxidase or alkaline phosphatase).

[000550] Detection of the label in a sample that has been incubated with the labeled, activatable antibody indicates that the sample contains the target and contains a protease that is specific for the CM of the activatable antibody. In some embodiments, the presence of the protease can be confirmed using broad spectrum protease inhibitors such as those described herein, and/or by using an agent that is specific for the protease, for example, an antibody such as A11, which is specific for the protease matriptase and inhibits the proteolytic activity of matriptase; see e.g., International Publication Number WO 2010/129609, published 11 November 2010. The same approach of using broad spectrum protease inhibitors such as those described herein, and/or by using a more selective inhibitory agent can be used to identify a protease or class of proteases specific for the CM of the activatable antibody. In some embodiments, the presence of the target can be confirmed using an agent that is specific for the target, *e.g.*, another antibody, or the detectable label can be competed with unlabeled target. In some embodiments, unlabeled activatable antibody could be used, with detection by a labeled secondary antibody or more complex detection system.

[000551] Similar techniques are also useful for *in vivo* imaging where detection of the fluorescent signal in a subject, *e.g.*, a mammal, including a human, indicates that the disease site contains the target and contains a protease that is specific for the CM of the activatable antibody.

[000552] These techniques are also useful in kits and/or as reagents for the detection, identification or characterization of protease activity in a variety of cells, tissues, and organisms based on the protease-specific CM in the activatable antibody.

[000553] In some embodiments, *in situ* imaging and/or *in vivo* imaging are useful in methods to identify which patients to treat. For example, in *in situ* imaging, the activatable antibodies are used to screen patient samples to identify those patients having the appropriate protease(s) and target(s) at the appropriate location, *e.g.*, at a tumor site.

[000554] In some embodiments *in situ* imaging is used to identify or otherwise refine a patient population suitable for treatment with an activatable antibody of the disclosure. For example, patients that test positive for both the target and a protease that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody being tested (e.g., accumulate activated antibodies at the disease site) are identified as suitable candidates for treatment with such an activatable antibody comprising such a CM. Likewise, patients that test negative for either or both of the target and the protease that cleaves the substrate in the CM in the activatable antibody being tested using these methods are identified as suitable candidates for another form of therapy (*i.e.*, not suitable for treatment with the activatable antibody being tested). In some embodiments, such patients that test negative with respect to a first activatable antibody can be tested with other activatable antibodies comprising different CMs until a suitable activatable antibody for treatment is identified (e.g., an activatable antibody comprising a CM that is cleaved by the patient at the site of disease).

[000555] In some embodiments *in vivo* imaging is used to identify or otherwise refine a patient population suitable for treatment with an activatable antibody of the disclosure. For example, patients that test positive for both the target and a protease that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody being tested (e.g., accumulate activated antibodies at the disease site) are identified as suitable candidates for treatment with such an activatable antibody comprising such a CM. Likewise, patients that test negative are identified as suitable candidates for another form of therapy (*i.e.*, not suitable for treatment with the activatable antibody being tested). In some embodiments, such patients that test negative with respect to a first activatable antibody can be tested with other activatable antibodies comprising different CMs until a suitable activatable antibody for treatment is identified (e.g., an activatable antibody comprising a CM that is cleaved by the patient at the site of disease).

Pharmaceutical compositions

[000556] The antibodies, conjugated antibodies, activatable antibodies and/or conjugated activatable antibodies of the disclosure (also referred to herein as "active compounds"), and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the antibody, the conjugated antibody, activatable antibody and/or conjugated activatable antibody and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Suitable examples of such carriers or diluents include, but are not limited to, water, saline, ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[000557] A pharmaceutical composition of the disclosure is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[000558] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In some embodiments, it will be desirable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

[000559] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[000560] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is

applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[000561] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser that contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[000562] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[000563] The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[000564] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[000565] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[000566] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[000567] The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

Examples

EXAMPLE 1. Selection of human ScFvs of the embodiments that bind human and/or mouse PDL1

[000568] This Example demonstrates that ScFvs (single-chain variable fragments) of the embodiments that bind PDL1 can be selected from a phage display library of ScFvs with diverse CDR sequences, and that such binding can inhibit PDL1 binding to PD1 and B7-1.

[000569] ScFvs were selected from a fully human ScFv library displayed on M13 bacteriophage; ScFv phage selection was conducted under contract with Creative Biolabs, Shirley, NY. A fusion protein comprised of the extracellular domain (ECD) of human PDL1 or mouse PDL1, with a carboxy terminal His6 tag (Sino Biological, Cat. No. 10084-H02H-200) was used as the antigen in three alternating rounds of selection for ScFvs displayed on M13 bacteriophage that bind PDL1. In the first round, bound phage were released by trypsin digestion, and in subsequent rounds, phage were eluted by human PD1 fusion protein (R&D Systems; Cat. No. 1080-PD-050) competition. Five (5) unique ScFvs that bind PDL1 were isolated. Table 1 lists the 5 ScFvs and SEQ ID NOs of their respective nucleic acid sequences and amino acid sequences.

Table 1. SEQ ID NOs of selected ScFvs

ScFv	Nucleic acid sequence	Amino acid sequence
PDL1 c8	SEQ ID NO: 1	SEQ ID NO: 2
PDL1 c12	SEQ ID NO: 3	SEQ ID NO: 4
PDL1 c16	SEQ ID NO: 5	SEQ ID NO: 6
PDL1 c20	SEQ ID NO: 7	SEQ ID NO: 8
PDL1 c60	SEQ ID NO: 9	SEQ ID NO: 10

[000570] The nucleic acid and amino acid sequences of each of the anti-PDL1 ScFvs (with CDRs underlined) are shown below:

SEQ ID NO: 1

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT
GGAGTGGGTCTCAGATATTACTGCGTCGGGTTAGAGGACAACGTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCCGTATATTACTGTGCGAGATCGAAGATTGCTTTTGACTACTGGGGCCAGGGAACCCTGGT
CACCGTCTCGAGCGGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCGGTGGCGGGTCGACGGACATC
CAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGG
CAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCT
GATCTATAAGGCATCCCGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
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SEQ ID NO: 2

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QMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYKASRLQSGVPSRFSGSGSGT
DFTLTISSLQPEDFATYYCQQRALKPVTFGQGTKVEIKR

SEQ ID NO: 3

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTATCCTGTG
CAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT
GGAGTGGGTCTCAAGTATTAATAAGGATGGTCATTATACAAGTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCCGTATATTACTGTGCGAAAAATCTTGATGAGTTTGACTACTGGGGCCAGGGAACCCTGGT

CACCGTCTCGAGCGGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCGGTGGCGGGTCGACGGACATC
CAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGG
CAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCT
GATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTT
ACAGTACCCCTAATACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGG

SEQ ID NO: 4
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QMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGT
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SEQ ID NO: 5
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CACCGTCTCGAGCGGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCGGTGGCGGGTCGACGGACATC
CAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGG
CAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCT
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SEQ ID NO: 6
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SEQ ID NO: 7
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CACCGTCTCGAGCGGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCGGTGGCGGGTCGACGGACATC
CAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGG
CAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCT
GATCTATAATGCATCCTCCTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
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SEQ ID NO: 8
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DFTLTISSLQPEDFATYYCQQYTYGPGTFGQGTKVEIKR

SEQ ID NO: 9
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GGAGTGGGTCTCAAGTATTTATTCTACTGGTGGTGCTACAGCTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
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CAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGG
CAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCT
GATCTATTATGCATCCACTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGGATA
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SEQ ID NO: 10
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYADSVKGR
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QMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYYASTLQSGVPSRFSGSGSGT
DFTLTISSLQPEDFATYYCQQDNGYPSTFGQGTKVEIKR

[000571] Figure 1 shows by ELISA-based binding that PDL1 c60 ScFv-phage binds specifically to human and mouse PDL1. Briefly, human PDL1, mouse PDL1, human PD1, mouse PD1, human 41BBLig or Notch1 (R&D Systems) were adsorbed to separate wells of a 96-well ELISA plate. Phage were applied to the plate and allowed to bind. Bound phage were visualized with an anti-M13-HRP conjugate (GE Healthcare, Piscataway, NY) and

developed with the chromogenic substrate tetramethyl benzidine (TMB) (Thermo Scientific, Rockford, IL).

[000572] Figure 2 shows that inclusion of PD1 can inhibit the binding of PDL1 ScFv-phage binding to PDL1 in an ELISA binding assay. Briefly, human PDL1 or mouse PDL1 (R&D Systems) was adsorbed to separate wells of a 96-well ELISA plate. Phage were applied to the plate in the presence or absence of human or mouse PD1-Fc (R&D Systems) and allowed to bind. Bound phage were visualized with an anti-M13-HRP conjugate (GE Healthcare, Piscataway, NY) and developed with the chromogenic substrate tetramethyl benzidine (TMB) (Thermo Scientific, Rockford, IL). Phage binding was reduced in the presence of PD1-Fc, demonstrating that the binding epitope of the phage overlaps with that of PD1 binding.

EXAMPLE 2. Production and testing of fully human anti-PDL1 c60 IgG antibodies

[000573] This Example demonstrates that PDL1 c60 ScFv-phage that bind human and mouse PDL1 can be converted into fully human IgG antibodies that retain human and mouse PDL1 binding.

[000574] Production of fully human IgGs comprising the variable domains of PDL1 c60 was accomplished using techniques similar to those described in PCT Publication No. WO 2010/081173. DNA molecules encoding the variable domains of PDL1 c60 ScFv-phage were cloned into expression vectors for the expression of fully human IgGs. Light chain (Lc) variable domains were amplified from the ScFv templates. Vector (LcpOP (modified from pCDNA3, Invitrogen, Carlsbad, CA)) and amplified DNA were cut with *Bsi*WI and *Eco*RI overnight, combined by ligation and transformed into *E. coli* MC1061 cells. Heavy chain (Hc) variable domains were amplified from ScFv templates. Fully human IgGs anti-PDL1 c60 were expressed from transiently transfected HEK-293 cells and purified from the culture supernatant by Protein A chromatography.

[000575] Figure 3 shows that the fully human anti-PDL1 c60 IgG binds human and mouse PDL1, with affinity for human PDL1 of 1 nM and for mouse PDL1 of 30 nM. Briefly human PDL1-Fc or mouse PDL1-Fc (R & D Systems, Minneapolis, MN) was adsorbed to the wells of a 96-well ELISA plate. Purified anti-PDL1 c60 was applied to the plate and allowed to bind. Bound antibody was visualized with an anti-human IgG-HRP conjugate, Fab specific, (Sigma, St Louis, MO; Cat # A0293-1ML) and developed with the chromogenic substrate TMB.

EXAMPLE 3. Affinity maturation of anti-PDL1 antibodies of the embodiments

[000576] This Example demonstrates the isolation of antibodies of the embodiments with improved binding kinetics and manufacturability profile.

[000577] Anti-PDL1 antibodies were isolated from libraries with CDRs modified from anti-PDL1 c60. Such libraries were designed as shown in Tables 2 and 3. Four libraries of Fab-Phage, based on the sequence of anti-PDL1 c60, were constructed using degenerate synthetic DNA. Residues were either varied by randomization at each indicated nucleotide. In addition, within libraries 3 and 6, additional randomized residues were added to CDR3 of the heavy chain. Libraries were transfected into *E. coli* strain TG1 and phage were prepared following super-infection with M13KO7 (Invitrogen).

[000578] Three rounds of selection were performed for each library with increasing stringency. Three alternating rounds of selection were done by mixing biotinylated Human PDL1 or Biotinylated mouse PDL1 with phage that were blocked with 100 µg/mL pooled human IgG (huIgG, or hIgG) and 2% non-fat dried milk (NFDM) in Tris-buffered saline (TBS; 40 mM Tris, 129 mM NaCl, pH 7.4). Following incubation at room temperature Bio-PDL1 bound to phage were captured with streptavidin magnetic beads which were washed extensively at 37^0C and the remaining bound phage eluted with 100 mM triethanolamine (TEA) (Sigma, St. Louis, MO) and expanded through *E. coli* TG1. Selected Lc libraries were combined with Hc libraries and each subjected to an additional seven rounds of selection.

Table 2. CDR sequences for affinity maturation variable heavy chain libraries

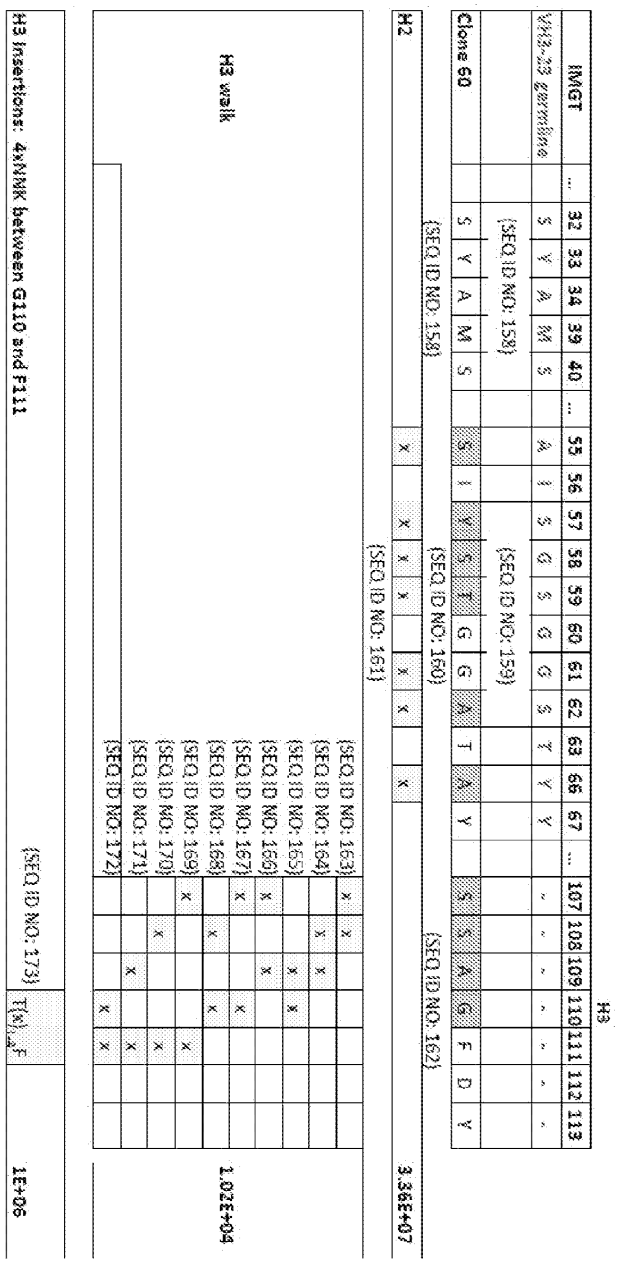

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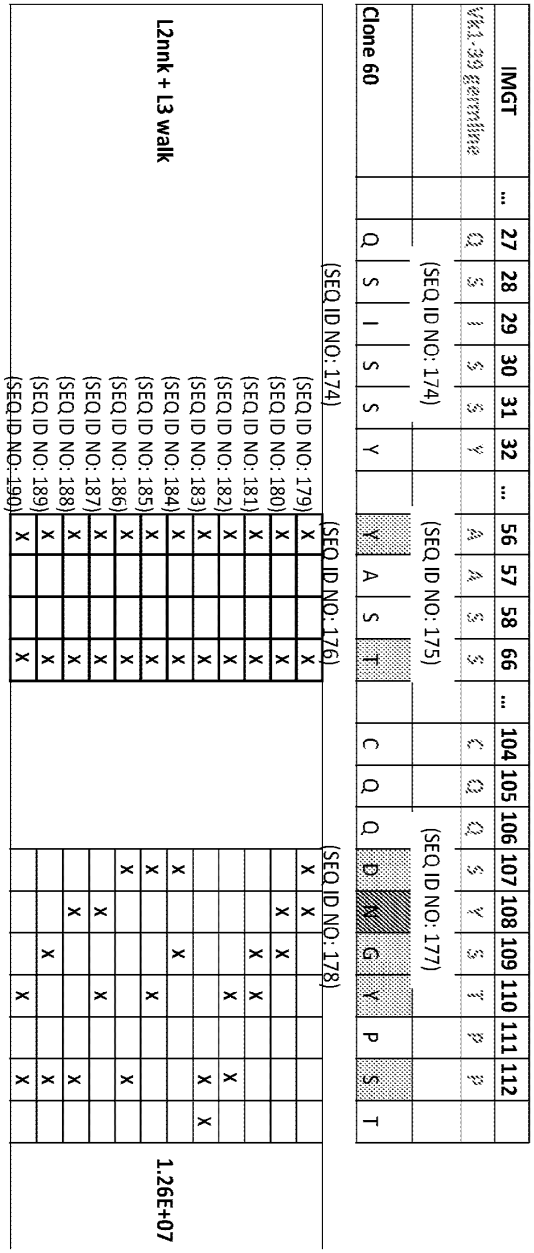
Table 3. CDR sequences for affinity maturation variable light chain libraries



[000579] Forty seven (47) individual isolates were chosen from the final pool. Phage were derived from each isolate and assayed for binding to human PDL1-Fc, human B7-1-Fc

and human CD28-Fc antigens (R&D Systems). Briefly, the antigens were adsorbed to the wells of a 96-well ELISA plate, each ligand on a separate plate. Phage were applied to correlative wells on each plate and allowed to bind. Bound phage were visualized with an anti-M13-HRP conjugate and developed with the chromogenic substrate TMB. Based on the results of the ELISA and DNA sequence, 6 unique clones with matured heavy variable CDR3 domains and 10 heavy variable CDR2 domains were chosen for further study. Thirteen phage clones, 10 CDR2 and 3 CDR3 were analyzed for their ability to bind mouse and human PDL1, and the results of a phage ELISA are shown in Figure 4A and Figure 4B. All phage clones bind human PDL1 but not B7-1, in addition, CDR2 clones C05 and G12 show strong binding to mouse PDL1 and CDR3 clone H9 showed weak binding to mouse PDL1. Table 4 lists the heavy variable domains encoded by the clones and SEQ ID NOs for the nucleic acid sequences and amino acid sequences of their respective heavy chains. The light variable domains for each are identical to that of c60 (SEQ ID NO 11 and 12).

SEQ ID NO: 11
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTT
GCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAA
GCTCCTGATCTATTATGCATCCACTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCT
GGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAAC
AGGATAATGGTTATCCTTCTACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGG

SEQ ID NO: 12
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYYASTLQSGVPSRFSGSGS
GTDFTLTISSLQPEDFATYYCQQDNGYPSTFGQGTKVEIKR

Table 4: Matured anti-PDL1 variable domains

VH	Nucleic acid sequence	Amino acid sequence
PDL1 c1	SEQ ID NO: 13	SEQ ID NO: 14
PDL1 d1	SEQ ID NO: 15	SEQ ID NO: 16
PDL1 g7	SEQ ID NO: 17	SEQ ID NO: 18
PDL1 h9	SEQ ID NO: 19	SEQ ID NO: 20
PDL1 b10	SEQ ID NO: 21	SEQ ID NO: 22
PDL1 E10	SEQ ID NO: 23	SEQ ID NO: 24
PDL1 A05	SEQ ID NO: 25	SEQ ID NO: 26

PDL1 C05	SEQ ID NO: 27	SEQ ID NO: 28
PDL1 C10	SEQ ID NO: 29	SEQ ID NO: 30
PDL1 D08	SEQ ID NO: 31	SEQ ID NO: 32
PDL1 G09	SEQ ID NO: 33	SEQ ID NO: 34
PDL1 G10	SEQ ID NO: 35	SEQ ID NO: 36
PDL1 G12	SEQ ID NO: 37	SEQ ID NO: 38
PDL1 E11	SEQ ID NO: 39	SEQ ID NO: 40
PDL1 D01	SEQ ID NO: 41	SEQ ID NO: 42
PDL1 H06	SEQ ID NO: 43	SEQ ID NO: 44

SEQ ID NO: 13

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GGAGTGGGTCTCAAGTATTTATTCTACTGGTGGTGCTACAGCTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
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GGGAACCCTGGTCACCGTCTCGAGC

SEQ ID NO: 14

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SEQ ID NO: 15

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CAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT
GGAGTGGGTCTCAAGTATTTATTCTACTGGTGGTGCTACAGCTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCCGTATATTACTGTGCGAAATCTTCTGCTGGTTCGTGGCCGGGTTTTGACTACTGGGGCCA
GGGAACCCTGGTCACCGTCTCGAGC

SEQ ID NO: 16

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SEQ ID NO: 17
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GGAGTGGGTCTCAAGTATTTATTCTACTGGTGGTGCTACAGCTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCCGTATATTACTGTGCGAAATCTTCTGCTGGTCAGTCGTTTCCGGGTTTTGACTACTGGGG
CCAGGGAACCCTGGTCACCGTCTCGAGC

SEQ ID NO: 18
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYADSVKGR
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SEQ ID NO: 19
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GGAGTGGGTCTCAAGTATTTATTCTACTGGTGGTGCTACAGCTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCCGTATATTACTGTGCGAAATGGTCTGCTGCTTTTGACTACTGGGGCCAGGGAACCCTGGT
CACCGTCTCGAGC

SEQ ID NO: 20
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SEQ ID NO: 21
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GGAGTGGGTCTCAAGTATTTATTCTACTGGTGGTGCTACAGCTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCCGTATATTACTGTGCGAAATGGTCTGCTGCTTTTGACTACTGGGGCCAGGGAACCCTGGT
CACCGTCTCGAGC

SEQ ID NO: 22
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SEQ ID NO: 23
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TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
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CACCGTCTCGAGC

SEQ ID NO: 24
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SEQ ID NO: 25
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GAGGACACGGCCGTATATTACTGTGCGAAATCTTCTGCTGGTTTTGACTACTGGGGCCAGGGAACCC
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SEQ ID NO: 26
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SEQ ID NO: 28
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SEQ ID NO: 29
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SEQ ID NO: 30
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SEQ ID NO: 32
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SEQ ID NO: 33
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SEQ ID NO: 35
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SEQ ID NO: 36
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SEQ ID NO: 37
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SEQ ID NO: 39
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT
GGAGTGGGTCTCAGATATTTGGAAGTAGGGTTTTGCTACAGCGAGCTTACGCAGACTCCGTGAAGGG
CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCC
GAGGACACGGCCGTATATTACTGTGCGAAATCTTCTGCTGGTTTTGACTACTGGGGCCAGGGAACCC
TGGTCACCGTCTCGAGC

SEQ ID NO: 40
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSDIWKQGFATADSVKGRFT
ISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS

SEQ ID NO: 41
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT

GGAGTGGGTCTCAAGTATTTGGAAGTAGGGTATTGTGACAGTGAGCTTACGCAGACTCCGTGAAGGG
CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCC
GAGGACACGGCCGTATATTACTGTGCGAAATCTTCTGCTGGTTTTGACTACTGGGGCCAGGGAACCC
TGGTCACCGTCTCGAGC

SEQ ID NO: 42
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWKQGIVTVYDSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS

SEQ ID NO: 43
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT
GGAGTGGGTCTCATCGATTTGGAGGTAGGGTCTGGCGACAGCGAGCTTACGCAGACTCCGTGAAGGG
CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCC
GAGGACACGGCCGTATATTACTGTGCGAAATCTTCTGCTGGTTTTGACTACTGGGGCCAGGGAACCC
TGGTCACCGTCTCGAGC

SEQ ID NO: 44
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRQGLATAYDSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS

EXAMPLE 4. Isolation and testing of affinity matured anti-PDL1 antibodies of the embodiments

[000580] This Example demonstrates that matured PDL1 Fab-phage that bind human and mouse PDL1 can be converted into fully human IgG antibodies that retain human and show enhanced mouse PDL1 binding.

[000581] Production of fully human IgGs comprising the variable domains of the matured clones was accomplished using techniques similar to those described in PCT Publication No. WO 2010/081173. DNA encoding the variable domains of anti-PDL1 Fab-Phage clones C1, D1, G7, C05, G12 and combinations of heavy variable CDR3 domains of anti-PDL1 Fab-Phage clones C05 or G12 with anti-PDL1 Fab-Phage clones heavy variable CDR2 domains of B10, E10 or H9 were cloned into expression vectors for the expression of fully human IgGs. The light variable domains for each are identical to that of c60 (SEQ ID NO: 10). Light chain (Lc) variable domains were amplified from the Fab templates. Vector (LcpOP (modified from pCDNA3, Invitrogen, Carlsbad, CA)) and amplified DNA were cut

with *Bsi*WI and *Eco*RI overnight, combined by ligation and transformed into *E. coli* MC1061 cells. Heavy chain (Hc) variable domains were amplified from Fab templates. Fully human IgGs anti-PDL1 antibodies were expressed from transiently transfected HEK-293 cells and purified from the culture supernatant by Protein A chromatography.

[000582] The affinity matured anti-PDL1 antibodies were analyzed for their ability to bind human and mouse PDL1. Briefly human PDL1-Fc or mouse PDL1-Fc (R & D Systems, Minneapolis, MN) was adsorbed to the wells of a 96-well ELISA plate. Purified anti-PDL1 antibodies were applied to the plate and allowed to bind. Bound antibody was visualized with an anti-human IgG-HRP conjugate, Fab specific, (Sigma, St Louis, MO; Cat # A0293-1ML) and developed with the chromogenic substrate TMB. Figure 5 shows that combining the matured heavy variable CDR3 domains with the heavy variable CDR2 domains results in antibodies with enhanced affinity towards human PDL1. In addition, the combination of the matured heavy variable CDR2 domain C05 with the heavy variable CD3 domain H9 results in an antibody with high affinity for both human and mouse PDL1. Table 5 provides the SEQ ID NOs for the nucleic acid sequences and amino acids sequences of the respective variable heavy chains.

[000583] The anti-PDL1 antibodies C5H9, C5B10 and C5E10 were further characterized for their species specificity towards binding to PDL1 and other closely related proteins. Briefly human PDL1-Fc, mouse PDL1-Fc (R & D Systems, Minneapolis, MN) or cynomolgus monkey (cyno) PDL1-Fc (Sino biological) were adsorbed to the wells of a 96-well ELISA plate. Purified anti-PDL1 antibodies were applied to the plate and allowed to bind. Bound antibody was visualized with an anti-human IgG-HRP conjugate, Fab specific, (Sigma, St Louis, MO; Cat # A0293-1ML) and developed with the chromogenic substrate TMB. Figures 6A and 6B show that C5H9 binds to human and mouse PDL1 with near equal affinity and C5B10 and C5E10 only bind human PDL1. None of the antibodies tested bind to either human B7-1 (Figure 6D) or human CD28 (Figure 6C), two closely related proteins. Figure 7 shows that anti-PDL1 antibody C5H9 binds with high and equal affinity to human, mouse and cyno PDL1, but not to human B7H3 (also referred to as B7-H3) (R&D Systems, Minneapolis, MN).

Table 5: Variable heavy domains of PDL1 antibodies of the embodiments

VH	Nucleic acid sequence	Amino acid sequence
PDL1 C5H9	SEQ ID NO: 45	SEQ ID NO: 46

PDL1 C5B10	SEQ ID NO: 47	SEQ ID NO: 48
PDL1 C5E10	SEQ ID NO: 49	SEQ ID NO: 50
PDL1 G12H9	SEQ ID NO: 51	SEQ ID NO: 52
PDL1 G12B10	SEQ ID NO: 53	SEQ ID NO: 54
PDL1 G12E10	SEQ ID NO: 55	SEQ ID NO: 56

SEQ ID NO: 45

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT
GGAGTGGGTCTCAAGTATTTGGCGGAATGGTATTGTTACAGTTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCCGTATATTACTGTGCGAAATGGTCTGCTGCTTTTGACTACTGGGGCCAGGGAACCCTGGT
CACCGTCTCGAGC

SEQ ID NO: 46

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRNGIVTVYADSVKGR
FTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAAFDYWGQGTLVTVSS

SEQ ID NO: 47

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT
GGAGTGGGTCTCAAGTATTTGGCGGAATGGTATTGTTACAGTTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCCGTATATTACTGTGCGAAATGGTCTGCTGGTTATGACTACTGGGGCCAGGGAACCCTGGT
CACCGTCTCGAGC

SEQ ID NO: 48

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRNGIVTVYADSVKGR
FTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAGYDYWGQGTLVTVSS

SEQ ID NO: 49

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT
GGAGTGGGTCTCAAGTATTTGGCGGAATGGTATTGTTACAGTTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG

ACACGGCCGTATATTACTGTGCGAAATGGTCTAAGGGTTTTGACTACTGGGGCCAGGGAACCCTGGT
CACCGTCTCGAGC

SEQ ID NO: 50
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRNGIVTVYADSVKGR
FTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSKGFDYWGQGTLVTVSS

SEQ ID NO: 51
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT
GGAGTGGGTCTCATCTATTTGGTATCAGGGTCTGGTGACAGTTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCCGTATATTACTGTGCGAAATGGTCTGCTGCTTTTGACTACTGGGGCCAGGGAACCCTGGT
CACCGTCTCGAGC

SEQ ID NO: 52
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMetSWVRQAPGKGLEWVSSIWYQGLVTVYADSVK
GRFTISRDNSKNTLYLQMetNSLRAEDTAVYYCAKWSAAFDYWGQGTLVTVSS

SEQ ID NO: 53
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT
GGAGTGGGTCTCAAGTATTTGGCGGAATGGTATTGTTACAGTTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCCGTATATTACTGTGCGAAATGGTCTGCTGGTTATGACTACTGGGGCCAGGGAACCCTGGT
CACCGTCTCGAGC

SEQ ID NO: 54
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWYQGLVTVYADSVKGR
FTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAGYDYWGQGTLVTVSS

SEQ ID NO: 55
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT
GGAGTGGGTCTCAAGTATTTGGCGGAATGGTATTGTTACAGTTTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG

ACACGGCCGTATATTACTGTGCGAAATGGTCTAAGGGTTTTGACTACTGGGGCCAGGGAACCCTGGT
CACCGTCTCGAGC

SEQ ID NO: 56
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWYQGLVTVYADSVKGR
FTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSKGFDYWGQGTLVTVSS

EXAMPLE 5. Germline revisions in anti-PDL1 antibody C5H9 light variable domain enhances manufacturability and reduces potential immunogenicity.

[000584] This example describes an additional anti-PDL1 antibody embodiment that exhibits reduced predicted immunogenicity, reduced aggregation, and improved expression.

[000585] In silico immunogenicity prediction, using ProPred revealed a strong potential epitope in CDR2 of the light variable domain of C5H9; anti-PDL1 antibody C5H9, also referred to herein as C5H9, comprises a light chain comprising SEQ ID NO: 12 and a heavy chain comprising SEQ ID NO: 46. Alignment of the light variable domain of C5H9 with human germline antibodies revealed two amino acids in CDR2 that when reverted to germline eliminated the predicted epitope. The residues are underlined and in bold text in the sequence shown below in SEQ ID NO: 12.

[000586] Furthermore, framework 4 of the C5H9 light chain was modified as indicated with bold italics in SEQ ID NO: 58 substituting glycine (G) for glutamine (Q) in the J region. This modification was aimed at increasing the flexibility of the junction between variable and constant regions, potentially reducing the tendency of C5H9 to form aggregates. The resultant DNA and amino acid sequences are described by SEQ ID NO: 57 and SEQ ID NO: 58, respectively. The resultant anti-PDL1 antibody, referred to herein as anti-PDL1 antibody C5H9v2, also referred to herein as C5H9v2, comprises a light chain comprising SEQ ID NO: 58 and a heavy chain comprising SEQ ID NO: 46.

SEQ ID NO: 12
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY**Y**AS**T**LQSGVPSRFSGSGS
GTDFTLTISSLQPEDFATYYCQQDNGYPSTFGQGTKVEIKR

SEQ ID NO: 57
GATATTCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCGAGCGTGGGCGATCGCGTGACCATTACCT
GCCGCGCGAGCCAGAGCATTAGCAGCTATCTGAACTGGTATCAGCAGAAACCGGGCAAAGCGCCGAA
ACTGCTGATTTATGCGGCGAGCAGCCTGCAGAGCGGCGTGCCGAGCCGCTTTAGCGGCAGCGGCAGC

GGCACCGATTTTACCCTGACCATTAGCAGCCTGCAGCCGGAAGATTTTGCGACCTATTATTGCCAGC
AGGATAACGGCTATCCGAGCACCTTTGGCGGCGGCACCAAAGTGGAAATTAAACGC

SEQ ID NO: 58
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGS
GTDFTLTISSLQPEDFATYYCQQDNGYPSTFG***G***GTKVEIKR

[000587] Figures 8A and 8B show that germline revisions described in SEQ ID NO: 58 reduces the predicted immunogenicity (top panel) and aggregation potential, and increases the expression level by three fold.

EXAMPLE 6. *In vitro* characterization of anti-PDL1 antibodies C5H9 and C5H9v2.

[000588] This Example demonstrates the binding specificities and biological activities of anti-PDL1 antibodies of the disclosure.

[000589] Production of fully human IgGs comprising the variable domains of the matured clones was accomplished using techniques similar to those described in PCT Publication No. WO 2010/081173. DNA encoding the heavy variable domains of anti-PDL1 antibody C5H9 (nucleic acid SEQ ID NO: 45; amino acid SEQ ID NO: 46) were amplified and cloned into vectors for the expression of fully human IgGs. DNA encoding the light variable domains of anti-PDL1 antibody C5H9 (nucleic acid SEQ ID NO: 11; amino acid SEQ ID NO: 12) were amplified and cloned into vectors for the expression of fully human IgGs. Similarly, DNA encoding the heavy variable domains of anti-PDL1 antibody C5H9 (nucleic acid SEQ ID NO: 45; amino acid SEQ ID NO: 46) were amplified and cloned into vectors for the expression of chimeric mouse IgG2as. DNA encoding the light variable domains of anti-PDL1 antibody C5H9v2 (nucleic acid SEQ ID NO: 57; amino acid SEQ ID NO: 58) were amplified and cloned into vectors for the expression of chimeric mouse IgG2as. Fully human IgGs or chimeric mouse IgG2a anti-PDL1 antibodies were expressed from transiently transfected HEK-293 cells and purified from the culture supernatant by Protein A chromatography. Anti-PDL1 IgG antibody C5H9 comprises a heavy chain comprising a heavy variable domain comprising SEQ ID NO: 46 and a light chain comprising a light variable domain comprising SEQ ID NO: 12. Anti-PDL1 IgG antibody C5H9v2 comprises a heavy chain comprising a heavy variable domain comprising SEQ ID NO: 46 and a light chain comprising a light variable domain comprising SEQ ID NO: 58.

[000590] Anti-PDL1 antibody C5H9v2 was characterized for species specific binding to PDL1 and whether that antibody would bind a panel of human proteins. Briefly human PDL1-Fc, mouse PDL1-Fc (R & D Systems, Minneapolis, MN) or cynomolgus monkey (cyno) PDL1-Fc (Sino biological) was adsorbed to the wells of a 96-well ELISA plate. Purified anti-PDL1 antibodies C5H9 and C5H9v2 were applied to the plate and allowed to bind. Bound antibody was visualized with an anti-human IgG-HRP conjugate, Fab specific, (Sigma, St Louis, MO; Cat # A0293-1ML) and developed with the chromogenic substrate TMB. Figure 9 shows that both anti-PDL1 antibodies C5H9 and C5H9v2 bind human, mouse and cyno PDL1 with near equal affinity. EC_{50} values for anti-PDL1 antibody C5H9v2 to human, cyno, rat and mouse PDL1 were also similar: 0.25 nM, 0.28 nM, 0.31 nM, and 0.30 nM, respectively.

[000591] In a similar experiment the binding of anti-PDL1 antibody C5H9 was evaluated for binding to a panel of human and mouse proteins. Briefly, each protein, listed in Figure 10, was absorbed to the wells of an ELISA plate. Purified anti-PDL1 antibodies C5H9 and C5H9v2 were applied to the plate and allowed to bind. Bound antibody was visualized with an anti-human IgG-HRP conjugate, Fab specific, (Sigma, St Louis, MO; Cat # A0293-1ML) and developed with the chromogenic substrate TMB. Anti-PDL1 antibody C5H9v2 binds only to human and mouse PDL1 demonstrating specificity toward PDL1.

[000592] The biological activity of PDL1 is mediated through binding to PD1 and/or to B7-1. Blockade of binding is a desired characteristic for a therapeutic antibody; therefore, the potency of blocking the interaction of PDL1 with PD1 and B7-1 was measured. Briefly, human, mouse or cyno PDL1 was adsorbed to the well of an ELISA plate. Biotinylated human, mouse or cyno PD1 or B7-1 was applied to the wells in the absence or the presence of an increasing concentration of either anti-PDL1 antibody C5H9 or anti-PDL1 antibody C5H9v2 and allowed to bind. Bound biotinylated PD1 or B7-1 was visualized with a Streptavidin-HRP conjugate (Thermo Scientific, Cat. No. 21126) and developed with the chromogenic substrate TMB. Figure 11 shows that both anti-PDL1 antibody C5H9 and anti-PDL1 antibody C5H9v2 are potent blockers of either B7-1 or PD1 binding to PDL1, and that blockade includes all three species human, cyno and mouse with single digit nM EC_{50}'s.

EXAMPLE 7: An anti-PDL1 antibody of the disclosure accelerates induction of diabetes in NOD mice

[000593] In this Example, anti-PDL1 antibody C5H9 was analyzed for the ability to induce diabetes in NOD mice.

[000594] The NOD mice, substrain NOD/ShiLtJ, were obtained from Jackson Laboratory at 6 weeks and acclimated on site for 1 week. At 7 weeks, mice were checked for diabetes prior to enrollment, grouped, and dosed as set forth in Table 6.

Table 6. Groups and Doses for NOD study

Group	Count	Treatment	Dose (mg/kg) Loading/regular	Dose volume (mL/kg)	Schedule	Route
1	6	Control	20/10	10	q2dx6	IP
2	6	Anti-PDL1(C5H9)	20/10	10	q2dx6	IP
3	6	Anti-PDL1 (10F9G2)	20/10	10	q2dx6	IP

[000595] Figure 12, which plots % non-diabetic versus number of days post initial dose, shows that anti-PDL1 antibody C5H9 antibody (Gr2-C5H9) induces diabetes in NOD mice similar to positive control anti-PDL1 antibody 10F9G2 antibody. A control rat IgG2b did not induce diabetes in NOD mice.

[000596] Anti-PDL1 antibody C5H9v2 was also tested for the ability to induce diabetes in NOD mice in a dose dependent matter, using a method similar to that described above, with 9-week old animals using the groups and doses set forth in Table 7.

Table 7. Groups and doses for dose dependent NOD study

Group	Count	Gender	Treatment	Dose (mg/kg)	Dose volume (mL/kg)	Schedule	Route
1	8	F	mIgG2a (C1.18.4)	25	10	Single dose	IP
2	8	F	Anti-PDL1(C5H9v2)	25	10	Single dose	IP
3	8	F	Anti-PDL1(C5H9v2)	5	10	Single dose	IP
4	8	F	Anti-PDL1(C5H9v2)	1	10	Single dose	IP
5	8	F	Anti-PDL1(C5H9v2)	0.2	10	Single dose	IP

[000597] Figure 13, which plots % non-diabetic versus number of days post initial dose, demonstrates that anti-PDL1 antibody C5H9v2 induces diabetes in NOD mice in a dose dependent matter.

EXAMPLE 8. An anti-PDL1 antibody of the embodiments reduces MC38 tumors in mice

[000598] In this Example, anti-PDL1 antibody C5H9v2 was analyzed for the ability to reduce the growth of MC38 syngeneic tumors.

[000599] The mouse colon carcinoma cell line MC38 was obtained from ATCC (American Type Culture Collection, Manassas, VA). MC38 cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum at 37°C in an atmosphere of 5% CO_2 in air. Cells were harvested during the logarithmic growth period, resuspended in PBS, and kept on ice for tumor induction.

[000600] Each mouse was inoculated subcutaneously at the right flank with $1X10^6$ of MC38 cells in PBS for tumor development. The treatments were started when the mean tumor size reached approximately 100-200 mm^3 (no more than 200 mm^3). Tumor sizes were measured twice weekly in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$ where a and b are the long and short diameters of the tumor, respectively.

[000601] The mice were grouped and dosed as set forth in Table 8.

Table 8. Groups and doses for MC38 syngeneic study

Group	Count	Treatment	Dose (mg/kg)	Dose volume (mL/kg)	Schedule	Route
1	10	Anti-PDL1 (10F9G2)	10	10	t.i.w. for 2 weeks (MWF)	IP
2	10	Anti-PDL1(C5H9v2)	10	10	t.i.w. for 2 weeks (MWF)	IP
3	10	mIgG2a + Hamster IgG1	10	10	t.i.w. for 2 weeks (MWF)	IP

[000602] Figure 14, which plots tumor volume versus number of days post initial dose, demonstrates that anti-PDL1 antibody C5H9v2 inhibits the growth of MC38 syngeneic tumors similar to positive control anti-PDL1 antibody 10F9G2.

EXAMPLE 9. Anti-PDL1 activatable antibody C5H9v2 masking moieties

[000603] This Example describes identification of masking moieties (MM) to reduce binding of activatable antibodies of anti-PDL1 antibody C5H9v2 to their target.

[000604] Anti-PDL1 antibody C5H9v2 and Fab were used to screen the libraries using a method similar to that described in PCT International Publication Number WO 2010/081173, published 15 July 2010. The screening consisted of one round of MACS and three rounds of FACS sorting. The initial MACS was done with protein-A Dynabeads (Invitrogen) and the anti-PDL1 antibody C5H9v2 at a concentration of 100 nM. For MACS, approximately $1x10^{11}$ cells were screened for binding and $6x10^{6}$ cells were collected. Anti-PDL1 antibody C5H9v2, directly labeled with Alexafluor-488, was used as a probe for all FACS selections. In the first round of FACS, the cells were labeled with 100 nM AlexaFluor- anti-PDL1 antibody C5H9v2. In the second round of FACS, the cells were labeled with 10 nM AlexaFluor- anti-PDL1 antibody C5H9v2. In the third round of FACS, the cells were labeled with 1 nM AlexaFluor- anti-PDL1 antibody C5H9v2. The positive population from the third FACS round was verified to be inhibited by recombinant human PDL1 protein from binding to the anti-PDL1 antibody C5H9v2 and Fab. Individual peptide clones were identified by sequence analysis and subsequently verified for their ability to bind the anti-PDL1 antibody C5H9v2 and Fab.

[000605] The sequences of the anti-PDL1 antibody C5H9v2 masking moieties are listed in Table 9.

Table 9. anti-PDL1 antibody C5H9v2 masking moieties (MM)

MM	Amino Acid Sequence	SEQ ID NO
PL01	YCEVSELFVLPWCMG	SEQ ID NO: 208
PL02	SCLMHPHYAHDYCYV	SEQ ID NO: 426
PL03	LCEVLMLLQHPWCMG	SEQ ID NO: 59
PL04	IACRHFMEQLPFCHH	SEQ ID NO: 60
PL05	FGPRCGEASTCVPYE	SEQ ID NO: 61
PL06	ILYCDSWGAGCLTRP	SEQ ID NO: 62
PL07	GIALCPSHFCQLPQT	SEQ ID NO: 63
PL08	DGPRCFVSGECSPIG	SEQ ID NO: 64
PL09	LCYKLDYDDRSYCHI	SEQ ID NO: 65

PL10	PCHPHPYDARPYCNV	SEQ ID NO: 66
PL11	PCYWHPFFAYRYCNT	SEQ ID NO: 67
PL12	VCYYMDWLGRNWCSS	SEQ ID NO: 68
PL13	LCDLFKLREFPYCMG	SEQ ID NO: 69
PL14	YLPCHFVPIGACNNK	SEQ ID NO: 70
PL15	IFCHMGVVVPQCANY	SEQ ID NO: 71
PL16	ACHPHPYDARPYCNV	SEQ ID NO: 72
PL17	PCHPAPYDARPYCNV	SEQ ID NO: 73
PL18	PCHPHAYDARPYCNV	SEQ ID NO: 74
PL19	PCHPHPADARPYCNV	SEQ ID NO: 75
PL20	PCHPHPYAARPYCNV	SEQ ID NO: 76
PL21	PCHPHPYDAAPYCNV	SEQ ID NO: 77
PL22	PCHPHPYDARPACNV	SEQ ID NO: 78
PL23	PCHPHPYDARPYCAV	SEQ ID NO: 79
PL24	PCHAHPYDARPYCNV	SEQ ID NO: 80
PL25	PCHPHPYDARAYCNV	SEQ ID NO: 81

EXAMPLE 10. Activatable antibodies comprising anti-PDL1 antibody C5H9v2

[000606] This Example describes examples of activatable antibodies of the disclosure comprising anti-PDL1 antibody C5H9v2.

[000607] Anti-PDL1 activatable antibodies comprising an anti-PDL1 antibody C5H9v2 masking moiety, a cleavable moiety, and anti-PDL1 antibody C5H9v2 were produced according to methods similar to those described in PCT Publication Nos. WO 2009/025846 and WO 2010/081173. Quality control of the resultant activatable antibodies indicated that most comprised at least 95% monomer. The amino acid and nucleic acid sequences of several activatable antibodies comprising anti-PDL1 antibody C5H9v2 are provided below. Each activatable antibody comprises anti-PDL1 antibody C5H9v2 having the variable heavy chain amino acid sequence shown in SEQ ID NO: 46 and a light chain amino acid sequence selected from the group consisting of the amino acid sequences shown in SEQ ID NO: 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959,

961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1144-1191, 1200, and 1201 below.

[000608] While the sequences shown below include the spacer sequence of SEQ ID NO: 923, those of ordinary skill in the art appreciate that the activatable anti-PDL1 antibodies of the disclosure can include any suitable spacer sequence, such as, for example, a spacer sequence selected from the group consisting of QGQSGS (SEQ ID NO: 923); GQSGS (SEQ ID NO: 1192); QSGS (SEQ ID NO: 1193); SGS (SEQ ID NO: 1194); GS (SEQ ID NO: 1195); S; QGQSGQG (SEQ ID NO: 924); GQSGQG (SEQ ID NO: 395); QSGQG (SEQ ID NO: 925); SGQG (SEQ ID NO: 926); GQG (SEQ ID NO: 927); QG (SEQ ID NO: 928); G; QGQSGQ (SEQ ID NO: 1196); GQSGQ (SEQ ID NO: 1197); QSGQ (SEQ ID NO: 1198); SGQ (SEQ ID NO: 1198); GQ (SEQ ID NO: 1199); and Q. While the sequences shown below include the spacer sequence of SEQ ID NO: 923, those of ordinary skill in the art will also appreciate that activatable anti-PDL1 antibodies of the disclosure in some embodiments do not include a spacer sequence.

C5H9v2 VH amino acid sequence
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRNGIVTVYADSVKGR
FTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAAFDYWGQGTLVTVSS (SEQ ID NO: 46)

[spacer (SEQ ID NO: 929)][PL01-0003 LC (SEQ ID NO: 930)]
[CAAGGTCAGTCTGGATCC][TATTGCGAGGTTAGTGAGCTGTTTGTTCTTCCTTGGTGCATGGGTG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 82)

[spacer (SEQ ID NO: 923)][PL01-0003 LC (SEQ ID NO: 931)]
[QGQSGS][YCEVSELFVLPWCMGGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 83)

PL01-0003 LC nucleotide sequence
TATTGCGAGGTTAGTGAGCTGTTTGTTCTTCCTTGGTGCATGGGTGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 930)

PL01-0003 LC amino acid sequence
YCEVSELFVLPWCMGGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 931)

[spacer (SEQ ID NO: 929)][PL02-0003 LC (SEQ ID NO: 932)]
[CAAGGTCAGTCTGGATCC][TCTTGCCTTATGCATCCGCATTATGCTCATGATTATTGCTATGTTG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 84)

[spacer (SEQ ID NO: 923)][PL02-0003 LC (SEQ ID NO: 933)]
[QGQSGS][SCLMHPHYAHDYCYVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 85)

PL02-0003 LC nucleotide sequence
TCTTGCCTTATGCATCCGCATTATGCTCATGATTATTGCTATGTTGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC

CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 932)

PL02-0003 LC amino acid sequence
SCLMHPHYAHDYCYVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 933)

[spacer (SEQ ID NO: 929)][PL03-0003 LC (SEQ ID NO: 934)]
[CAAGGTCAGTCTGGATCC][TTGTGCGAGGTTTTGATGTTGTTGCAGCATCCGTGGTGCATGGGGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 86)

[spacer (SEQ ID NO: 923)][PL03-0003 LC (SEQ ID NO: 935)]
[QGQSGS][LCEVLMLLQHPWCMGGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 87)

PL03-0003 LC nucleotide sequence
TTGTGCGAGGTTTTGATGTTGTTGCAGCATCCGTGGTGCATGGGGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 934)

PL03-0003 LC amino acid sequence
LCEVLMLLQHPWCMGGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 935)

[spacer (SEQ ID NO: 929)][PL04-0003 LC (SEQ ID NO: 936)]
[CAAGGTCAGTCTGGATCC][ATTGCGTGCCGGCATTTTATGGAGCAGTTGCCGTTTTGCCATCATG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 88)

[spacer (SEQ ID NO: 923)][PL04-0003 LC (SEQ ID NO: 937)]
[QGQSGS][IACRHFMEQLPFCHHGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 89)

PL04-0003 LC nucleotide sequence
ATTGCGTGCCGGCATTTTATGGAGCAGTTGCCGTTTTGCCATCATGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 936)

PL04-0003 LC amino acid sequence
IACRHFMEQLPFCHHGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 937)

[spacer (SEQ ID NO: 929)][PL05-0003 LC (SEQ ID NO: 938)]
[CAAGGTCAGTCTGGATCC][TTTGGTCCTAGGTGCGGTGAGGCTTCTACTTGCGTTCCGTATGAGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 90)

[spacer (SEQ ID NO: 923)][PL05-0003 LC (SEQ ID NO: 939)]
[QGQSGS][FGPRCGEASTCVPYEGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 91)

PL05-0003 LC nucleotide sequence
TTTGGTCCTAGGTGCGGTGAGGCTTCTACTTGCGTTCCGTATGAGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 938)

PL05-0003 LC amino acid sequence
FGPRCGEASTCVPYEGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 939)

[spacer (SEQ ID NO: 929)][PL06-0003 LC (SEQ ID NO: 940)]
[CAAGGTCAGTCTGGATCC][ATTCTTTATTGCGATAGTTGGGGGGCGGGGTGCTTGACGCGGCCGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT

TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 92)

[spacer (SEQ ID NO: 923)][PL06-0003 LC (SEQ ID NO: 941)]
[QGQSGS][ILYCDSWGAGCLTRPGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 93)

PL06-0003 LC nucleotide sequence
ATTCTTTATTGCGATAGTTGGGGGGCGGGGTGCTTGACGCGGCCGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 940)

PL06-0003 LC amino acid sequence
ILYCDSWGAGCLTRPGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 941)

[spacer (SEQ ID NO: 929)][PL07-0003 LC (SEQ ID NO: 942)]
[CAAGGTCAGTCTGGATCC][GGGATTGCGTTGTGCCCGTCTCATTTTTGCCAGCTGCCTCAGACTG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 94)

[spacer (SEQ ID NO: 923)][PL07-0003 LC (SEQ ID NO: 943)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 95)

PL07-0003 LC nucleotide sequence
GGGATTGCGTTGTGCCCGTCTCATTTTTGCCAGCTGCCTCAGACTGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 942)

PL07-0003 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 943)

[spacer (SEQ ID NO: 929)][PL08-0003 LC (SEQ ID NO: 944)]
[CAAGGTCAGTCTGGATCC][GATGGGCCGCGTTGCTTTGTGTCGGGGGAGTGCTCTCCGATTGGTG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 96)

[spacer (SEQ ID NO: 923)][PL08-0003 LC (SEQ ID NO: 945)]
[QGQSGS][DGPRCFVSGECSPIGGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 97)

PL08-0003 LC nucleotide sequence
GATGGGCCGCGTTGCTTTGTGTCGGGGGAGTGCTCTCCGATTGGTGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 944)

PL08-0003 LC amino acid sequence
DGPRCFVSGECSPIGGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 945)

[spacer (SEQ ID NO: 929)][PL09-0003 LC (SEQ ID NO: 946)]
[CAAGGTCAGTCTGGATCC][TTGTGCTATAAGCTGGATTATGATGATAGGTCTTATTGCCATATTG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 98)

[spacer (SEQ ID NO: 923)][PL09-0003 LC (SEQ ID NO: 947)]
[QGQSGS][LCYKLDYDDRSYCHIGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 99)

PL09-0003 LC nucleotide sequence
TTGTGCTATAAGCTGGATTATGATGATAGGTCTTATTGCCATATTGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC

CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 946)

PL09-0003 LC amino acid sequence
LCYKLDYDDRSYCHIGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 947)

[spacer (SEQ ID NO: 929)][PL10-0003 LC (SEQ ID NO: 948)]
[CAAGGTCAGTCTGGATCC][CCGTGCCATCCGCATCCTTATGATGCTCGTCCTTATTGCAATGTGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 100)

[spacer (SEQ ID NO: 923)][PL10-0003 LC (SEQ ID NO: 949)]
[QGQSGS][PCHPHPYDARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR](SEQ ID NO: 101)

PL10-0003 LC nucleotide sequence
CCGTGCCATCCGCATCCTTATGATGCTCGTCCTTATTGCAATGTGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 948)

PL10-0003 LC amino acid sequence
PCHPHPYDARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR(SEQ ID NO: 949)

[spacer (SEQ ID NO: 929)][PL11-0003 LC (SEQ ID NO: 950)]
[CAAGGTCAGTCTGGATCC][CCTTGCTATTGGCATCCTTTTTTTGCGTATAGGTATTGCAATACTG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 102)

[spacer (SEQ ID NO: 923)][PL11-0003 LC (SEQ ID NO: 951)]
[QGQSGS][PCYWHPFFAYRYCNTGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 103)

PL11-0003 LC nucleotide sequence
CCTTGCTATTGGCATCCTTTTTTTGCGTATAGGTATTGCAATACTGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 950)

PL11-0003 LC amino acid sequence
PCYWHPFFAYRYCNTGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 951)

[spacer (SEQ ID NO: 929)][PL12-0003 LC (SEQ ID NO: 952)]
[CAAGGTCAGTCTGGATCC][GTTTGCTATTATATGGATTGGTTGGGGCGGAATTGGTGCTCTTCGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 104)

[spacer (SEQ ID NO: 923)][PL12-0003 LC (SEQ ID NO: 953)]
[QGQSGS][VCYYMDWLGRNWCSSGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 105)

PL12-0003 LC nucleotide sequence
GTTTGCTATTATATGGATTGGTTGGGGCGGAATTGGTGCTCTTCGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 952)

PL12-0003 LC amino acid sequence
VCYYMDWLGRNWCSSGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 953)

[spacer (SEQ ID NO: 929)][PL13-0003 LC (SEQ ID NO: 954)]
[CAAGGTCAGTCTGGATCC][CTGTGCGATCTGTTTAAGTTGCGTGAGTTTCCTTATTGCATGGGGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT

TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 106)

[spacer (SEQ ID NO: 923)][PL13-0003 LC (SEQ ID NO: 955)]
[QGQSGS][LCDLFKLREFPYCMGGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 107)

PL13-0003 LC nucleotide sequence
CTGTGCGATCTGTTTAAGTTGCGTGAGTTTCCTTATTGCATGGGGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 954)

PL13-0003 LC amino acid sequence
LCDLFKLREFPYCMGGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 955)

[spacer (SEQ ID NO: 929)][PL14-0003 LC (SEQ ID NO: 956)]
[CAAGGTCAGTCTGGATCC][TATCTTCCGTGCCATTTTGTTCCGATTGGGGCTTGCAATAATAAGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 108)

[spacer (SEQ ID NO: 923)][PL14-0003 LC (SEQ ID NO: 957)]
[QGQSGS][YLPCHFVPIGACNNKGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 109)

PL14-0003 LC nucleotide sequence
TATCTTCCGTGCCATTTTGTTCCGATTGGGGCTTGCAATAATAAGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 956)

PL14-0003 LC amino acid sequence
YLPCHFVPIGACNNKGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 957)

[spacer (SEQ ID NO: 929)][PL15-0003 LC (SEQ ID NO: 958)]
[CAAGGTCAGTCTGGATCC][ATTTTTTGCCATATGGGTGTTGTGGTTCCTCAGTGCGCGAATTATG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 110)

[spacer (SEQ ID NO: 923)][PL15-0003 LC (SEQ ID NO: 959)]
[QGQSGS][IFCHMGVVVPQCANYGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 111)

PL15-0003 LC nucleotide sequence
ATTTTTTGCCATATGGGTGTTGTGGTTCCTCAGTGCGCGAATTATGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 958)

PL15-0003 LC amino acid sequence
IFCHMGVVVPQCANYGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 959)

[spacer (SEQ ID NO: 929)][PL16-0003 LC (SEQ ID NO: 960)]
[CAAGGTCAGTCTGGATCC][GCGTGCCATCCGCATCCTTATGATGCTCGTCCTTATTGCAATGTGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 112)

[spacer (SEQ ID NO: 923)][PL16-0003 LC (SEQ ID NO: 961)]
[QGQSGS][ACHPHPYDARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 113)

PL16-0003 LC nucleotide sequence
GCGTGCCATCCGCATCCTTATGATGCTCGTCCTTATTGCAATGTGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC

CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 960)

PL16-0003 LC amino acid sequence
ACHPHPYDARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 961)

[spacer (SEQ ID NO: 929)][PL17-0003 LC (SEQ ID NO: 962)]
[CAAGGTCAGTCTGGATCC][CCGTGCCATCCGGCTCCTTATGATGCTCGTCCTTATTGCAATGTGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 114)

[spacer (SEQ ID NO: 923)][PL17-0003 LC (SEQ ID NO: 963)]
[QGQSGS][PCHPAPYDARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 115)

PL17-0003 LC nucleotide sequence
CCGTGCCATCCGGCTCCTTATGATGCTCGTCCTTATTGCAATGTGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 962)

PL17-0003 LC amino acid sequence
PCHPAPYDARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 963)

[spacer (SEQ ID NO: 929)][PL18-0003 LC (SEQ ID NO: 964)]
[CAAGGTCAGTCTGGATCC][CCGTGCCATCCGCATGCTTATGATGCTCGTCCTTATTGCAATGTGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 116)

[spacer (SEQ ID NO: 923)][PL18-0003 LC (SEQ ID NO: 965)]
[QGQSGS][PCHPHAYDARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 117)

PL18-0003 LC nucleotide sequence
CCGTGCCATCCGCATGCTTATGATGCTCGTCCTTATTGCAATGTGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 964)

PL18-0003 LC amino acid sequence
PCHPHAYDARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 965)

[spacer (SEQ ID NO: 929)][PL19-0003 LC (SEQ ID NO: 966)]
[CAAGGTCAGTCTGGATCC][CCGTGCCATCCGCATCCTGCTGATGCTCGTCCTTATTGCAATGTGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 118)

[spacer (SEQ ID NO: 923)][PL19-0003 LC (SEQ ID NO: 967)]
[QGQSGS][PCHPHPADARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 119)

PL19-0003 LC nucleotide sequence
CCGTGCCATCCGCATCCTGCTGATGCTCGTCCTTATTGCAATGTGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 966)

PL19-0003 LC amino acid sequence
PCHPHPADARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 967)

[spacer (SEQ ID NO: 929)][PL20-0003 LC (SEQ ID NO: 968)]
[CAAGGTCAGTCTGGATCC][CCGTGCCATCCGCATCCTTATGCTGCTCGTCCTTATTGCAATGTGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT

TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 120)

[spacer (SEQ ID NO: 923)][PL20-0003 LC (SEQ ID NO: 969)]
[QGQSGS][PCHPHPYAARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 121)

PL20-0003 LC nucleotide sequence
CCGTGCCATCCGCATCCTTATGCTGCTCGTCCTTATTGCAATGTGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 968)

PL20-0003 LC amino acid sequence
PCHPHPYAARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 969)

[spacer (SEQ ID NO: 929)][PL21-0003 LC (SEQ ID NO: 970)]
[CAAGGTCAGTCTGGATCC][CCGTGCCATCCGCATCCTTATGATGCTGCTCCTTATTGCAATGTGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 122)

[spacer (SEQ ID NO: 923)][PL21-0003 LC (SEQ ID NO: 971)]
[QGQSGS][PCHPHPYDAAPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 123)

PL21-0003 LC nucleotide sequence
CCGTGCCATCCGCATCCTTATGATGCTGCTCCTTATTGCAATGTGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 970)

PL21-0003 LC amino acid sequence
PCHPHPYDAAPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 971)

[spacer (SEQ ID NO: 929)][PL22-0003 LC (SEQ ID NO: 972)]
[CAAGGTCAGTCTGGATCC][CCGTGCCATCCGCATCCTTATGATGCTCGTCCTGCTTGCAATGTGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 124)

[spacer (SEQ ID NO: 923)][PL22-0003 LC (SEQ ID NO: 973)]
[QGQSGS][PCHPHPYDARPACNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 125)

PL22-0003 LC nucleotide sequence
CCGTGCCATCCGCATCCTTATGATGCTCGTCCTGCTTGCAATGTGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 972)

PL22-0003 LC amino acid sequence
PCHPHPYDARPACNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 973)

[spacer (SEQ ID NO: 929)][PL23-0003 LC (SEQ ID NO: 974)]
[CAAGGTCAGTCTGGATCC][CCGTGCCATCCGCATCCTTATGATGCTCGTCCTTATTGCGCTGTGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 126)

[spacer (SEQ ID NO: 923)][PL23-0003 LC (SEQ ID NO: 975)]
[QGQSGS][PCHPHPYDARPYCAVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 127)

PL23-0003 LC nucleotide sequence
CCGTGCCATCCGCATCCTTATGATGCTCGTCCTTATTGCGCTGTGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC

CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 974)

PL23-0003 LC amino acid sequence
PCHPHPYDARPYCAVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 975)

[spacer (SEQ ID NO: 929)][PL24-0003 LC (SEQ ID NO: 976)]
[CAAGGTCAGTCTGGATCC][CCGTGCCATGCGCATCCTTATGATGCTCGTCCTTATTGCAATGTGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 128)

[spacer (SEQ ID NO: 923)][PL24-0003 LC (SEQ ID NO: 977)]
[QGQSGS][PCHAHPYDARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 129)

PL24-0003 LC nucleotide sequence
CCGTGCCATGCGCATCCTTATGATGCTCGTCCTTATTGCAATGTGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 976)

PL24-0003 LC amino acid sequence
PCHAHPYDARPYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 977)

[spacer (SEQ ID NO: 929)][PL25-0003 LC (SEQ ID NO: 978)]
[CAAGGTCAGTCTGGATCC][CCGTGCCATCCGCATCCTTATGATGCTCGTGCTTATTGCAATGTGG
GAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCC
ACGTGGCGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGAC
AGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGC
CCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATT
TTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCA
AGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTG
T] (SEQ ID NO: 130)

[spacer (SEQ ID NO: 923)][PL25-0003 LC (SEQ ID NO: 979)]
[QGQSGS][PCHPHPYDARAYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASV
GDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED
FATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 131)

PL25-0003 LC nucleotide sequence
CCGTGCCATCCGCATCCTTATGATGCTCGTGCTTATTGCAATGTGGGAGGTGGCTCGAGCGGTGGCA
GCGGTGGCTCTGGTGGTACTAGCACCTCTGGTCGTTCCGCTAACCCACGTGGCGGCGGTTCTGACAT
CCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGA
GCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGC
TGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCAC
CGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGAC
AACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACA
AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT (SEQ ID NO: 978)

PL25-0003 LC amino acid sequence
PCHPHPYDARAYCNVGGGSSGGSGGSGGTSTSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQD
NGYPSTFGGGTKVEIKR (SEQ ID NO: 979)

[spacer (SEQ ID NO: 929)][PL03-2001 LC (SEQ ID NO: 980)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCA
GTGGTCTGTTAAGCGGTCGTAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAG
CAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGC
TACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTC
TGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAG
CTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTT
GGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTG
TCAAGAGCTTCAACAGGAATGAGTGT] (SEQ ID NO: 132)

[spacer (SEQ ID NO: 923)][PL03-2001 LC (SEQ ID NO: 981)]
[QGQSGS][LCEVLMLLQHPWCMGGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 133)

PL03-2001 LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCAGTGGTCTGTTAAGCGGTCGTA
GCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGG
CGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG
AAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCA
GATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTT
CGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAA
ATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATG
AGTGT (SEQ ID NO: 980)

PL03-2001 LC amino acid sequence
LCEVLMLLQHPWCMGGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 981)

[spacer (SEQ ID NO: 923)][PL04-2001 LC (SEQ ID NO: 1183)]
[QGQSGS][IACRHFMEQLPFCHHGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1182)

PL04-2001 LC amino acid sequence
IACRHFMEQLPFCHHGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1183)

[spacer (SEQ ID NO: 929)][PL06-2001-mk LC (SEQ ID NO: 982)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCA
GTGGTCTGTTAAGCGGTCGTAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAG
CAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGC
TACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTC
TGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAG
CTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTT
GGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTG
TCAAGAGCTTCAACAGGAATGAGTGT] (SEQ ID NO: 134)

[spacer (SEQ ID NO: 923)][PL06-2001-mk LC (SEQ ID NO: 983)]
[QGQSGS][ILYCDSWGAGCLTRPGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 135)

PL06-2001-mk LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCAGTGGTCTGTTAAGCGGTCGTA
GCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGG
CGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG
AAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCA
GATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTT
CGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAA
ATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATG
AGTGT (SEQ ID NO: 982)

PL06-2001-mk LC amino acid sequence
ILYCDSWGAGCLTRPGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 983)

[spacer (SEQ ID NO: 929)][PL07-2001 LC (SEQ ID NO: 984)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCA
GTGGTCTGTTAAGCGGTCGTAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAG
CAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGC
TACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTC
TGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAG
CTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTT
GGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTG
TCAAGAGCTTCAACAGGAATGAGTGT] (SEQ ID NO: 136)

[spacer (SEQ ID NO: 923)][PL07-2001 LC (SEQ ID NO: 985)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 137)

PL07-2001 LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCAGTGGTCTGTTAAGCGGTCGTA
GCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGG
CGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG
AAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCA
GATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTT
CGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAA
ATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATG
AGTGT (SEQ ID NO: 984)

PL07-2001 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 985)

[spacer (SEQ ID NO: 929)][PL10-2001 LC (SEQ ID NO: 986)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCA
GTGGTCTGTTAAGCGGTCGTAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAG
CAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGC
TACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTC
TGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAG
CTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTT

GGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTG
TCAAGAGCTTCAACAGGAATGAGTGT] (SEQ ID NO: 138)

[spacer (SEQ ID NO: 923)][PL10-2001 LC (SEQ ID NO: 987)]
[QGQSGS][PCHPHPYDARPYCNVGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 139)

PL10-2001 LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCAGTGGTCTGTTAAGCGGTCGTA
GCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGG
CGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG
AAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCA
GATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTT
CGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAA
ATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATG
AGTGT (SEQ ID NO: 986)

PL10-2001 LC amino acid sequence
PCHPHPYDARPYCNVGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 987)

[spacer (SEQ ID NO: 923)][PL11-2001 LC (SEQ ID NO: 1185)]
[QGQSGS][PCYWHPFFAYRYCNTGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1184)

PL11-2001 LC amino acid sequence
PCYWHPFFAYRYCNTGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1185)

[spacer (SEQ ID NO: 923)][PL12-2001 LC (SEQ ID NO: 1187)]
[QGQSGS][VCYYMDWLGRNWCSSGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1186)

PL12-2001 LC amino acid sequence
VCYYMDWLGRNWCSSGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1187)

[spacer (SEQ ID NO: 929)][PL14-2001 LC (SEQ ID NO: 988)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCA
GTGGTCTGTTAAGCGGTCGTAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAG
CAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGC
TACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTC
TGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAG
CTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTT
GGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTG
TCAAGAGCTTCAACAGGAATGAGTGT] (SEQ ID NO: 140)

[spacer (SEQ ID NO: 923)][PL14-2001 LC (SEQ ID NO: 989)]
[QGQSGS][YLPCHFVPIGACNNKGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 141)

PL14-2001 LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCAGTGGTCTGTTAAGCGGTCGTA
GCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGG
CGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG
AAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCA
GATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTT
CGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAA
ATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATG
AGTGT (SEQ ID NO: 988)

PL14-2001 LC amino acid sequence
YLPCHFVPIGACNNKGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 989)

[spacer (SEQ ID NO: 929)][PL15-2001 LC (SEQ ID NO: 990)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCA
GTGGTCTGTTAAGCGGTCGTAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAG
CAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGC
TACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTC
TGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAG
CTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTT
GGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTG
TCAAGAGCTTCAACAGGAATGAGTGT] (SEQ ID NO: 142)

[spacer (SEQ ID NO: 923)][PL15-2001 LC (SEQ ID NO: 991)]
[QGQSGS][IFCHMGVVVPQCANYGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 143)

PL15-2001 LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCAGTGGTCTGTTAAGCGGTCGTA
GCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGG
CGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG
AAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCA
GATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTT
CGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAA
ATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATG
AGTGT (SEQ ID NO: 990)

PL15-2001 LC amino acid sequence
IFCHMGVVVPQCANYGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 991)

[spacer (SEQ ID NO: 929)][PL18-2001 LC (SEQ ID NO: 992)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCA
GTGGTCTGTTAAGCGGTCGTAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAG
CAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGC
TACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTC
TGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAG
CTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTT

GGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTG
TCAAGAGCTTCAACAGGAATGAGTGT] (SEQ ID NO: 144)

[spacer (SEQ ID NO: 923)][PL18-2001 LC (SEQ ID NO: 993)]
[QGQSGS][PCHPHAYDARPYCNVGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 145)

PL18-2001 LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATTAGCAGTGGTCTGTTAAGCGGTCGTA
GCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGTGGG
CGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG
AAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCAGCA
GATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGACTT
CGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTGGAA
ATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATG
AGTGT (SEQ ID NO: 992)

PL18-2001 LC amino acid sequence
PCHPHAYDARPYCNVGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 993)

[spacer (SEQ ID NO: 923)][PL19-2001 LC (SEQ ID NO: 1189)]
[QGQSGS][PCHPHPADARPYCNVGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1188)

PL19-2001 LC amino acid sequence
PCHPHPADARPYCNVGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1189)

[spacer (SEQ ID NO: 923)][PL20-2001 LC (SEQ ID NO: 1191)]
[QGQSGS][PCHPHPYAARPYCNVGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1190)

PL20-2001 LC amino acid sequence
PCHPHPYAARPYCNVGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1191)

[spacer (SEQ ID NO: 929)][PL03-1004/GG/0001 (also referred to herein as PL03-3001) LC (SEQ ID NO: 994)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGCTGTGGGTCTCCTGGCTC
CCCCGGGCGGCCTGTCCGGCCGCAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCC
CAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGC
AGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCT
CTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCAT
CAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACC
TTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCA
TTGTCAAGAGCTTCAACAGGAATGAGTGT] (SEQ ID NO: 146)

[spacer (SEQ ID NO: 923)][PL03-1004/GG/0001 LC (SEQ ID NO: 995)]
[QGQSGS][LCEVLMLLQHPWCMGGGGSSGGSGAVGLLAPPGGLSGRSDNHGGSDIQMTQSPSSLSA
SVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQP
EDFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 147)

PL03-1004/GG/0001 LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGCTGTGGGTCTCCTGGCTCCCCCGGGCGGCCTGTCCGGCC
GCAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGT
GGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAG
CAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCA
GCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGA
CTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTG
GAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGA
ATGAGTGT (SEQ ID NO: 994)

PL03-1004/GG/0001 LC amino acid sequence
LCEVLMLLQHPWCMGGGGSSGGSGAVGLLAPPGGLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTIT
CRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
QDNGYPSTFGGGTKVEIKR (SEQ ID NO: 995)

[spacer (SEQ ID NO: 929)][PL06-1004/GG/0001 (also referred to herein as PL06-3001) LC (SEQ ID NO: 996)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGCTGTGGGTCTCCTGGCTC
CCCCGGGCGGCCTGTCCGGCCGCAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCC
CAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGC
AGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCT
CTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCAT
CAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACC
TTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCG
TCACAAAGAGCTTCAACAGGGGAGAGTGT] (SEQ ID NO: 148)

[spacer (SEQ ID NO: 923)][PL06-1004/GG/0001 LC (SEQ ID NO: 997)]
[QGQSGS][ILYCDSWGAGCLTRPGGGSSGGSGAVGLLAPPGGLSGRSDNHGGSDIQMTQSPSSLSA
SVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQP
EDFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 149)

PL06-1004/GG/0001 LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGCTGTGGGTCTCCTGGCTCCCCCGGGCGGCCTGTCCGGCC
GCAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGT
GGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAG
CAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCA
GCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGA
CTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTG
GAAATCAAGCGTTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGG
GAGAGTGT (SEQ ID NO: 996)

PL06-1004/GG/0001 LC amino acid sequence
ILYCDSWGAGCLTRPGGGSSGGSGAVGLLAPPGGLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTIT
CRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
QDNGYPSTFGGGTKVEIKR (SEQ ID NO: 997)

[spacer (SEQ ID NO: 929)][PL07-1004/GG/0001 (also referred to herein as PL07-3001) LC (SEQ ID NO: 998)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGCTGTGGGTCTCCTGGCTC
CCCCGGGCGGCCTGTCCGGCCGCAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCC
CAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGC
AGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCT

CTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCAT
CAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACC
TTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCG
TCACAAAGAGCTTCAACAGGGGAGAGTGT] (SEQ ID NO: 150)

[spacer (SEQ ID NO: 923)][PL01-1004/GG/0001 LC (SEQ ID NO: 999)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGAVGLLAPPGGLSGRSDNHGGSDIQMTQSPSSLSA
SVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQP
EDFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 151)

PL07-1004/GG/0001 LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGCTGTGGGTCTCCTGGCTCCCCCGGGCGGCCTGTCCGGCC
GCAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGT
GGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAG
CAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCA
GCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGA
CTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTG
GAAATCAAGCGTTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGG
GAGAGTGT (SEQ ID NO: 998)

PL07-1004/GG/0001 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGAVGLLAPPGGLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTIT
CRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
QDNGYPSTFGGGTKVEIKR (SEQ ID NO: 999)

[spacer (SEQ ID NO: 929)][PL14-1004/GG/0001 (also referred to
herein as PL14-3001) LC (SEQ ID NO: 1000)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGCTGTGGGTCTCCTGGCTC
CCCCGGGCGGCCTGTCCGGCCGCAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCC
CAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGC
AGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCT
CTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCAT
CAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACC
TTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCG
TCACAAAGAGCTTCAACAGGGGAGAGTGT] (SEQ ID NO: 152)

[spacer (SEQ ID NO: 923)][PL14-1004/GG/0001 LC (SEQ ID NO: 1001)]
[QGQSGS][YLPCHFVPIGACNNKGGGSSGGSGAVGLLAPPGGLSGRSDNHGGSDIQMTQSPSSLSA
SVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQP
EDFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 153)

PL14-1004/GG/0001 LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGCTGTGGGTCTCCTGGCTCCCCCGGGCGGCCTGTCCGGCC
GCAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGT
GGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAG
CAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCA
GCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGA
CTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTG
GAAATCAAGCGTTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGG
GAGAGTGT (SEQ ID NO: 1000)

PL14-1004/GG/0001 LC amino acid sequence
YLPCHFVPIGACNNKGGGSSGGSGAVGLLAPPGGLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTIT
CRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
QDNGYPSTFGGGTKVEIKR (SEQ ID NO: 1001)

[spacer (SEQ ID NO: 929)][PL15-1004/GG/0001 (also referred to
herein as PL15-3001) LC (SEQ ID NO: 1002)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGCTGTGGGTCTCCTGGCTC
CCCCGGGCGGCCTGTCCGGCCGCAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCC
CAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGC
AGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCT
CTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCAT
CAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACC
TTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCA
TTGTCAAGAGCTTCAACAGGAATGAGTGT] (SEQ ID NO: 154)

[spacer (SEQ ID NO: 923)][PL15-1004/GG/0001 LC (SEQ ID NO: 1003)]
[QGQSGS][IFCHMGVVVPQCANYGGGSSGGSGAVGLLAPPGGLSGRSDNHGGSDIQMTQSPSSLSA
SVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQP
EDFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 155)

PL15-1004/GG/0001 LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGCTGTGGGTCTCCTGGCTCCCCCGGGCGGCCTGTCCGGCC
GCAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGT
GGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAG
CAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCA
GCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGA
CTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTG
GAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGA
ATGAGTGT (SEQ ID NO: 1002)

PL15-1004/GG/0001 LC amino acid sequence
IFCHMGVVVPQCANYGGGSSGGSGAVGLLAPPGGLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTIT
CRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
QDNGYPSTFGGGTKVEIKR (SEQ ID NO: 1003)

[spacer (SEQ ID NO: 929)][PL18-1004/GG/0001 (also referred to
herein as PL18-3001) LC (SEQ ID NO: 1004)]
[CAAGGTCAGTCTGGATCC][GGAGGTGGCTCGAGCGGTGGCAGCGGTGCTGTGGGTCTCCTGGCTC
CCCCGGGCGGCCTGTCCGGCCGCAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCC
CAGCAGCCTGTCTGCTAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGC
AGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCT
CTCTGCAGTCTGGCGTGCCCAGCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCAT
CAGCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACC
TTTGGCGGAGGTACCAAGGTGGAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCA
TTGTCAAGAGCTTCAACAGGAATGAGTGT] (SEQ ID NO: 156)

[spacer (SEQ ID NO: 923)][PL18-1004/GG/0001 LC (SEQ ID NO: 1005)]
[QGQSGS][PCHPHAYDARPYCNVGGGSSGGSGAVGLLAPPGGLSGRSDNHGGSDIQMTQSPSSLSA
SVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQP
EDFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 157)

PL18-1004/GG/0001 LC nucleotide sequence
GGAGGTGGCTCGAGCGGTGGCAGCGGTGCTGTGGGTCTCCTGGCTCCCCCGGGCGGCCTGTCCGGCC
GCAGCGATAATCATGGCGGTTCTGACATCCAGATGACCCAGAGCCCCAGCAGCCTGTCTGCTAGCGT
GGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAG
CAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGCCAGCTCTCTGCAGTCTGGCGTGCCCA
GCAGATTTTCCGGCAGCGGCTCTGGCACCGACTTCACCCTGACCATCAGCTCCCTGCAGCCCGAGGA

CTTCGCCACCTACTACTGCCAGCAGGACAACGGCTACCCCAGCACCTTTGGCGGAGGTACCAAGGTG
GAAATCAAGCGTTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGA
ATGAGTGT (SEQ ID NO: 1004)

PL18-1004/GG/0001 LC amino acid sequence
PCHPHAYDARPYCNVGGGSSGGSGAVGLLAPPGGLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTIT
CRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
QDNGYPSTFGGGTKVEIKR (SEQ ID NO: 1005)

PL07-0001
[spacer (SEQ ID NO: 923)][PL07-0001 LC (SEQ ID NO: 1145)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGLSGRSDNHGGSDIQMTQSPSSLSASVGDRV
TITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY
YCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1144)

PL07-0001 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITCRASQS
ISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYP
STFGGGTKVEIKR (SEQ ID NO: 1145)

PL07-0002
[spacer (SEQ ID NO: 923)][PL07-0002 LC (SEQ ID NO: 1147)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGLSGRSGNHGGSDIQMTQSPSSLSASVGDRV
TITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY
YCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1146)

PL07-0002 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGLSGRSGNHGGSDIQMTQSPSSLSASVGDRVTITCRASQS
ISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYP
STFGGGTKVEIKR (SEQ ID NO: 1147)

PL07-1001
[spacer (SEQ ID NO: 923)][PL07-1001 LC (SEQ ID NO: 1149)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSSGGSDIQMTQSPSSLSASVGDRV
TITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY
YCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1148)

PL07-1001 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSSGGSDIQMTQSPSSLSASVGDRVTITCRASQS
ISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYP
STFGGGTKVEIKR (SEQ ID NO: 1149)

PL07-1002
[spacer (SEQ ID NO: 923)][PL07-1002 LC (SEQ ID NO: 1151)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGQNQALRMAGGSDIQMTQSPSSLSASVGDRV
TITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY
YCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1150)

PL07-1002 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGQNQALRMAGGSDIQMTQSPSSLSASVGDRVTITCRASQS
ISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYP
STFGGGTKVEIKR (SEQ ID NO: 1151)

PL07-1003
[spacer (SEQ ID NO: 923)][PL07-1003 LC (SEQ ID NO: 1153)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGVHMPLGFLGPGGSDIQMTQSPSSLSASVGD
RVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1152)

PL07-1003 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGVHMPLGFLGPGGSDIQMTQSPSSLSASVGDRVTITCRAS
QSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQDNG
YPSTFGGGTKVEIKR (SEQ ID NO: 1153)

PL07-1004
[spacer (SEQ ID NO: 923)][PL07-1004 LC (SEQ ID NO: 1201)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGAVGLLAPPGGSDIQMTQSPSSLSASVGDRV
TITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY
YCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1200)

PL07-1004 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGAVGLLAPPGGSDIQMTQSPSSLSASVGDRVTITCRASQS
ISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYP
STFGGGTKVEIKR (SEQ ID NO: 1201)

PL07-2002
[spacer (SEQ ID NO: 923)][PL07-2002 LC (SEQ ID NO: 1155)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSGNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1154)

PL07-2002 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSGNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1155)

PL07-2003
[spacer (SEQ ID NO: 923)][PL07-2003 LC (SEQ ID NO: 1157)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSANPRGGGSDIQMTQSPSSLS
ASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQ
PEDFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1156)

PL07-2003 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSANPRGGGSDIQMTQSPSSLSASVGDRVTI
TCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
QQDNGYPSTFGGGTKVEIKR (SEQ ID NO: 1157)

PL07-2004
[spacer (SEQ ID NO: 923)][PL07-2004 LC (SEQ ID NO: 1159)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGAVGLLAPPTSGRSANPRGGGSDIQMTQSPS
SLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1158)

PL07-2004 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGAVGLLAPPTSGRSANPRGGGSDIQMTQSPSSLSASVGDR
VTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFAT
YYCQQDNGYPSTFGGGTKVEIKR (SEQ ID NO: 1159)

PL07-2005
[spacer (SEQ ID NO: 923)][PL07-2005 LC (SEQ ID NO: 1161)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGAVGLLAPPSGRSANPRGGGSDIQMTQSPSS
LSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISS
LQPEDFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1160)

PL07-2005 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGAVGLLAPPSGRSANPRGGGSDIQMTQSPSSLSASVGDRV
TITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY
YCQQDNGYPSTFGGGTKVEIKR (SEQ ID NO: 1161)

PL07-2006
[spacer (SEQ ID NO: 923)][PL07-2006 LC (SEQ ID NO: 1163)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDDHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1162)

PL07-2006 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDDHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1163)

PL07-2007
[spacer (SEQ ID NO: 923)][PL07-2007 LC (SEQ ID NO: 1165)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDIHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1164)

PL07-2007 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDIHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1165)

PL07-2008
[spacer (SEQ ID NO: 923)][PL07-2008 LC (SEQ ID NO: 1167)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDQHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1166)

PL07-2008 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDQHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1167)

PL07-2009
[spacer (SEQ ID NO: 923)][PL07-2009 LC (SEQ ID NO: 1169)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDTHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1168)

PL07-2009 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDTHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1169)

PL07-2010
[spacer (SEQ ID NO: 923)][PL07-2010 LC (SEQ ID NO: 1171)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDYHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1170)

PL07-2010 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDYHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1171)

PL07-2011
[spacer (SEQ ID NO: 923)][PL07-2011 LC (SEQ ID NO: 1173)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDNPGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1172)

PL07-2011 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDNPGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1173)

PL07-2012
[spacer (SEQ ID NO: 923)][PL07-2012 LC (SEQ ID NO: 1175)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSANPGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1174)

PL07-2012 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSANPGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1175)

PL07-2013
[spacer (SEQ ID NO: 923)][PL07-2013 LC (SEQ ID NO: 1177)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSANIGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1176)

PL07-2013 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSANIGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1177)

PL07-2014
[spacer (SEQ ID NO: 923)][PL07-2014 LC (SEQ ID NO: 1179)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDNIGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1178)

PL07-2014 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDNIGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKR (SEQ ID NO: 1179)

PL07-1004/GG/0003
[spacer (SEQ ID NO: 923)][PL07-1004/GG/0003 LC (SEQ ID NO: 1181)]
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGAVGLLAPPGGTSTSGRSANPRGGGSDIQMT
QSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQDNGYPSTFGGGTKVEIKR] (SEQ ID NO: 1180)

PL07-1004/GG/0003 LC amino acid sequence
GIALCPSHFCQLPQTGGGSSGGSGGSGGAVGLLAPPGGTSTSGRSANPRGGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKR (SEQ ID NO: 1181)

EXAMPLE 11. Anti-PDL1 activatable antibodies of the disclosure

[000609] This example demonstrates that anti-PDL1 activatable antibodies can be made in a variety of combinations of MM, CM, VL, and VH domains as well as in a variety of distinct human isotypes.

Table 18. Anti-PDL1 Activatable Antibody Components

Mask Sequence (MM)	Substrate Sequence (CM)	VL	VH
YCEVSELFVLPWCMG (SEQ ID NO: 208)	LSGRSDNH (SEQ ID NO: 341)	SEQ ID NO: 12	SEQ ID NO: 14

SCLMHPHYAHDYCYV (SEQ ID NO: 426)	TGRGPSWV (SEQ ID NO: 338)	SEQ ID NO: 58	SEQ ID NO: 16
LCEVLMLLQHPWCMG (SEQ ID NO: 59)	PLTGRSGG (SEQ ID NO: 344)		SEQ ID NO: 18
IACRHFMEQLPFCHH (SEQ ID NO: 60)	TARGPSFK (SEQ ID NO: 340)		SEQ ID NO: 20
FGPRCGEASTCVPYE (SEQ ID NO: 61)	NTLSGRSENHSG (SEQ ID NO: 435)		SEQ ID NO: 22
ILYCDSWGAGCLTRP (SEQ ID NO: 62)	NTLSGRSGNHGS (SEQ ID NO: 436)		SEQ ID NO: 24
GIALCPSHFCQLPQT (SEQ ID NO: 63)	TSTSGRSANPRG (SEQ ID NO: 437)		SEQ ID NO: 26
DGPRCFVSGECSPIG (SEQ ID NO: 64)	TSGRSANP (SEQ ID NO: 438)		SEQ ID NO: 28
LCYKLDYDDRSYCHI (SEQ ID NO: 65)	VHMPLGFLGP (SEQ ID NO: 352)		SEQ ID NO: 30
PCHPHPYDARPYCNV (SEQ ID NO: 66)	AVGLLAPP (SEQ ID NO: 372)		SEQ ID NO: 32
PCYWHPFFAYRYCNT (SEQ ID NO: 67)	AQNLLGMV (SEQ ID NO: 360)		SEQ ID NO: 34
VCYYMDWLGRNWCSS (SEQ ID NO: 68)	QNQALRMA (SEQ ID NO: 359)		SEQ ID NO: 36
LCDLFKLREFPYCMG (SEQ ID NO: 69)	LAAPLGLL (SEQ ID NO: 371)		SEQ ID NO: 38
YLPCHFVPIGACNNK (SEQ ID NO: 70)	STFPFGMF (SEQ ID NO: 361)		SEQ ID NO: 40
IFCHMGVVVPQCANY (SEQ ID NO: 71)	ISSGLLSS (SEQ ID NO: 364)		SEQ ID NO: 42
ACHPHPYDARPYCNV (SEQ ID NO: 72)	PAGLWLDP (SEQ ID NO: 374)		SEQ ID NO: 44
PCHPAPYDARPYCNV (SEQ ID NO: 73)	VAGRSMRP (SEQ ID NO: 439)		SEQ ID NO: 46
PCHPHAYDARPYCNV (SEQ ID NO: 74)	VVPEGRRS (SEQ ID NO: 440)		SEQ ID NO: 48

PCHPHPADARPYCNV (SEQ ID NO: 75)	ILPRSPAF (SEQ ID NO: 441)		SEQ ID NO: 50
PCHPHPYAARPYCNV (SEQ ID NO: 76)	MVLGRSLL (SEQ ID NO: 442)		SEQ ID NO: 52
PCHPHPYDAAPYCNV (SEQ ID NO: 77)	QGRAITFI (SEQ ID NO: 443)		SEQ ID NO: 54
PCHPHPYDARPACNV (SEQ ID NO: 78)	SPRSIMLA (SEQ ID NO: 444)		SEQ ID NO: 56
PCHPHPYDARPYCAV (SEQ ID NO: 79)	SMLRSMPL (SEQ ID NO: 445)		
PCHAHPYDARPYCNV (SEQ ID NO: 80)	ISSGLLSGRSDNH (SEQ ID NO: 377)		
PCHPHPYDARAYCNV (SEQ ID NO: 81)	AVGLLAPPGGLSGRSDNH (SEQ ID NO: 383)		
	ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 378)		
	LSGRSGNH (SEQ ID NO: 883)		
	SGRSANPRG (SEQ ID NO: 884)		
	LSGRSDDH (SEQ ID NO: 885)		
	LSGRSDIH (SEQ ID NO: 886)		
	LSGRSDQH (SEQ ID NO: 887)		
	LSGRSDTH (SEQ ID NO: 888)		
	LSGRSDYH (SEQ ID NO: 889)		
	LSGRSDNP (SEQ ID NO: 890)		
	LSGRSANP (SEQ ID NO: 891)		
	LSGRSANI (SEQ ID NO: 892)		

	LSGRSDNI (SEQ ID NO: 893)		
	MIAPVAYR (SEQ ID NO: 894)		
	RPSPMWAY (SEQ ID NO: 895)		
	WATPRPMR (SEQ ID NO: 896)		
	FRLLDWQW (SEQ ID NO: 897)		
	ISSGL (SEQ ID NO: 898)		
	ISSGLLS (SEQ ID NO: 899)		
	ISSGLL (SEQ ID NO: 900)		
	ISSGLLSGRSANPRG (SEQ ID NO: 901)		
	AVGLLAPPTSGRSANPRG (SEQ ID NO: 902)		
	AVGLLAPPSGRSANPRG (SEQ ID NO: 903)		
	ISSGLLSGRSDDH (SEQ ID NO: 904)		
	ISSGLLSGRSDIH (SEQ ID NO: 905)		
	ISSGLLSGRSDQH (SEQ ID NO: 906)		
	ISSGLLSGRSDTH (SEQ ID NO: 907)		
	ISSGLLSGRSDYH (SEQ ID NO: 908)		
	ISSGLLSGRSDNP (SEQ ID NO: 909)		
	ISSGLLSGRSANP (SEQ ID NO: 910)		

	ISSGLLSGRSANI (SEQ ID NO: 911)		
	AVGLLAPPGGLSGRSDDH (SEQ ID NO: 912)		
	AVGLLAPPGGLSGRSDIH (SEQ ID NO: 913)		
	AVGLLAPPGGLSGRSDQH (SEQ ID NO: 914)		
	AVGLLAPPGGLSGRSDTH (SEQ ID NO: 915)		
	AVGLLAPPGGLSGRSDYH (SEQ ID NO: 916)		
	AVGLLAPPGGLSGRSDNP (SEQ ID NO: 917)		
	AVGLLAPPGGLSGRSANP (SEQ ID NO: 918)		
	AVGLLAPPGGLSGRSANI (SEQ ID NO: 919)		
	ISSGLLSGRSDNI (SEQ ID NO: 920)		
	AVGLLAPPGGLSGRSDNI (SEQ ID NO: 921)		
	GLSGRSDNHGGAVGLLAPP (SEQ ID NO: 1009)		
	GLSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 1010)		

[000610] Any of the combinations described in Table 18 can be combined with human immunoglobulin constant regions to result in fully human IgGs including IgG1, IgG2, IgG4 or mutated constant regions to result in human IgGs with altered functions such as IgG1 N297A, IgG1 N297Q, or IgG4 S228P. The combinations described in Table 18 are not limited by the particular combinations shown in any given row and include any mask sequence from column 1 matched with any substrate sequence from column 2 matched with any VL sequence from column 3 matched with any VH sequence from column 4. In

addition to the substrate sequences disclosed in column 2, any CM disclosed herein can be used.

[000611] As an example, a spacer sequence (SEQ ID NO: 923) and Mask SEQ ID NO: 63 can be combined with substrate ISSGLLSGRSDNH (SEQ ID NO: 377), VL SEQ ID NO: 58, and combined with human kappa constant domain to give SEQ ID NO: 428; or Mask SEQ ID NO: 63 can be combined with substrate ISSGLLSGRSDNH (SEQ ID NO: 377), VL SEQ ID NO: 58, and combined with human kappa constant domain to give SEQ ID NO: 1008. Furthermore, VH SEQ ID NO: 46 can be combined with human immunoglobulin heavy chain constant domains to give human IgG1 (SEQ ID NO: 430), mutated human IgG4 S228P (SEQ ID NO: 432), mutated human IgG1 N297A (SEQ ID NO: 434), or mutated human IgG1 N297Q (SEQ ID NO: 1202). Co-expression of SEQ ID NO: 427 with SEQ ID NO: 429 will yield a fully human IgG1 anti-PDL1 activatable antibody. Co-expression of SEQ ID NO: 427 with SEQ ID NO: 431 will yield a fully human IgG4S228P anti-PDL1 activatable antibody. Co-expression of SEQ ID NO: 427 with SEQ ID NO: 433 will yield a fully human IgG1 N297A anti-PDL1 activatable antibody. Co-expression of SEQ ID NO: 427 with a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 1202 will yield a fully human IgG1 N297Q anti-PDL1 activatable antibody.

[Light chain sequence (SEQ ID NO: 1007) with spacer (SEQ ID NO: 1006)] (SEQ ID NO: 427)

[CAGGGCCAGTCCGGCTCA][TATCTGCCCTGCCACTTCGTGCCAATCGGGGCCTGTAACAATAAGG
GCGGTGGATCTAGTGGTGGCTCAGGCGGGTCTGGCGGCATTTCCAGTGGACTCTTGTCAGGACGATC
CGATAATCATGGCGGGTCCGACATCCAGATGACACAGAGCCCTTCTTCCCTCTCCGCAAGCGTTGGC
GACAGGGTCACCATTACCTGTAGGGCTTCTCAGAGCATCTCAAGCTATCTGAACTGGTACCAGCAGA
AACCTGGAAAGGCTCCAAAACTGCTGATTTACGCTGCCTCCAGTCTTCAGTCAGGCGTCCCCTCCAG
ATTTAGCGGATCAGGTAGTGGAACTGATTTTACCCTTACAATATCTTCTCTGCAGCCAGAGGACTTC
GCCACATACTATTGTCAGCAAGACAATGGTTACCCCAGTACATTTGGCGGAGGGACAAAGGTCGAGA
TCAAAAGGACCGTAGCAGCACCAAGCGTCTTTATTTTCCCCCCCAGTGACGAACAGCTGAAGAGCGG
AACAGCTTCAGTGGTGTGTCTCCTGAATAACTTCTATCCACGCGAGGCAAAGGTGCAGTGGAAGGTG
GACAATGCACTGCAGTCTGGTAATTCCCAAGAAAGTGTTACTGAGCAGGATTCCAAGGATTCAACTT
ACTCTCTGTCTAGCACCCTGACTCTTTCTAAAGCAGATTATGAGAAGCATAAGGTCTACGCTTGCGA
GGTGACCCACCAGGGGCTTTCCTCTCCAGTTACCAAGTCATTCAACCGGGGTGAGTGTTGATGAGAA
TTC] (SEQ ID NO: 427)

Light chain sequence without spacer
TATCTGCCCTGCCACTTCGTGCCAATCGGGGCCTGTAACAATAAGGGCGGTGGATCTAGTGGTGGCT
CAGGCGGGTCTGGCGGCATTTCCAGTGGACTCTTGTCAGGACGATCCGATAATCATGGCGGGTCCGA
CATCCAGATGACACAGAGCCCTTCTTCCCTCTCCGCAAGCGTTGGCGACAGGGTCACCATTACCTGT
AGGGCTTCTCAGAGCATCTCAAGCTATCTGAACTGGTACCAGCAGAAACCTGGAAAGGCTCCAAAAC
TGCTGATTTACGCTGCCTCCAGTCTTCAGTCAGGCGTCCCCTCCAGATTTAGCGGATCAGGTAGTGG
AACTGATTTTACCCTTACAATATCTTCTCTGCAGCCAGAGGACTTCGCCACATACTATTGTCAGCAA
GACAATGGTTACCCCAGTACATTTGGCGGAGGGACAAAGGTCGAGATCAAAAGGACCGTAGCAGCAC
CAAGCGTCTTTATTTTCCCCCCCAGTGACGAACAGCTGAAGAGCGGAACAGCTTCAGTGGTGTGTCT
CCTGAATAACTTCTATCCACGCGAGGCAAAGGTGCAGTGGAAGGTGGACAATGCACTGCAGTCTGGT
AATTCCCAAGAAAGTGTTACTGAGCAGGATTCCAAGGATTCAACTTACTCTCTGTCTAGCACCCTGA
CTCTTTCTAAAGCAGATTATGAGAAGCATAAGGTCTACGCTTGCGAGGTGACCCACCAGGGGCTTTC
CTCTCCAGTTACCAAGTCATTCAACCGGGGTGAGTGTTGATGAGAATTC (SEQ ID NO: 1007)

[Light chain sequence (SEQ ID NO: 1008) with spacer (SEQ ID NO: 923)] (SEQ ID NO: 428)
[QGQSGS][GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSAS
VGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQDNGYPSTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW
KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC]
(SEQ ID NO: 428)

Light chain sequence without spacer
GIALCPSHFCQLPQTGGGSSGGSGGSGGISSGLLSGRSDNHGGSDIQMTQSPSSLSASVGDRVTITC
RASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
DNGYPSTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG
NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 1008)

SEQ ID NO: 429
GAAGTGCAGCTGCTCGAAAGCGGCGGAGGCTTGGTGCAGCCAGGAGGGAGCCTGCGACTGTCTTGCG
CAGCCAGCGGATTCACTTTCTCTTCCTATGCCATGAGCTGGGTTCGACAGGCACCCGGCAAAGGTCT
CGAGTGGGTGTCTAGCATCTGGCGAAACGGAATAGTTACAGTGTATGCCGATAGCGTGAAGGGTCGC
TTTACTATTTCACGGGATAATTCTAAGAACACCCTCTACCTGCAAATGAATAGCCTTAGGGCAGAAG
ATACCGCCGTGTACTACTGTGCCAAATGGTCCGCAGCCTTTGACTACTGGGGCCAGGGGACACTGGT
GACCGTGTCCTCTGCATCAACCAAGGGGCCATCAGTGTTCCCACTCGCCCCATCTTCCAAGAGTACT
TCCGGCGGAACCGCAGCCCTTGGCTGCCTTGTTAAGGACTATTTCCCAGAACCCGTGACCGTAAGTT

GGAACTCTGGCGCCCTTACTTCTGGGGTGCACACCTTCCCAGCAGTGTTGCAGTCCAGTGGCCTTTA
CTCTCTGTCTAGTGTAGTGACTGTGCCTTCCTCTAGTCTCGGTACCCAGACCTATATTTGTAATGTT
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SEQ ID NO: 430

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FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO: 431

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SEQ ID NO: 432

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SEQ ID NO: 433

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AGAATTCATTGATCATAATCAGCCATACCAC

SEQ ID NO: 434
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SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
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SEQ ID NO: 1202
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DPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

[000612] Additional examples of heavy chain constant domains include, but are not limited to, a heavy chain constant region having a mutation or deletion at one or more of the following amino acids: Ser 228, Leu234, Leu235, Gly236, Gly237, Asp265, Asp270, Asn297, Lys 320, Lys 322, Glu328, Pro329, Pro331 as numbered in the EU index as set forth in Kabat, such that the Fc domain has less affinity for a Fc-gamma receptor.

EXAMPLE 12. Evaluation of efficiency of masking moieties

[000613] This assay measures the ability of the masking peptide to block binding of the activatable antibody to antigen as compared to the binding of the unmodified antibody to antigen.

[000614] The general outline for this assay is as follows: Nunc, Maxisorp plates are coated overnight at 4°C with 100 µl/well of a 1 µg/mL solution of human PDL1 (R & D

Systems) in PBS, pH 7.4. Plates are washed 3 times with PBST (PBS, pH 7.4, 0.05% Tween-20), and wells are blocked with 200 ml/well of 10 mg/mL BSA in PBST for 2 hours at RT. Plates are washed 3 times with PBST (PBS, pH 7.4, 0.05% Tween-20). Dilution curves are prepared, in 10 mg/mL BSA in PBST, as shown below in Table 10.

Table 10. Plate layout for masking efficiency assay, one plate for each time point.

	[Antibody] = nM Columns 1-3	[activatable antibody 1] = nM Columns 4-6	[activatable antibody 2] = nM Columns 7-9	[activatable antibody 3] = nM Columns 10-12
A	**37**	**1000**	**1000**	**1000**
B	**12.3**	**333**	**333**	**333**
C	**4.1**	**111**	**111**	**111**
D	**1.34**	**37**	**37**	**37**
E	**0.45**	**12.3**	**12.3**	**12.3**
F	**0.15**	**4.1**	**4.1**	**4.1**
G	**0.03**	**1.34**	**1.34**	**1.34**
H	**0.01**	**0.45**	**0.45**	**Blank**

[000615] The binding solutions are added to the plates, which are then are incubated for 1 hour at room temperature (RT), and then washed 3 times with PBST (PBS, pH 7.4, 0.05% Tween-20). One hundred (100) µl/well 1:4000 dilution goat-anti-human IgG (Fab specific, Sigma cat # A0293) in 10 mg/mL BSA in PBST is added, and the plate is incubated for 1 hour at RT. The plate is developed with TMB and 1N HCl. Shown in Figures 15-17 are plots of binding isotherms for anti-PDL1 activatable antibodies that include the anti-PDL1 antibody C5H9v2 described herein. Plots are generated in Prizm (Sigma Plot) and the data are fit to a model of single site saturation and a Kd is determined. In Figure 17, PL03+ is an effector positive activatable antibody, namely wild type IgG1. PL03- is an effector negative activatable antibody, namely mutated IgG1 N297Q.

[000616] Masking efficiency is calculated by dividing the Kd for binding of the activatable antibodies by the Kd of the parental antibody. Masking efficiencies are shown in Figures 15-17.

EXAMPLE 13. Anti-PDL1 activatable antibodies of the disclosure delay induction of diabetes in NOD mice

[000617] In this Example, anti-PDL1 activatable antibodies PL15-0003-C5H9v2 and PL18-0003-C5H9v2 were analyzed for the ability to induce diabetes in NOD mice. The NOD mice, substrain NOD/ShiLtJ, were obtained from Jackson Laboratory at 6 weeks and acclimated on site. At 9.5 weeks, mice were checked for diabetes prior to enrollment, grouped, and dosed as set forth in Table 19.

Table 19. Groups and doses for diabetes study with anti-PDL1 activatable antibodies

Group	Count	Treatment	Dose (mg/kg)	Dose volume (mL/kg)	Schedule	Route
1	8	mIgG2a (C1.18.4)	3	10	Day 0	IP
2	8	Anti-PDL1(C5H9v2)	1	10	Day 0	IP
3	8	Anti-PDL1(C5H9v2)	0.3	10	Day 0	IP
4	8	Activatable Antibody PL15-0003-C5H9v2	1	10	Day 0	IP
5	8	Activatable Antibody PL18-0003-C5H9v2	1	10	Day 0	IP

[000618] Figure 18A, which plots % non-diabetic versus number of days post initial dose, shows that anti-PDL1 antibody C5H9v2 induced diabetes in NOD mice at 1 mg/kg while the anti-PDL1 activatable antibody PL15-0003-C5H9v2 (labeled herein as Gr4 - PFl5) did not induce diabetes and the anti-PDL1 activatable antibody PL18-0003-C5H9v2 (labeled in Figure 18 as Gr 5 - PL18) exhibited delayed diabetes at 1 mg/kg. Control mIgG2a and anti-PDL1 antibody C5H9v2 at 0.3 mg/kg did not induce diabetes in NOD mice. In an additional group, a single 3 mg/kg dose of anti-PDL1 activatable antibody PL15-0003-C5H9v2 administered IP at a dose volume of 10 mL/kg induced diabetes in 2 of 8 mice (75% non-diabetic), providing a greater than three-fold safety margin relative to anti-PDL1 antibody C5H9v2 (Group 2). Results are shown in Figure 18B.

EXAMPLE 14. Anti-PDL1 activatable antibodies of the disclosure reduce MC38 tumors in mice

[000619] In this Example, anti-PDL1 activatable antibodies PL15-0003-C5H9v2 and PL18-0003-C5H9v2 were analyzed for the ability to reduce the growth of MC38 syngeneic tumors.

[000620] The mouse colon carcinoma cell line MC38 was obtained from ATCC. MC38 were grown in RPMI-1640 supplemented with 10% fetal bovine serum at 37°C in an atmosphere of 5% CO_2 in air. Cells were harvested during the logarithmic growth period, resuspended in PBS with proper cell concentration, and kept on ice for tumor induction.

[000621] Each mouse was inoculated subcutaneously in the right flank with $0.5X10^6$ of MC38 cells in PBS for tumor development. The treatments were started when the mean tumor size reached approximately 100-200 mm^3 (no more than 200 mm^3). Tumor sizes were measured twice weekly in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$ where a and b are the long and short diameters of the tumor, respectively.

[000622] The mice were grouped and dosed as set forth in Table 20.

Table 20. Groups and doses for MC38 syngeneic study with anti-PDL1 activatable antibodies

Group	Count	Treatment	Dose (mg/kg)	Dose volume (mL/kg)	Schedule	Route
1	9	mIgG2a (C1.18.4)	10	10	t.i.w. for 2 weeks	IP
2	9	Anti-PDL1(C5H9v2)	10	10	t.i.w. for 2 weeks	IP
4	9	PL18-0003-C5H9v2	10	10	t.i.w. for 2 weeks	IP
6	9	PL15-0003-C5H9v2	10	10	t.i.w. for 2 weeks	IP

[000623] Figure 19, which plots tumor volume versus number of days post initial dose, demonstrates that anti-PDL1 activatable antibodies PL15-0003-C5H9v2 and PL18-0003-C5H9v2 inhibited the growth of MC38 syngeneic tumors similar to positive control anti-PDL1 antibody C5H9v2.

EXAMPLE 15. An anti-PDL1 activatable antibody of the disclosure demonstrates reduced PDL1 occupancy in blood and spleen from C57Bl/6 mice

[000624] Unlike an antibody that would bind target antigen indiscriminately, an activatable antibody would be a molecule that is inert unless it is activated in the tumor microenvironment. Once activated, the cleaved activatable antibody would bind the targeted antigen within the tumor only, sparing the antigens in the peripheral areas. PDL1 is expressed in tumor cells as well as T cells (CD4+ and CD8+) in circulation and the spleen. This example demonstrates that animals treated with anti-PDL1 activatable antibody did not have detectable activatable antibody on T cells in the periphery, while animals treated with anti-PDL1 antibody showed a detectable dose dependent presence of antibody on T cells in the periphery.

[000625] To measure whether anti-PDL1 activatable antibody PL15-0003-C5H9v2 is protected from binding T cells in circulation and the spleen compared to the anti-PDL1 antibody C5H9v2, 14-week old C57Bl/6 mice (with tumors ranging from 38-671mm3) were dosed as set forth in Table 21. Tumor sizes were measured in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$ where a and b are the long and short diameters of the tumor, respectively. After about 20 hours, animals were euthanized; and blood, spleen, and plasma were harvested from each animal.

Table 21. Groups and doses for PDL1 occupancy study

Group	Count	Treatment	Dose (mg/kg)	Dose volume (mL/kg)	Schedule	Route
1	2	mIgG2a (C1.18.4)	1	10	Day 0	IP
2	2	Anti-PDL1(C5H9v2)	1	10	Day 0	IP
3	2	Anti-PDL1(C5H9v2)	0.3	10	Day 0	IP
4	2	Anti-PDL1(C5H9v2)	0.1	10	Day 0	IP
5	2	PL15-0003--C5H9v2	1	10	Day 0	IP
6	2	PL15-0003-C5H9v2	0.3	10	Day 0	IP

[000626] Whole blood was processed as follows: One volume of blood was added to 10 volumes of RBC Lysis Solution (130-094-183 Miltenyi Biotec) and processed using

manufacturer's protocol. Lymphocytes were resuspended in staining buffer (HBSS 2% FBS). Spleen was processed as follows: Spleen was placed in a 70uM cell strainer (352350 Corning/BD Falcon) with 1 mL of serum free RPMI-1640. The back of a 3-mL syringe was used to mash the spleen in a circular motion. The strainer was washed with media. Splenocytes were pelleted using a centrifuge and blood was lysed using 10 volumes of RBC Lysis Solution (130-094-183 Miltenyi Biotec) according to manufacturer's protocol. Splenocytes were refiltered after resuspending in staining buffer (HBSS 2% FBS). Lymphocytes from whole blood and lymphocytes from spleen were counted and were aliquoted to wells of a 96-well round bottom plates (5e5 cells/well). Cells were pelleted and mouse FcR Reagent was added 1:10 dilution according to manufacturer's protocol (130-092-575 Miltenyi Biotec). One set of cells was stained with a saturating amount, 100nM, of anti-PDL1 antibody C5H9v2 for 60 minutes to obtain a maximum saturation value. Cells were washed 3 times and are stained with a biotinylated 'a' allotype antibody, anti-mIgG2a(a) clone 8.3 (553502 BD Biosciences) at 300ng/mL for 60 minutes. Cells were washed 3 times and then stained with 1 ug/mL (also referred to herein as "μg/mL") of anti-CD4-Pacific Blue (558107 BD Bioscience), 1ug/mL of anti-CD8-APC (553035 BD Bioscience) and 1:500 dilution of SAPE (S-866 Life Technologies) for 30 minutes. Cells were washed 2 times and stained with 7-AAD according to manufacturer's protocol (559925 BD Bioscience). The MACSQuant flow cytometer was used to measure the amount of anti-PDL1 antibody C5H9v2 and anti-PDL1 activatable antibody PL15-0003-C5H9v2 bound to T cells from whole blood and the spleen. Briefly, at the most 30,000 events were collected in the lymphocyte gate. Live cells were gated from the 7AAD negative gate. CD4+ and CD8+ cells were gated separately and anti-PDL1 mAb and anti-PDL1 activatable antibody MFI were recorded for both CD4 and CD8. Percent occupancy was determined by taking the MFI of the sample compared to the average of the MFI for the maximum binding MFI. Figures 20A-20D demonstrate the percent of anti-PDL1 antibody C5H9v2 and anti-PDL1 activatable antibody PL15-0003-C5H9v2 bound to CD4+ and CD8+ T cells from peripheral blood (Figures 20A, 20B) or spleen (Figures 20C, 20D). In all graphs, the circle represents isotype, the square represents anti-PDL1 antibody C5H9v2, and the triangle represents anti-PDL1 activatable antibody PL15-0003-C5H9v2. Percent occupancy of the anti-PDL1 antibody C5H9v2 was reflective of the dose titration. Percent occupancy of the anti-PDL1 activatable antibody PL15-0003-C5H9v2 at 1 mg/kg was similar to isotype control mIgG2a at 1 mg/kg.

EXAMPLE 16. An anti-PDL1 activatable antibody of the disclosure demonstrates reduced PDL1 occupancy in blood and spleen from C57Bl/6 mice

[000627] This example demonstrates that animals treated with an anti-PDL1 activatable antibody of the disclosure exhibited reduced binding to blood T cells from tumor bearing mice compared to that exhibited by an anti-PDL1 antibody of the disclosure at a variety of doses.

[000628] The study was conducted in a manner similar to that described in Example 15. Briefly, mice bearing MC38 tumors between 100-200 mm^3 were treated with a single dose of anti-PDL1 antibody C5H9v2 or anti-PDL1 activatable antibody PL15-0003-C5H9v2 as indicated in Figures 21A and 21B, and blood was analyzed for surface bound antibody by flow cytometry four days after dosing. Plasma concentrations of the antibody and activatable antibody were determined by ELISA. Figures 21A and 21 B demonstrate that the presence of tumor-derived proteases capable of cleaving the activatable antibody did not lead to high levels of activated activatable antibody in the blood (Figure 21A); i.e., the activatable antibody (depicted by squares) demonstrated reduced peripheral PDL1 binding in tumor-bearing mice compared to the antibody (depicted by circles) or an isotype antibody (depicted by triangles) despite the plasma concentrations of the activatable antibody being higher than those of the antibody (Figure 21B).

EXAMPLE 17. Activity of an anti-PDL1 antibody and an anti-PDL1 activatable antibody of the embodiments in a human T-cell restimulation assay

[000629] In this example, peripheral blood mononuclear cells from a CMV-positive donor were incubated in the presence of CMV viral lysate and an anti-PDL1 antibody or an anti-PDL1 activatable antibody of the disclosure to assess the effect of such anti-PDL1 antibody or anti-PDL1 activatable antibody on interferon gamma (IFN-gamma) cytokine secretion.

[000630] PBMCs from a CMV-positive donor (Hemacare Donor C) were plated at 3.5 $\times 10^5$ cells per well in the presence of 4 μg/mL CMV viral lysate (Astarte) and either anti-PDL1 antibody C5H9v2, anti-PDL1 activatable antibody PL07-2001-C5H9v2, or an hIgG4 isotype control antibody. After four days, supernatant was removed from each well and IFN-gamma levels were assayed using IFN-gamma ELISA kit (Life Technologies, Carlsbad, CA). Figure 22 demonstrates that anti-PDL1 activatable antibody PL07-2001-

C5H9v2 exhibited increased CMV-stimulated IFN-gamma secretion compared with control hIgG4 but decreased potency relative to anti-PDL1 parental antibody C5H9v2 due to the activatable antibody being masked.

EXAMPLE 18. *In situ* imaging of an anti-PDL1 activatable antibody of the disclosure

[000631] This Example demonstrates the ability of anti-PDL1 activatable antibody PL15-0003-C5H9v2 to be activated and to bind frozen MC38 mouse cancer tissues using an *in situ* imaging method.

[000632] Fluorescently-labeled anti-PDL1 activatable antibody PL15-0003-C5H9v2 or anti-PDL1 antibody C5H9v2 was incubated on frozen PDL1+ MC38 tumor sections for 1 hour in a protease-compatible buffer in the presence or absence of a broad spectrum inhibitor cocktail as described in PCT International publication number WO 2014/107599, published July 10, 2014.

[000633] Results are shown in Figure 23. The tissue image in Figure 23A demonstrates binding of anti-PDL1 antibody C5H9v2 to the tumor section indicating the presence of PDL1 antigen in the tumor section. The tissue image in Figure 23B demonstrates that anti-PDL1 activatable antibody PL15-0003-C5H9v2 was activated by tumor-derived proteolytic cleavage of the anti-PDL1 activatable antibody to yield an anti-PDL1 antibody that bound to the PDL1 target in the tumor section. The tissue image in Figure 23D demonstrates that the fluorescent signal shown in Figure 23B was inhibited by pre-treatment of the tumor section with a 1:100 dilution of broad spectrum inhibitor cocktail set III and 50 mM EDTA, whereas no effect of broad spectrum protease inhibitors was detected on the binding of anti-PDL1 antibody C5H9v2 to the tumor section as demonstrated in the tissue image in Figure 23C. These results demonstrate that the tumor sections comprise sufficient protease activity to activate activatable antibodies of the disclosure.

EXAMPLE 19. Ability of an activatable antibody of the disclosure to demonstrate protease-dependent binding and blocking activity *in vitro*

[000634] This Example demonstrates the ability anti-PDL1 activatable antibody PL15-0003-C5H9v2 to exhibit protease-dependent binding and blocking activity *in vitro*.

[000635] Binding and blocking studies were conducted on anti-PDL1 antibody C5H9v2 and anti-PDL1 activatable antibody PL15-0003-C5H9v2 in a manner similar to

those described elsewhere in the Examples except that plates were coated with 0.5 μg/mL PDL1.

[000636] Figure 24 depicts that anti-PDL1 activatable antibody PL15-0003-C5H9v2 exhibited a higher EC_{50} for PDL1 binding and PD1 blocking than did the anti-PDL1 antibody as measured by ELISA. The figure further demonstrates that activation of anti-PDL1 activatable antibody PL15-0003-C5H9v2 by matriptase fully restored PDL1 binding and PD1 blocking activities to levels comparable to those of anti-PDL1 antibody C5H9v2.

EXAMPLE 20. Ability of an anti-PDL1 activatable antibody of the disclosure to reduce binding to PDL1 and to demonstrate protease-dependent blocking activity *in vitro*

[000637] This Example measures the ability of a masking peptide to reduce binding of an anti-PDL1 activatable antibody of the disclosure to PDL1 compared to the ability of the parental antibody to bind to PDL1 *in vitro*. This Example also demonstrates the ability of an anti-PDL1 activatable antibody of the disclosure to exhibit protease-dependent blocking activity *in vitro*.

[000638] The abilities of anti-PDL1 activatable antibodies PL07-2001-C5H9v2 and PF07-3001-C5H9v2 and anti-PDL1 antibody C5H9 to bind to human or murine PDL1 were tested in a manner similar to techniques described elsewhere in the Examples. Results are shown in Figure 25.

[000639] The abilities of anti-PDL1 activatable antibody PL07-2001-C5H9v2, uPA-activated anti-PDL1 activatable antibody PL07-2001-C5H9v2, MMP14-activated anti-PDL1 activatable antibody PL07-2001-C5H9v2, and anti-PDL1 antibody C5H9v2 to block PD1 (PD-1) or B7-1 binding to PDL1 were tested in a manner similar to techniques described elsewhere in the Examples.

[000640] Briefly, for the PD1 blockade assay, human, cyno or rat PDL1 was adsorbed to the well of an ELISA plate. Biotinylated human, cyno, or rat PD1 was applied to the wells in the absence or presence of an increasing concentration of either anti-PDL1 activatable antibody PL07-2001-C5H9v2, uPA-activated anti-PDL1 activatable antibody PL07-2001-C5H9v2, MMP14-activated anti-PDL1 activatable antibody PL07-2001-C5H9v2, or anti-PDL1 antibody C5H9v2. Results, shown in Figure 26, indicate that activatable antibody PF07-2001-C5H9v2 exhibited a higher EC_{50} for PD1 blocking than did the anti-PDL1 antibody as measured by ELISA. However, activation of the activatable

antibody by either uPA or MMP14 restored PD1 blocking activity to levels comparable to those of anti-PDL1 antibody C5H9v2.

[000641] Briefly, for the B7-1 blockade assay, human or cyno PDL1 was adsorbed to the well of an ELISA plate. Biotinylated human or cyno B7-1 was applied to the wells in the absence or presence of an increasing concentration of either anti-PDL1 activatable antibody PL07-2001-C5H9v2, uPA-activated anti-PDL1 activatable antibody PL07-2001-C5H9v2, MMP14-activated anti-PDL1 activatable antibody PL07-2001-C5H9v2, or anti-PDL1 antibody C5H9v2. Results, shown in Figure 27, indicate that activatable antibody PF07-2001-C5H9v2 exhibited a higher EC_{50} for B7-1 blocking than did the anti-PDL1 antibody as measured by ELISA. However, activation of the activatable antibody by either uPA or MMP14 restored B7-1 blocking activity to levels comparable to those of anti-PDL1 antibody C5H9v2.

EXAMPLE 21. Anti-PDL1 activatable antibodies of the disclosure reduce MC38 tumors in mice

[000642] In this Example, anti-PDL1 activatable antibodies PL15-0003-C5H9v2, PL15-2001-C5H9v2, and PL15-3001-C5H9v2 were analyzed for the ability to reduce the growth of MC38 syngeneic tumors.

[000643] The mouse colon carcinoma cell line MC38 was obtained from ATCC. MC38 were grown in RPMI-1640 supplemented with 10% fetal bovine serum at 37°C in an atmosphere of 5% CO_2 in air. Cells were harvested during the logarithmic growth period, resuspended in PBS with proper cell concentration, and kept on ice for tumor induction.

[000644] Each mouse was inoculated subcutaneously in the right flank with $0.5X10^6$ of MC38 cells in PBS for tumor development. The treatments were started when the mean tumor size reached approximately 100-200 mm^3 (no more than 200 mm^3). Tumor sizes were measured twice weekly in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$ where a and b are the long and short diameters of the tumor, respectively.

[000645] The mice were grouped and dosed as set forth in Table 24.

Table 24. Groups and doses for MC38 syngeneic study with anti-PDL1 activatable antibodies

Group	Count	Treatment	Dose (mg/kg)	Dose volume (mL/kg)	Schedule	Route
1	9	mIgG2a (C1.18.4)	5	10	b.i.w.; 4 doses	IP
2	9	Anti-PDL1 (C5H9v2)	5	10	b.i.w.; 4 doses	IP
3	9	PL15-0003- C5H9v2	5	10	b.i.w.; 4 doses	IP
4	9	PL15-2001- C5H9v2	5	10	b.i.w.; 4 doses	IP
5	9	PL15-3001- C5H9v2	5	10	b.i.w.; 4 doses	IP

[000646] Figure 28, which plots tumor volume versus number of days post initial dose, demonstrates that anti-PDL1 activatable antibodies PL15-0003-C5H9v2, PL15-2001-C5H9v2, and PL15-3001-C5H9v2 inhibited the growth of MC38 syngeneic tumors similar to positive control anti-PDL1 antibody C5H9v2.

EXAMPLE 22. An anti-PDL1 activatable antibody of the disclosure demonstrates reduced PDL1 occupancy in blood from C57Bl/6 mice

[000647] This example demonstrates that animals treated with anti-PDL1 activatable antibodies of the disclosure exhibited reduced binding to blood T cells from tumor bearing mice compared to that exhibited by an anti-PDL1 antibody of the disclosure.

[000648] The study was conducted in a manner similar to that described in Example 15. Briefly, mice bearing MC38 tumors between 100-200 mm^3 were treated with a single dose of anti-PDL1 antibody C5H9v2 or anti-PDL1 activatable antibody PL15-0003-C5H9v2, PL15-2001-C5H9v2, or PL15-3001-C5H9v2 as indicated in Table 25, and blood was analyzed for surface bound antibody by flow cytometry four and eight days after dosing. Percent occupancy was calculated as described in Example 15.

Table 25. Groups and doses for PDL1 occupancy study

Group	Count	Treatment	Dose (mg/kg)	Dose volume (mL/kg)	Schedule	Route
1	9	mIgG2a (C1.18.4)	5	10	b.i.w.; 4 doses	IP

2	9	Anti-PD-L1(C5H9v2)	5	10	b.i.w.; 4 doses	IP
3	9	Anti-PD-L1(C5H9v2)	3	10	b.i.w.; 4 doses	IP
4	9	PL15-0003-C5H9v2	5	10	b.i.w.; 4 doses	IP
5	9	PL15-0003-C5H9v2	3	10	b.i.w.; 4 doses	IP
6	9	PL15-2001-C5H9v2	5	10	b.i.w.; 4 doses	IP
7	9	PL15-2001-C5H9v2	3	10	b.i.w.; 4 doses	IP
8	9	PL15-3001-C5H9v2	5	10	b.i.w.; 4 doses	IP
9	9	PL15-3001-C5H9v2	3	10	b.i.w.; 4 doses	IP

[000649] Plasma concentrations of the antibody and activatable antibodies were determined by ELISA. Figure 29 demonstrates that the presence of tumor-derived proteases capable of cleaving the activatable antibodies did not lead to high levels of activated activatable antibodies in the blood.

EXAMPLE 23. *In vivo* imaging of an anti-PDL1 activatable antibody of the disclosure

[000650] This Example demonstrates the ability of proteases in a tumor implanted into a mouse to activate an anti-PDL1 activatable antibody of the disclosure.

[000651] A MDA-MB-231-luc2 orthotopic xenograft breast cancer model was used for these studies. Female nu/nu mice were purchased from Charles River Laboratories to arrive at 7 week old. After 1 week acclimation, three million MDA-MD-231-luc2-4D3LN cells (Perkin Elmer) in 30 microliters (ul, also referred to herein as "µl") serum-free RPMI (1:1 matrigel) were injected into the fourth abdominal left mammary fat pad. Tumors were grown to 110-159 mm^3. Three mice per group were administered intravenously 5 mg/kg Alexa750-conjugated anti-PDL1 antibody C5H9v2 or anti-PDL1 activatable antibody PL07-2001-C5H9v2. Four mice were administered intravenously 5 mg/kg Alexa750-conjugated anti-RSV antibody control palivizumab (Synagis). Ninety-six hours after administration, optical images were collected from each of the mice. Results are shown in Figure 30. A high-intensity fluorescent signal was detected only in the tumors of mice dosed with the anti-PDL1 antibody or anti-PDL1 activatable antibody PL07-2001-C5H9v2, suggesting that the anti-PDL1 activatable antibody was activated and accumulated in the tumor through PDL1 binding.

EXAMPLE 24. Activation of an activatable antibody of the disclosure in human plasma samples

[000652] This Example determines whether an activatable antibody of the disclosure is activated in human plasma samples.

[000653] Anti-PDL1 activatable antibody PL07-2001-C5H9v2 was conjugated with Oregon Green dye (ThermoFisher Cat# O6149). The concentration and degree of labeling was determined with a spectrophotometer. Three uM (also referred to herein as "μM") of labeled anti-PDL1 activatable antibody PL07-2001-C5H9v2 was added into plasma samples obtained from healthy donors, patients with melanoma or lung cancer patients in a final activatable antibody to plasma ratio of 30:70. The samples were incubated for 48 hours in a 37° C humidifying chamber. Reactions of labeled anti-PDL1 activatable antibody PL07-2001-C5H9v2 in plasma were stopped at either 0 or 48 hours by freezing samples in -80° C or denaturing in Wes running buffer (ProteinSimple Cat #PS-MK14). Samples were analyzed by the Wes capillary western blot system (ProteinSimple) using antibodies against Oregon Green dye (ThermoFisher Cat# A-11095 at 100μg/ml) followed by HRP conjugated secondary antibody (Jackson ImmunoResearch Cat# 705-035-147 at 1/40 dilution). Analysis of percent activation of the labeled anti-PDL1 activatable antibody PL07-2001-C5H9v2 was determined using Compass (ProteinSimple) software.

[000654] There was no detectable activatable antibody activation in five normal patient samples, in five melanoma patient samples (three metastases in lymph nodes, two in the brain, and one on the skin of the back), nor in five lung cancer patient samples (one metastasis in the lower lobe of the left lung (stage IV), one in the lower lobe of the right lung (stage IV), one in the upper lobe of the left lung (stage IA), and two in the upper lobe of the right lung (stages IIIA and IB).

Other Embodiments

[000655] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following.

What is claimed:

1. An isolated antibody or antigen binding fragment thereof (AB) that specifically binds to mammalian PDL1, wherein the AB has one or more of the following characteristics:
(a) the AB specifically binds to human PDL1 and murine PDL1;
(b) the AB specifically binds to human PDL1 and cynomolgus monkey PDL1;
(c) the AB specifically binds to human PDL1, murine PDL1, and cynomolgus monkey PDL1;
(d) the AB inhibits binding of human B7-1 and human PD1 to human PDL1 with an EC_{50} value less than 10 nM;
(e) the AB inhibits binding of murine B7-1 and murine PD1 to murine PDL1 with an EC_{50} value less than 10 nM; and
(f) the AB inhibits binding of cynomolgus monkey B7-1 and cynomolgus monkey PD1 to cynomolgus monkey PDL1 with an EC_{50} value less than 10 nM.

2. An activatable antibody that, in an activated state, specifically binds to mammalian PDL1, wherein said activatable antibody comprises:
an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian PDL1;
a masking moiety (MM) that inhibits the binding of the AB to mammalian PDL1 when the activatable antibody is in an uncleaved state; and
a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

3. An activatable antibody that, in an activated state,
(a) specifically binds to mammalian PDL1; and
(b) specifically blocks a natural ligand of PDL1 from binding to the mammalian PDL1,
wherein the activatable antibody comprises:
an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian PDL1;
a masking moiety (MM) that inhibits the binding of the AB to mammalian PDL1 when the activatable antibody is in an uncleaved state; and

a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

4. The activatable antibody of claim 2 or 3, wherein the activatable antibody in an uncleaved state specifically binds to the mammalian PDL1 with a dissociation constant of
1 to 2 nM, 1 to 5 nM, 1 to 10 nM, 1 to 15 nM, 1 to 20 nM, 1 to 25 nM,
2 nM to 5 nM, 2 nM to 10 nM, 2 nM to 15 nM, 2 to 20 nM, 2 to 25 nM,
5 nM to 10 nM, 5 nM to 15 nM, 5 to 20 nM, 5 to 25 nM,
10 nM to 15 nM, 10 to 20 nM, 10 to 25 nM,
15 to 20 nM, 15 to 25 nM, or
20 to 25 nM.

5. The activatable antibody of any one of claims 2 to 4, wherein the activatable antibody in an activated state specifically binds to the mammalian PDL1 with a dissociation constant of
0.01 nM to 1 nM, 0.05 nM to 1 nM, 0.1 nM to 1 nM, 0.2 nM to 1 nM, 0.3 nM to 1 nM, 0.4 nM to 1 nM, 0.5 nM to 1 nM, 0.75 nM to 1 nM,
0.01 nM to 0.75 nM, 0.05 nM to 0.75 nM, 0.1 nM to 0.75 nM, 0.2 nM to 0.75 nM, 0.3 nM to 0.75 nM, 0.4 nM to 0.75 nM, 0.5 nM to 0.75 nM,
0.01 nM to 0.5 nM, 0.05 nM to 0.5 nM, 0.1 nM to 0.5 nM, 0.2 nM to 0.5 nM, 0.3 nM to 0.5 nM, 0.4 nM to 0.5 nM,
0.01 nM to 0.4 nM, 0.05 nM to 0.4 nM, 0.1 nM to 0.4 nM, 0.2 nM to 0.4 nM, 0.3 nM to 0.4 nM,
0.01 nM to 0.3 nM, 0.05 nM to 0.3 nM, 0.1 nM to 0.3 nM, 0.2 nM to 0.3 nM,
0.01 nM to 0.2 nM, 0.05 nM to 0.2 nM, 0.1 nM to 0.2 nM,
0.01 nM to 0.1 nM, 0.05 nM to 0.1 nM, or
0.01 nM to 0.05 nM.

6. The antibody of claim 1 or the activatable antibody of any one of claims 2 to 5, wherein the AB specifically binds to the mammalian PDL1 with a dissociation constant of

0.01 nM to 1 nM, 0.05 nM to 1 nM, 0.1 nM to 1 nM, 0.2 nM to 1 nM, 0.3 nM to 1 nM, 0.4 nM to 1 nM, 0.5 nM to 1 nM, 0.75 nM to 1 nM,
0.01 nM to 0.75 nM, 0.05 nM to 0.75 nM, 0.1 nM to 0.75 nM, 0.2 nM to 0.75 nM, 0.3 nM to 0.75 nM, 0.4 nM to 0.75 nM, 0.5 nM to 0.75 nM,
0.01 nM to 0.5 nM, 0.05 nM to 0.5 nM, 0.1 nM to 0.5 nM, 0.2 nM to 0.5 nM, 0.3 nM to 0.5 nM, 0.4 nM to 0.5 nM,
0.01 nM to 0.4 nM, 0.05 nM to 0.4 nM, 0.1 nM to 0.4 nM, 0.2 nM to 0.4 nM, 0.3 nM to 0.4 nM,
0.01 nM to 0.3 nM, 0.05 nM to 0.3 nM, 0.1 nM to 0.3 nM, 0.2 nM to 0.3 nM,
0.01 nM to 0.2 nM, 0.05 nM to 0.2 nM, 0.1 nM to 0.2 nM,
0.01 nM to 0.1 nM, 0.05 nM to 0.1 nM, or
0.01 nM to 0.05 nM.

7. The antibody of claim 1 or claim 6, or the activatable antibody of any one of claims 2 to 6, wherein the mammalian PDL1 is selected from the group consisting of a human PDL1, a murine PDL1, a rat PDL1, and a cynomolgus monkey PDL1.

8. The antibody of any one of claims 1, 6 or 7, or the activatable antibody of any one of claims 2 to 7, wherein the AB specifically binds to human PDL1, murine PDL1 or cynomolgus monkey PDL1 with a dissociation constant of less than 1 nM.

9. The antibody of any one of claims 1, 6, 7, or 8 or the activatable antibody of any one of claims 2 to 8, wherein the mammalian PDL1 is a human PDL1.

10. The activatable antibody of any one of claims 2 to 9, wherein the AB has one or more of the following characteristics:
(a) the AB specifically binds to human PDL1 and murine PDL1;
(b) the AB specifically binds to human PDL1 and cynomolgus monkey PDL1; and
(c) the AB specifically binds to human PDL1, murine PDL1, and cynomolgus monkey PDL1.

11. The antibody of any one of claims 1, 6-8, or 9, or the activatable antibody of any one of claims 2 to 10, wherein:

(a) the AB specifically binds human PDL1, murine PDL1, and cynomolgus monkey PDL1;
(b) the AB inhibits binding of human B7-1 and human PD1 to human PDL1;
(c) the AB inhibits binding of murine B7-1 and murine PD1 to murine PDL1; and
(d) the AB inhibits binding of cynomolgus monkey B7-1 and cynomolgus monkey PD1 to cynomolgus monkey PDL1.

12. The antibody of any one of claims 1, 6-9, or 11 or the activatable antibody of any one of claims 2 to 11, wherein the AB blocks the ability of a natural ligand to bind to the mammalian PDL1 with an EC_{50} of
0.5 nM to 10 nM, 0.5 nM to 5 nM, 0.5 nM to 3 nM, 0.5 nM to 2 nM, 0.5 nM to 1 nM,
1 nM to 10 nM, 1 nM to 5 nM, 1 nM to 3 nM, 1 nM to 2 nM,
2 nM to 10 nM, 2 nM to 5 nM, 2 nM to 3 nM,
3 nM to 10 nM, 3 nM to 5 nM, or
5 nM to 10 nM.

13. The antibody or activatable antibody of claim 12, wherein the natural ligand is a mammalian PD1.

14. The antibody or activatable antibody of claim 12, wherein the natural ligand is selected from the group consisting of: a human PD1, a murine PD1, and a cynomolgus monkey PD1.

15. The antibody or activatable antibody of claim 12, wherein the natural ligand is a mammalian B7-1.

16. The antibody or activatable antibody of claim 12, wherein the natural ligand is selected from the group consisting of: a human B7-1, a murine B7-1, and a cynomolgus monkey B7-1.

17. The activatable antibody of any one of claims 2 to 16, wherein:
(a) the AB induces type 1 diabetes in a non-obese diabetic (NOD) mouse; and

(b) the activatable antibody in an uncleaved state inhibits the induction of type 1 diabetes in a NOD mouse.

18. The activatable antibody of claim 17, wherein the activatable antibody inhibits the induction of type 1 diabetes in the NOD mouse after administration of the activatable antibody at a single dose of
0.1 mg/kg to 3 mg/kg, 0.5 mg/kg to 3 mg/kg, 1 mg/kg to 3 mg/kg, 2 mg/kg to 3 mg/kg,
0.1 mg/kg to 2 mg/kg, 0.5 mg/kg to 2 mg/kg, 1 mg/kg to 2 mg/kg,
0.1 mg/kg to 1 mg/kg, 0.5 mg/kg to 1 mg/kg, or
0.1 mg/kg to 0.5 mg/kg.

19. The activatable antibody of any one of claims 2 to 18, wherein:
(a) the activatable antibody in an uncleaved state does not induce type 1 diabetes in greater than 50% of a population of non-obese diabetic (NOD) mice, and
(b) the AB induces type 1 diabetes in greater than 50% of a population of NOD mice.

20. The activatable antibody of claim 19, wherein the activatable antibody does not induce type 1 diabetes in greater than 50% of the population of NOD mice after administration to each mouse in the population a single dose of the activatable antibody at a dosage of:
0.1 mg/kg to 3 mg/kg, 0.5 mg/kg to 3 mg/kg, 1 mg/kg to 3 mg/kg, 2 mg/kg to 3 mg/kg,
0.1 mg/kg to 2 mg/kg, 0.5 mg/kg to 2 mg/kg, 1 mg/kg to 2 mg/kg,
0.1 mg/kg to 1 mg/kg, 0.5 mg/kg to 1 mg/kg, or
0.1 mg/kg to 0.5 mg/kg.

21. The activatable antibody of claim 19, wherein:
(a) the activatable antibody in an uncleaved state does not induce type 1 diabetes in greater than 50% of a population of non-obese diabetic (NOD) mice when administered at a single dose of 1 mg/kg; and
(b) the AB induces type 1 diabetes in greater than 50% of a population of NOD mice, when administered at a single dose of 1 mg/kg.

22. The activatable antibody of any one of claims 17 to 21, wherein the NOD mouse is a female NOD/ShiLtJ mouse substrain.

23. The activatable antibody of any one of claims 17 to 21, wherein the activatable antibody inhibits the induction of type 1 diabetes in a NOD mouse by at least 3-fold compared to the AB.

24. The activatable antibody of any one of claims 17 to 21, wherein the activatable antibody exhibits a safety margin that is at least a three-fold safety margin relative to the AB.

25. The activatable antibody of any one of claims 2 to 24, wherein the activatable antibody in an uncleaved state binds to a smaller percentage of a population of peripheral blood CD4+ CD8+ T lymphocytes than does the AB.

26. The activatable antibody of claim 25, wherein the peripheral blood CD4+ CD8+ T lymphocytes are murine.

27. The activatable antibody of claim 26, wherein the murine peripheral blood CD4+ CD8+ T lymphocytes are derived from a tumor-bearing mouse.

28. The antibody of any one of claims 1, 6-9, 11-15, or 16 or the activatable antibody of any one of claims 2 to 27,
wherein the AB comprises:
(a) a variable heavy chain complementarity determining region 1 (VH CDR1) comprising the amino acid sequence of SEQ ID NO: 212;
(b) a variable heavy chain complementarity determining region 2 (VH CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 213, 217, 221, 225, 229, 231, and 238-247;
(c) a variable heavy chain complementarity determining region 3 (VH CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 214, 218, 222, 226, 230, 232, and 233-237;
(d) a variable light chain complementarity determining region 1 (VL CDR1) comprising the amino acid sequence of SEQ ID NO: 209;

(e) a variable light chain complementarity determining region 2 (VL CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 210, 215, 219, 223, and 227; and

(f) a variable light chain complementarity determining region 3 (VL CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 211, 216, 220, 224, and 228.

29. The antibody of any one of claims 1, 6-9, 11-16, or 28 or the activatable antibody of any one of claims 2 to 28, wherein the AB comprises a VL CDR1 sequence comprising RASQSISSYLN (SEQ ID NO: 209); a VL CDR2 sequence comprising AASSLQS (SEQ ID NO: 215); a VL CDR3 sequence comprising DNGYPST (SEQ ID NO: 228); a VH CDR1 sequence comprising SYAMS (SEQ ID NO: 212); a VH CDR2 sequence comprising SSIWRNGIVTVYADS (SEQ ID NO: 246); and a VH CDR3 sequence comprising WSAAFDY (SEQ ID NO: 235).

30. The antibody of any one of claims 1, 6-9, 11-16, 28, or 29 or the activatable antibody of any one of claims 2 to 29, wherein the AB comprises a variable heavy chain (VH) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46, 48, 50, 52, 54, and 56, and a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 12 and SEQ ID NO: 58.

31. The antibody of any one of claims 1, 6-9, 11-16, 28, or 29 or the activatable antibody of any one of claims 2 to 30, wherein the AB comprises a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 46 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 58, or a VH comprising the amino acid sequence of SEQ ID NO: 46 and a VL comprising the amino acid sequence of SEQ ID NO: 12.

32. The activatable antibody of any one of claims 2 to 31, wherein the MM has a dissociation constant for binding to the AB that is greater than the dissociation constant of the AB to PDL1.

33. The activatable antibody of any one of claims 2 to 32, wherein the MM does not interfere or compete with the AB for binding to PDL1 when the activatable antibody is in a cleaved state.

34. The activatable antibody of any one of claims 2 to 33, wherein the MM is a polypeptide of no more than 40 amino acids in length.

35. The activatable antibody of any one of claims 2 to 34, wherein the MM polypeptide sequence is different from that of human PDL1.

36. The activatable antibody of any one of claims 2 to 35, wherein the MM polypeptide sequence is no more than 50% identical to any natural binding partner of the AB.

37. The activatable antibody of any one of claims 2 to 36, wherein the MM polypeptide sequence is no more than 25% identical to any natural binding partner of the AB.

38. The activatable antibody of any one of claims 2 to 37, wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 59-81, 208, and 426.

39. The activatable antibody of any one of claims 2 to 38, wherein the CM is a substrate for a protease that is active in diseased tissue.

40. The activatable antibody of any one of claims 2 to 39, wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 338-394, and 435-445, 883-921, 1009, and 1010.

41. The activatable antibody of any one of claims 2 to 40, wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 377-394, 883-921, 1009, and 1010.

42. The antibody of any one of claims 1, 6-9, 11-16, 28, 30, or 31 or the activatable antibody of any one of claims 2 to 41, wherein the antigen binding fragment thereof is

selected from the group consisting of a Fab fragment, a F(ab')2 fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

43. The activatable antibody of any one of claims 2 to 42, wherein the AB is linked to the CM.

44. The activatable antibody of claim 43, wherein the AB is linked directly to the CM.

45. The activatable antibody of claim 43, wherein the AB is linked to the CM via a linking peptide.

46. The activatable antibody of any one of claims 2 to 45, wherein the MM is linked to the CM such that the activatable antibody in an uncleaved state comprises the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM.

47. The activatable antibody of claim 46, wherein the activatable antibody comprises a linking peptide between the MM and the CM.

48. The activatable antibody of claim 46, wherein the activatable antibody comprises a linking peptide between the CM and the AB.

49. The activatable antibody of claim 46, wherein the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM.

50. The activatable antibody of claim 49, wherein the two linking peptides need not be identical to each other.

51. The activatable antibody of claim 49 or 50, wherein each of LP1 and LP2 is a peptide of about 1 to 20 amino acids in length.

52. The activatable antibody of any one of claims 2 to 51 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, and 157, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, and 1144-1191, 1200, and 1201.

53. The activatable antibody of any one of claims 2 to 51 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, and 1201

54. The activatable antibody of any one of claims 2 to 53 comprising the amino acid sequence of SEQ ID NO: 428 or SEQ ID NO: 1008.

55. The activatable antibody of any one of claims 2 to 54 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 430, 432, 434, and 1202.

56. A conjugated antibody or activatable antibody comprising the antibody of any one of claims 1, 6-9, 11-16, 28-31, or 42 or the activatable antibody of any one of claims 2 to 55 conjugated to an agent.

57. The conjugated antibody or conjugated activatable antibody of claim 56, wherein the agent is a toxin or fragment thereof.

58. The conjugated antibody or conjugated activatable antibody of claim 56 or 57, wherein the agent is a microtubule inhibitor.

59. The conjugated antibody or conjugated activatable antibody of claim 56 or 57, wherein the agent is a nucleic acid damaging agent.

60. The conjugated antibody or conjugated activatable antibody of claim 56 or 57, wherein the agent is selected from the group consisting of a dolastatin or a derivative thereof, an auristatin or a derivative thereof, a maytansinoid or a derivative thereof, a duocarmycin or a derivative thereof, and a calicheamicin or a derivative thereof.

61. The conjugated antibody or conjugated activatable antibody of any one of claims 56-58 and 60, wherein the agent is auristatin E or a derivative thereof.

62. The conjugated antibody or conjugated antibody or conjugated activatable antibody of any one of claims 56-58 and 60, wherein the agent is monomethyl auristatin E (MMAE).

63. The conjugated antibody or conjugated activatable antibody of any one of claims 56-58 and 60, wherein the agent is monomethyl auristatin D (MMAD).

64. The conjugated antibody or conjugated activatable antibody of any one of claims 56-58 and 60, wherein the agent is a maytansinoid selected from the group consisting of DM1 and DM4.

65. The conjugated antibody or conjugated activatable antibody of any one of claims 56 to 64, wherein the agent is conjugated to the activatable antibody via a linker.

66. The conjugated antibody or conjugated activatable antibody of claim 65, wherein the linker is a cleavable linker.

67. The conjugated antibody or conjugated activatable antibody of claim 65, wherein the linker is a non-cleavable linker.

68. The conjugated antibody or conjugated activatable antibody of claim 56, wherein the agent is a detectable moiety.

69. The conjugated antibody or conjugated activatable antibody of claim 68, wherein the detectable moiety is a diagnostic agent.

70. A pharmaceutical composition comprising the antibody of any one of claims 1, 6-9, 11-16, 28-31, or 42, the activatable antibody of any one of claims 2 to 55, or the conjugated antibody or conjugated activatable antibody of any one of claims 56 to 69 and a carrier.

71. The pharmaceutical composition of claim 70 comprising an additional agent.

72. The pharmaceutical composition of claim 71, wherein the additional agent is a therapeutic agent.

73. An isolated nucleic acid molecule encoding the antibody of any one of claims 1, 6-9, 11-16, 28-31, or 43 or the activatable antibody of any one of claims 2 to 53.

74. A vector comprising the isolated nucleic acid molecule of claim 73.

75. A method of producing an activatable antibody by culturing a cell under conditions that lead to expression of the activatable antibody, wherein the cell comprises the nucleic acid molecule of claim 73.

76. A method of manufacturing an activatable antibody that, in an activated state, binds PDL1, the method comprising:
(a) culturing a cell comprising a nucleic acid construct that encodes the activatable antibody under conditions that lead to expression of the activatable antibody of any one of claims 2 to 55; and
(b) recovering the activatable antibody.

77. A method of reducing PDL1 activity comprising administering an effective amount of the antibody of any one of claims 1, 6-9, 11-16, 28-31, or 42 or the activatable antibody of any one of claims 2 to 55 or the pharmaceutical composition of any one of claims 70 to 72 to a subject in need thereof.

78. A method of blocking binding of a natural ligand to PDL1 comprising administering an effective amount of the antibody of any one of claims 1, 6-9, 11-16, 28-31, or 42 or the activatable antibody of any one of claims 2 to 55 or the pharmaceutical composition of any one of claims 70 to 72 to a subject in need thereof.

79. A method of treating, alleviating a symptom of, or delaying the progression of a PDL1-mediated disorder or disease comprising administering a therapeutically effective amount of the antibody of any one of claims 1, 6-9, 11-16, 28-31, or 42 or the activatable antibody of any one of claims 2 to 55 or the pharmaceutical composition of any one of claims 70 to 72 to a subject in need thereof.

80. The method of claim 79, wherein the PDL1-mediated disorder or disease is cancer.

81. The method of claim 80, wherein the cancer is a bladder cancer, a bone cancer, a breast cancer, a carcinoid, a cervical cancer, a colon cancer, an endometrial cancer, a glioma, a head and neck cancer, a liver cancer, a lung cancer, a lymphoma, a melanoma, an ovarian cancer, a pancreatic cancer, a prostate cancer, a renal cancer, a sarcoma, a skin cancer, a stomach cancer, a testis cancer, a thyroid cancer, a urogenital cancer, or a urothelial cancer.

82. The method of claim 80, wherein the cancer is selected from the group consisting of melanoma (MEL), renal cell carcinoma (RCC), squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer (CRC), castration-resistant prostate cancer (CRPC), hepatocellular carcinoma (HCC), squamous cell carcinoma of the head and neck, carcinomas of the esophagus, ovary, gastrointestinal tract and breast, or a hematologic malignancy such as multiple myeloma, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma/primary mediastinal B-cell lymphoma, and chronic myelogenous leukemia.

83. The method of any one of claims 79 to 82, wherein the method comprises administering an additional agent.

84. The method of claim 83, wherein the additional agent is a therapeutic agent.

FIGURE 1

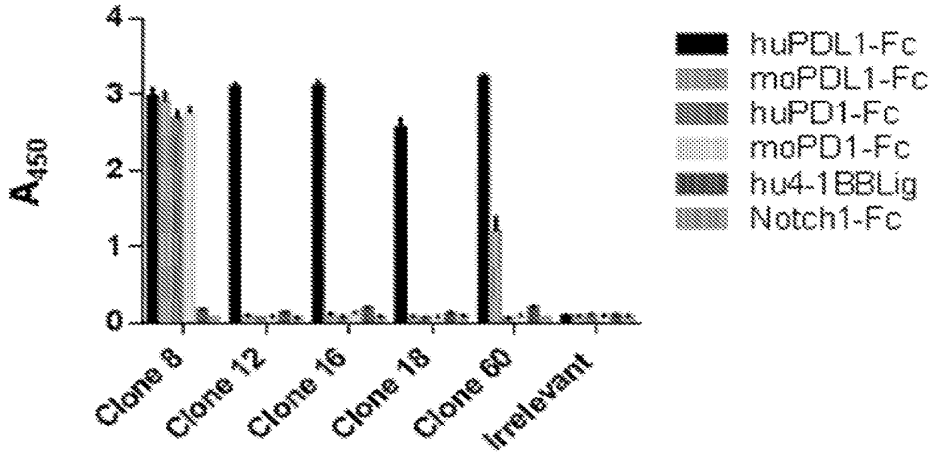


FIGURE 2

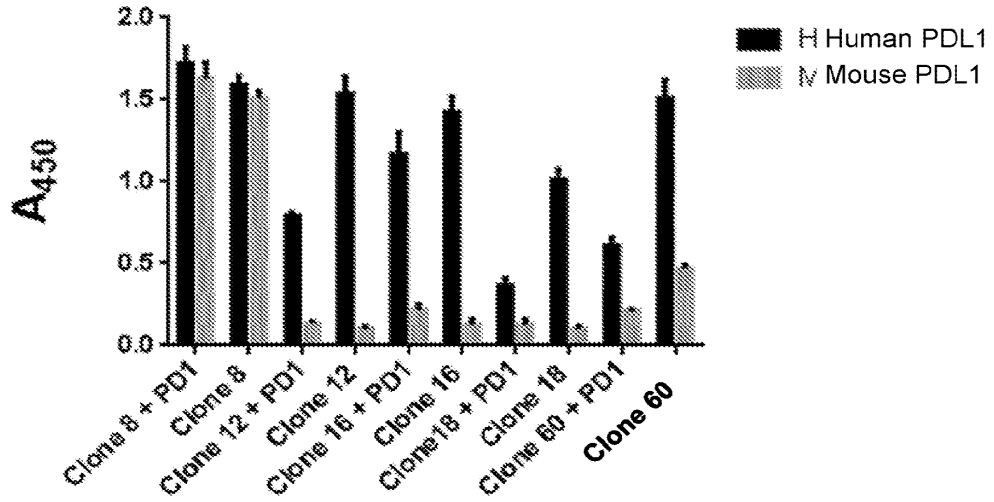


FIGURE 3

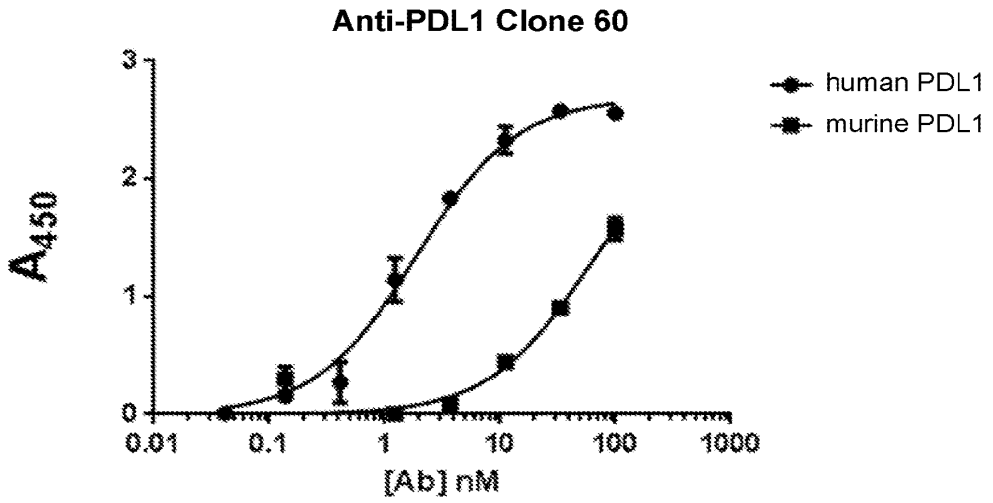


FIGURE 4A

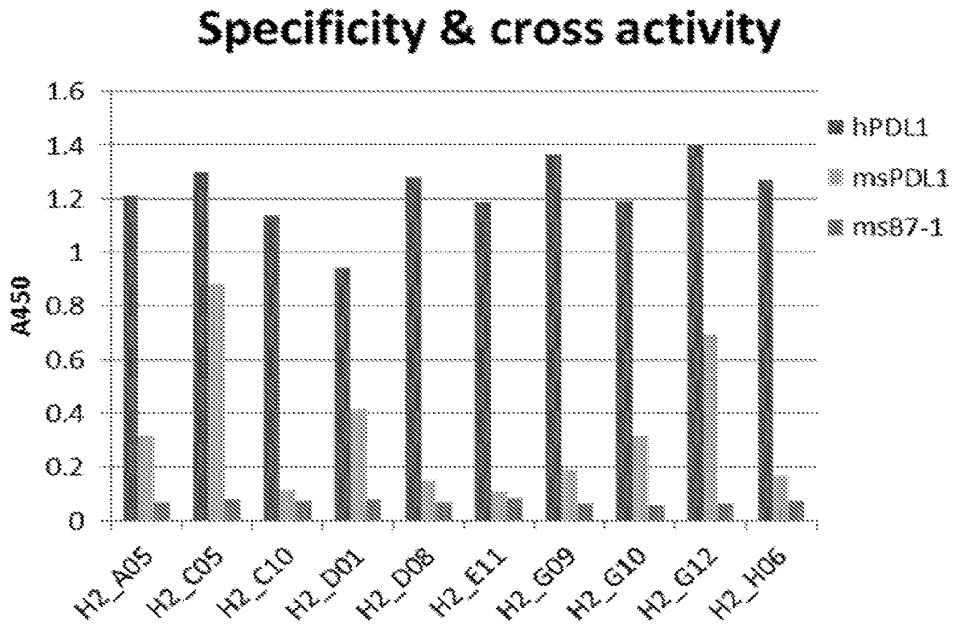


FIGURE 4B

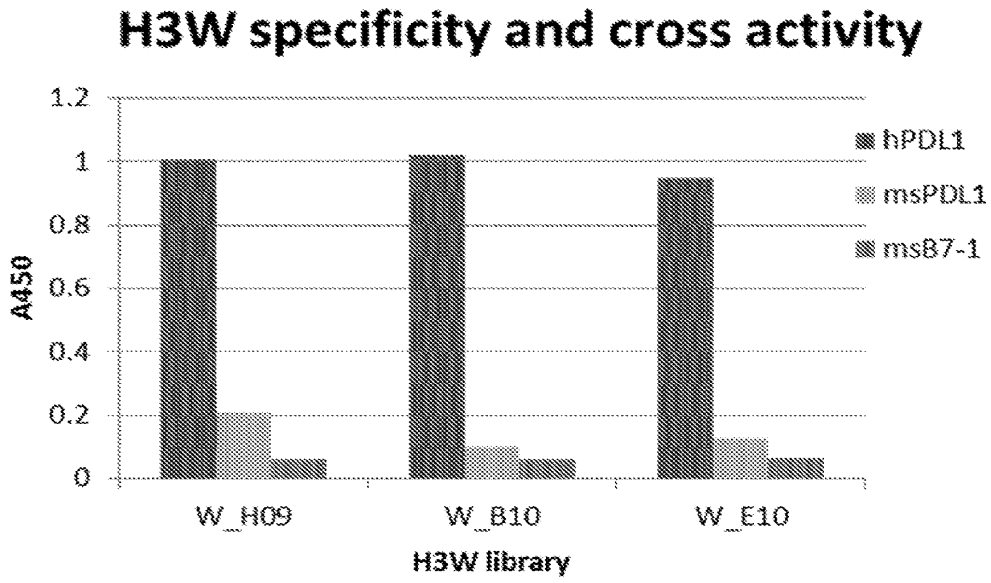


FIGURE 5A

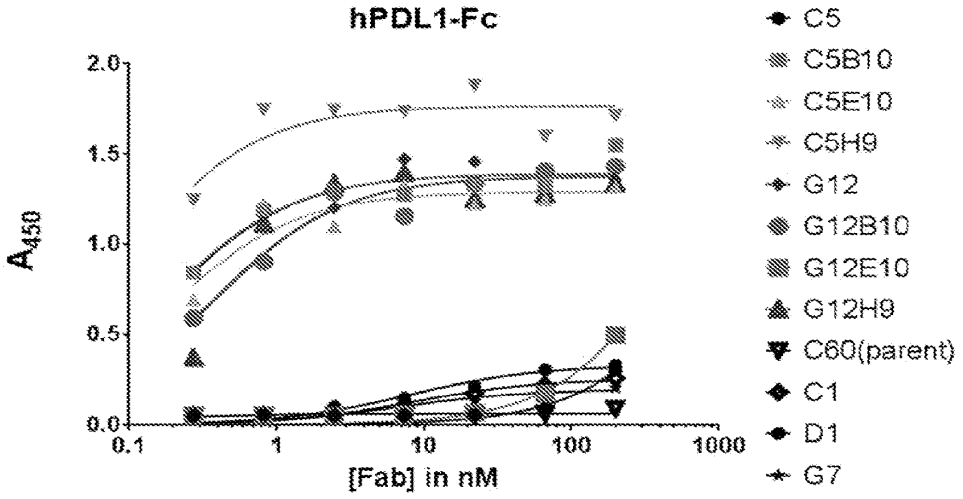


FIGURE 5B

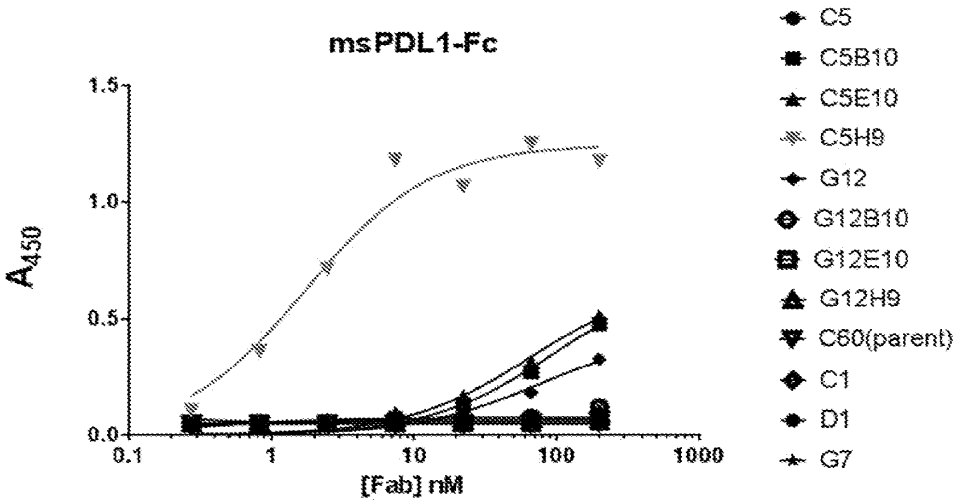


FIGURE 6A

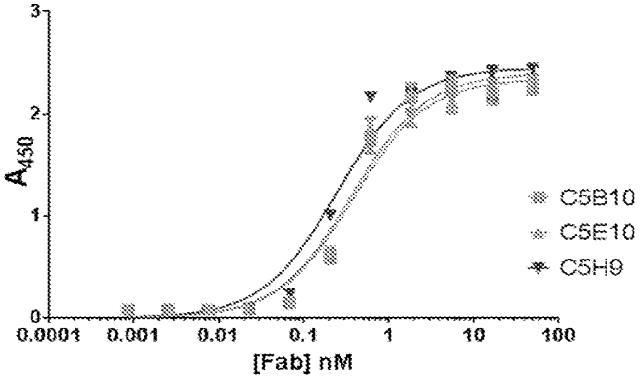


FIGURE 6B

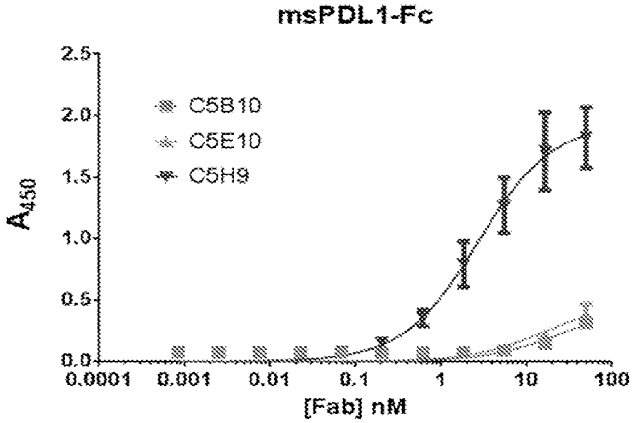


FIGURE 6C

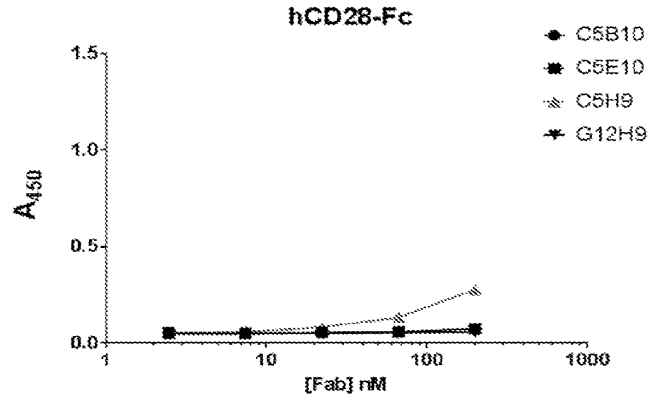


FIGURE 6D

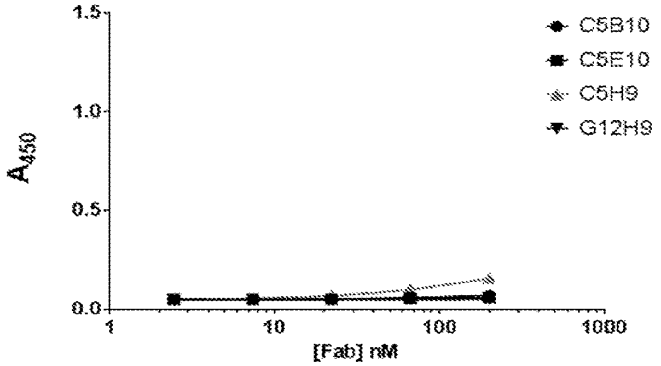
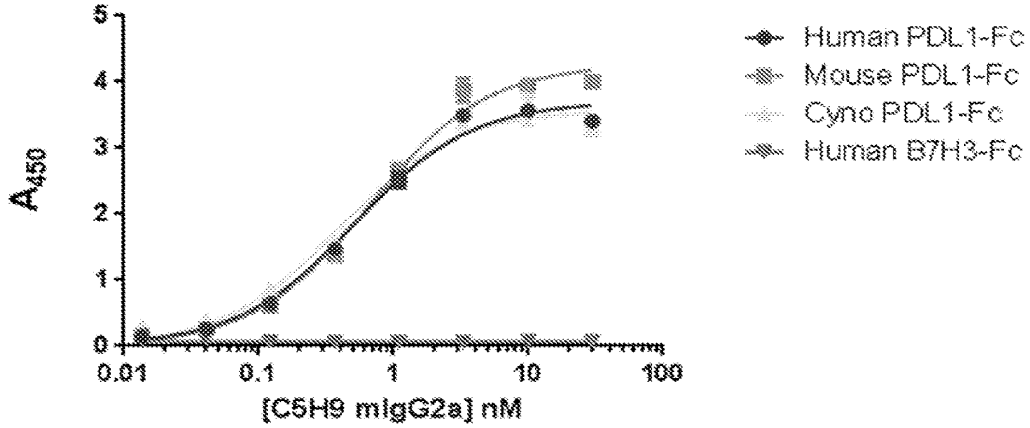


FIGURE 7



	Human	Mouse	Cynomolgus
K_d nM	0.5150	0.6657	0.3994

FIGURE 8A

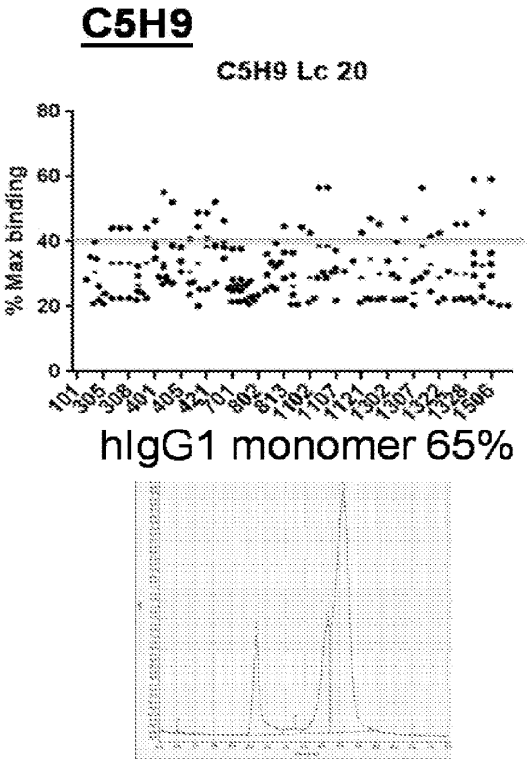


FIGURE 8B

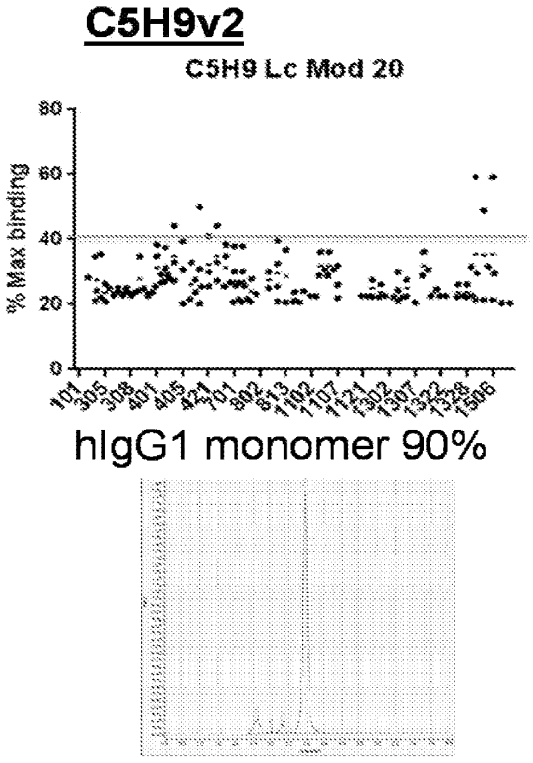


FIGURE 9A

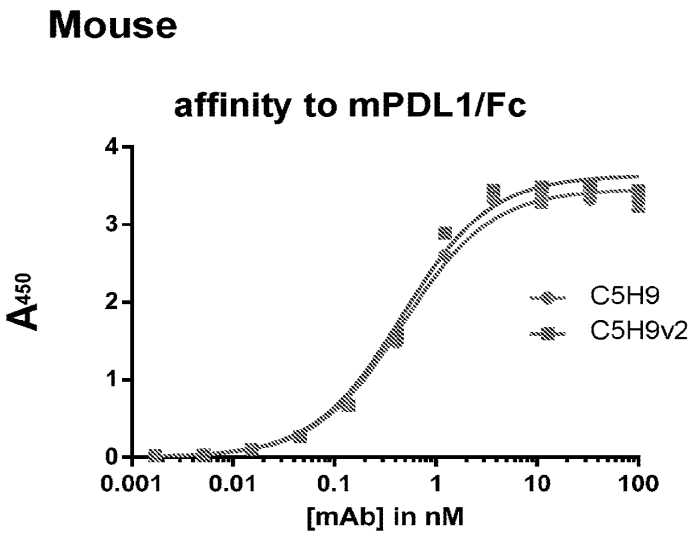


FIGURE 9B

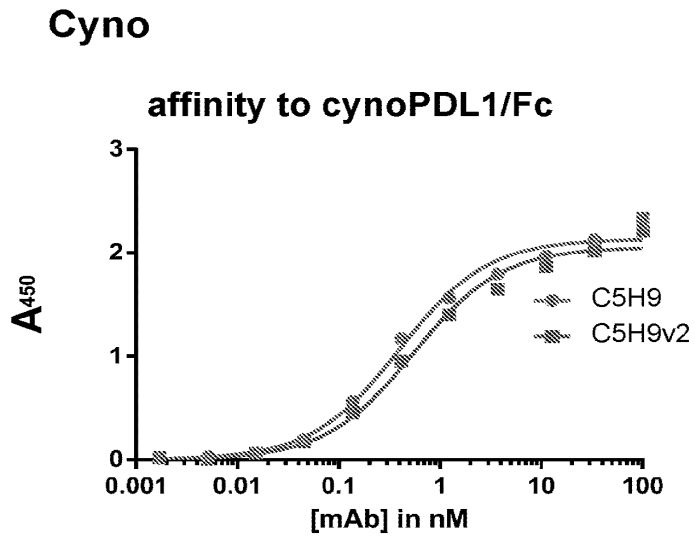
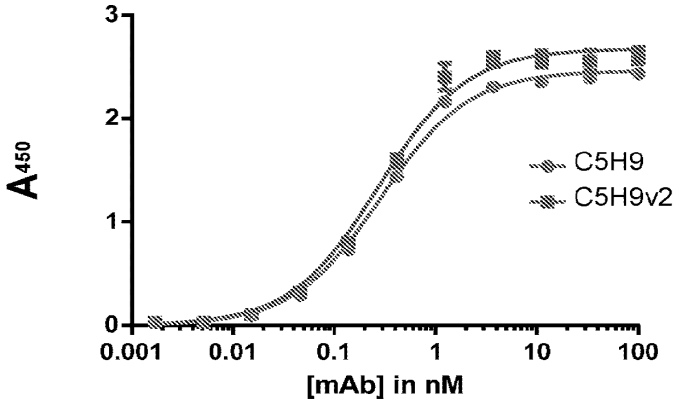


FIGURE 9C

Human

affinity to hPDL1/Fc



	Kd (nM)	
	C5H9	C5H9v2
Mouse	0.4754	0.4637
Cyno	0.3981	0.5439
Human	0.2865	0.2716

FIGURE 10

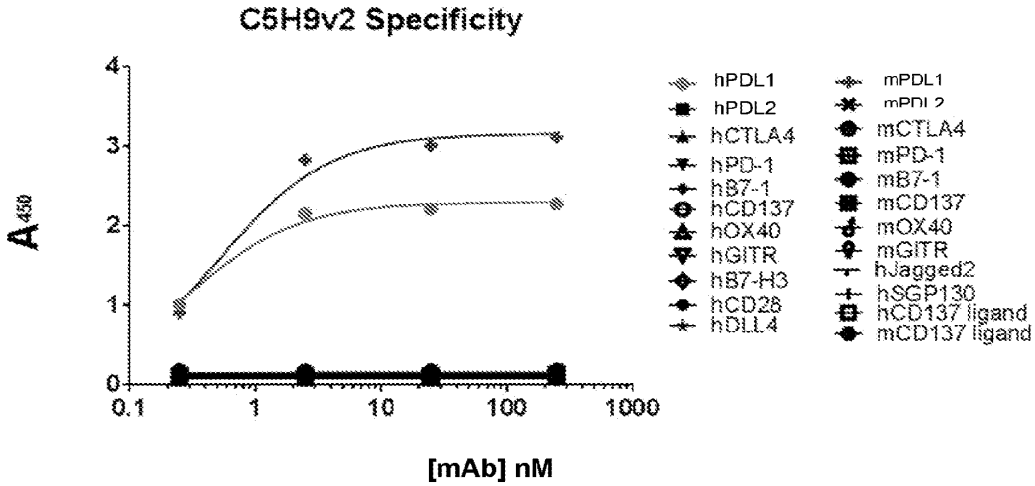
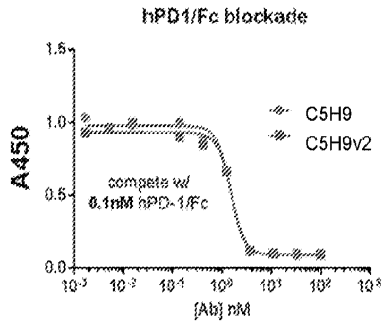
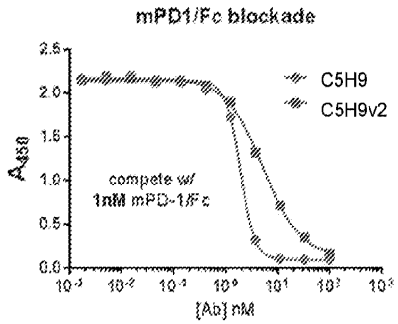
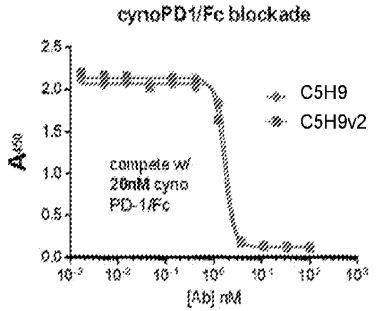

C5H9v2 Specificity
A450
4
3
2
1
0
0.1
1
10
100
1000
[mAb] nM
hPDL1
hPDL2
hCTLA4
hPD-1
hB7-1
hCD137
hOX40
hGITR
hB7-H3
hCD28
hDLL4
mPDL1
mPDL2
mCTLA4
mPD-1
mB7-1
mCD137
mOX40
mGITR
hJagged2
hSGP130
hCD137 ligand
mCD137 ligand

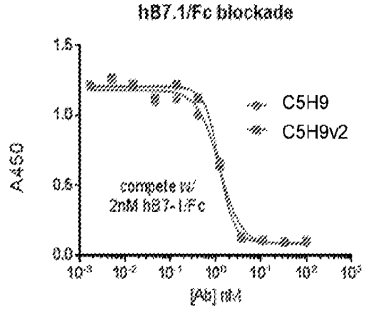
FIGURE 11

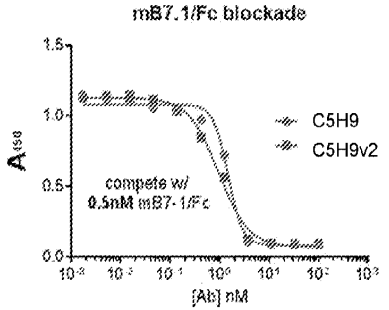






EC50 [nM]	hPD-1	mPD-1	cynoPD-1	hB7-1	mB7-1	cynoB7-1
C5H9v1	1.5	1.9	1.9	1.2	1.5	1.6
C5H9v2	1.6	5.2	1.6	1.2	0.9	1.4





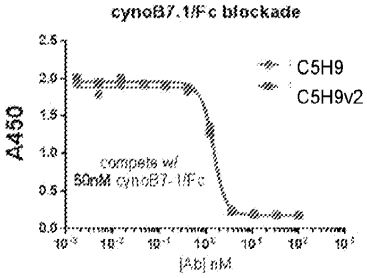


FIGURE 12

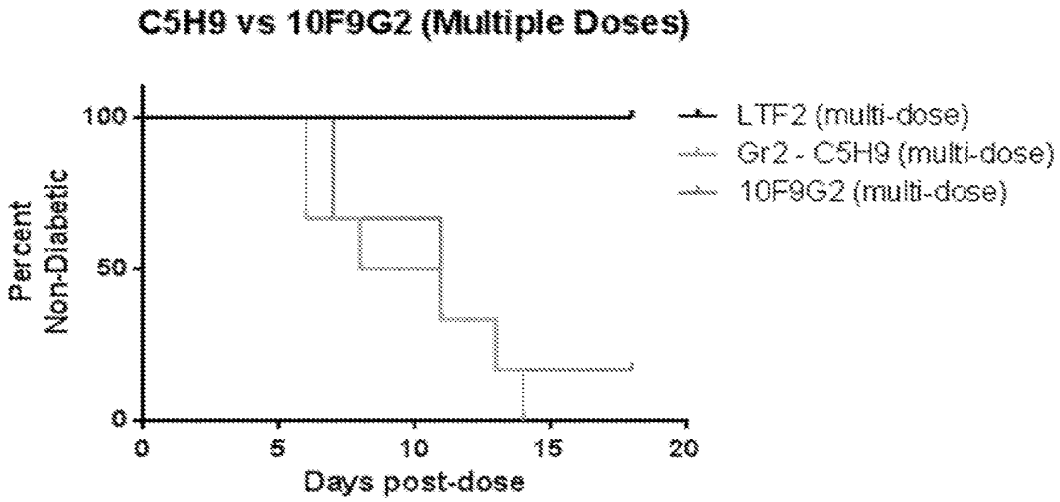


FIGURE 13

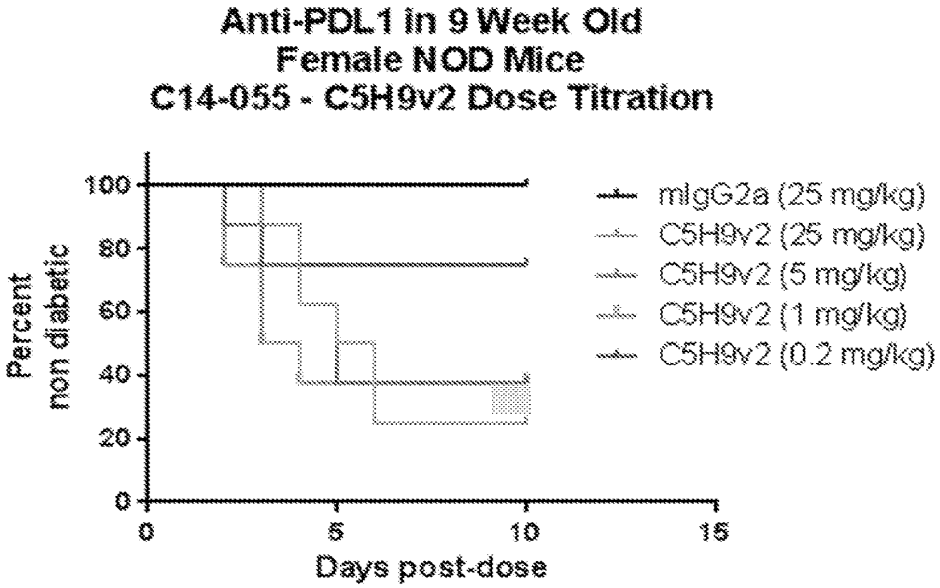


FIGURE 14

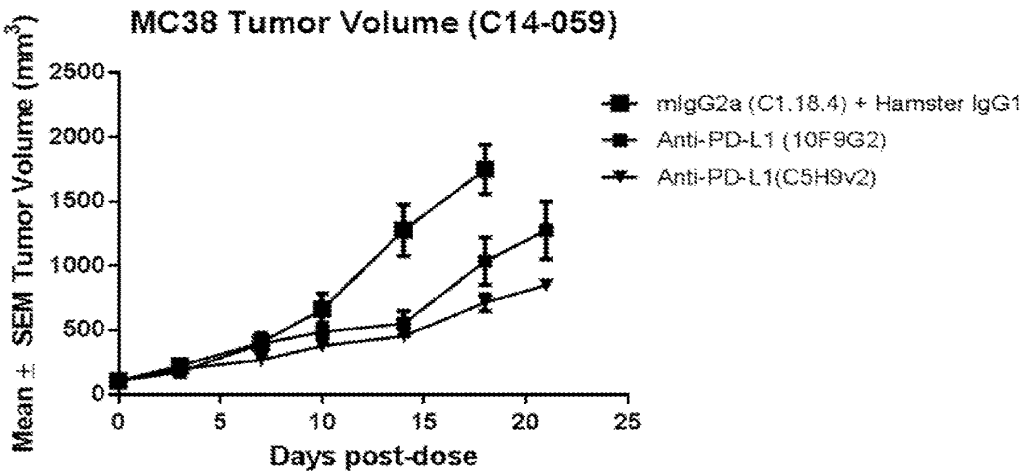


FIGURE 15A

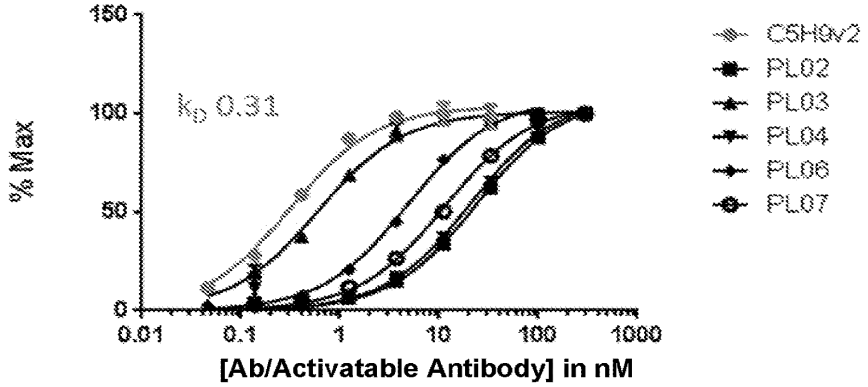
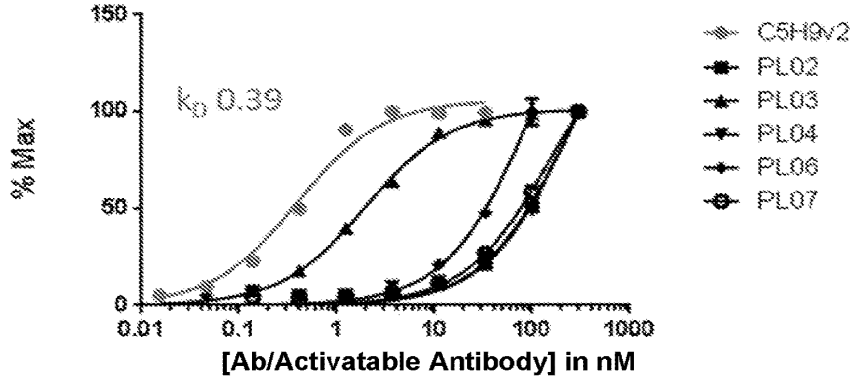
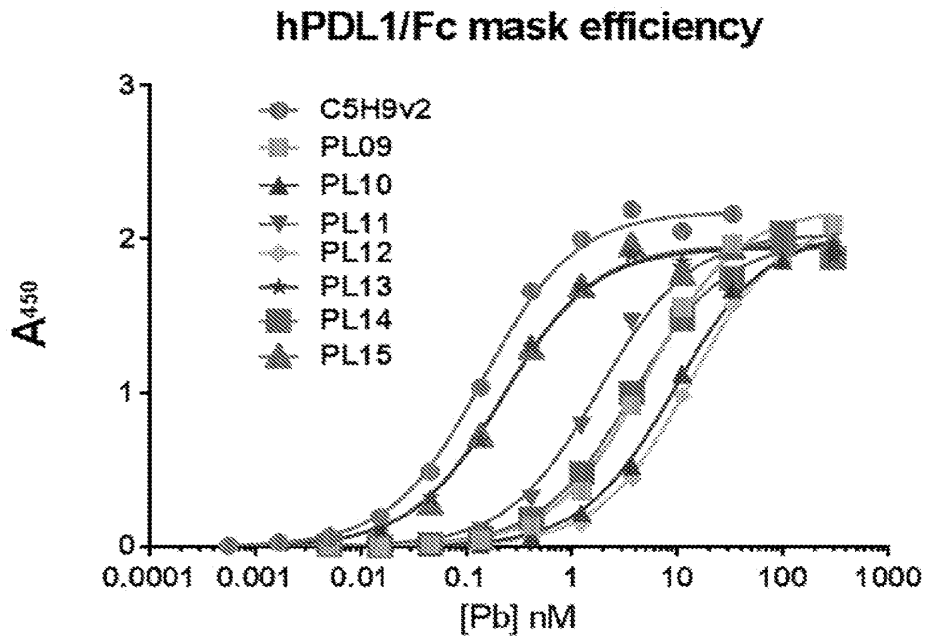


FIGURE 15B



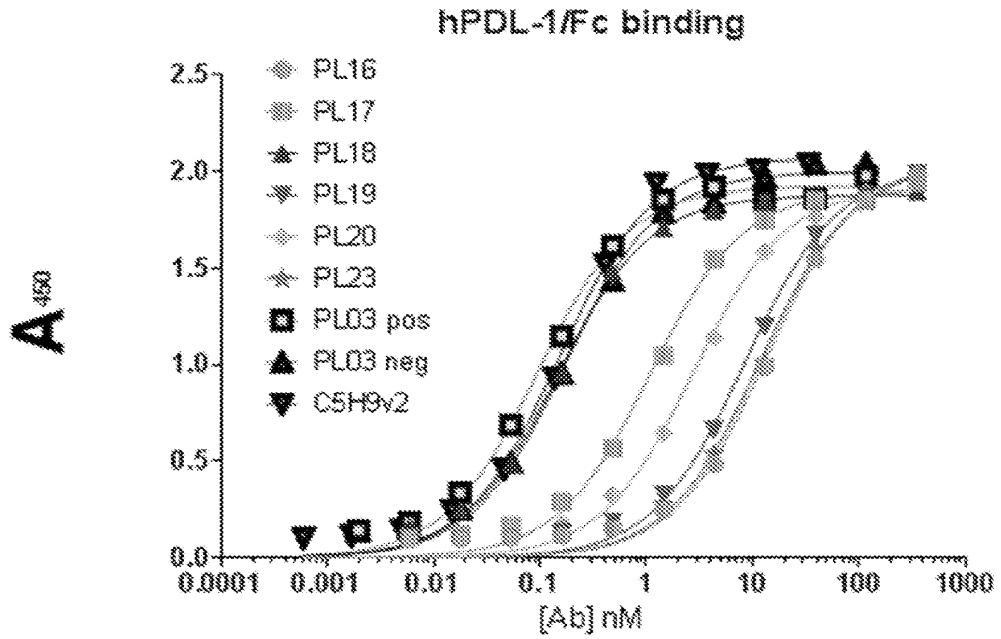
	Masking Efficiency		
	Media	Purified	
	Human	Human	Mouse
PL02	12	80	650
PL03	5	2	5
PL04	52	70	574
PL06	23	16	259
PL07	38	37	389

FIGURE 16



Mask efficiency	PL09	PL10	PL11	PL12	PL13	PL14	PL15
hPDL1	31	63	12	88	1.4	26	2
mPDL1	1518	2246	251	2940	4	1012	48

FIGURE 17



ME	PL16	PL17	PL18	PL19	PL20	PL23	PL03+	PL03-
hPDL1	88	7.2	1.0	56	18.3	77.7	0.6	1.1
mPDL1	n/a	421	25	954	645	n/a	1	2.2

FIGURE 18A

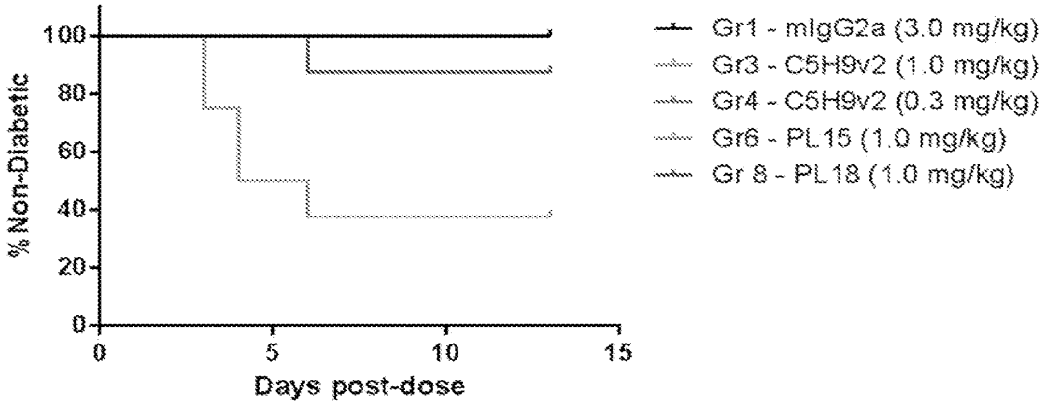


FIGURE 18B

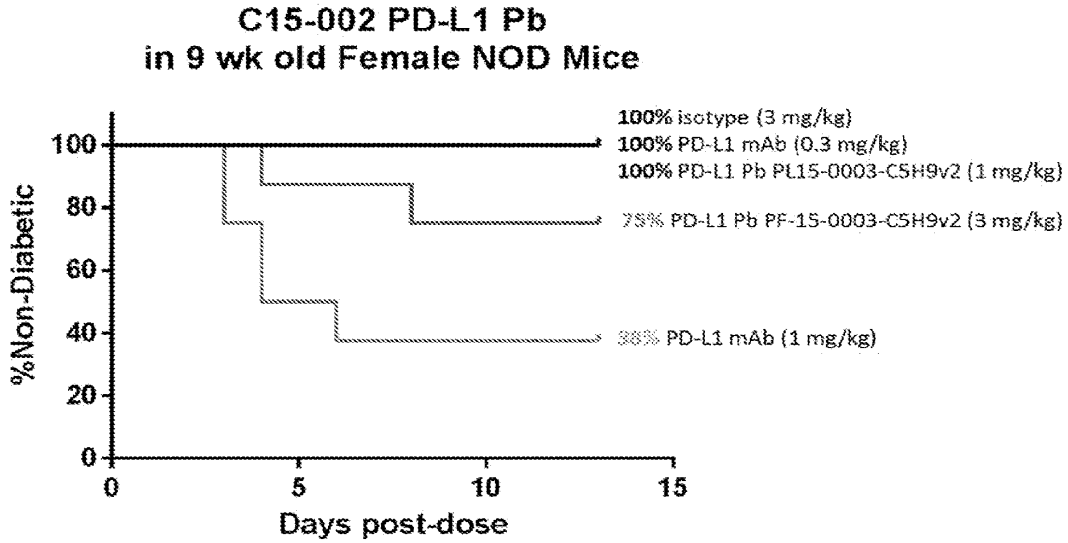


FIGURE 19

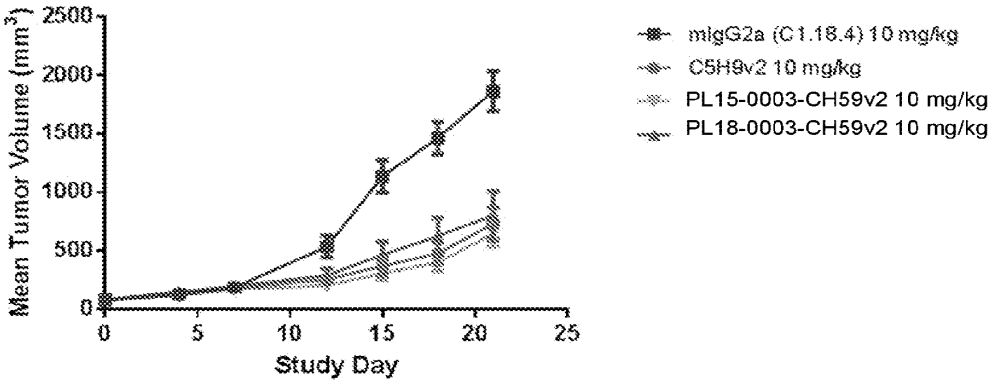


FIGURE 20A

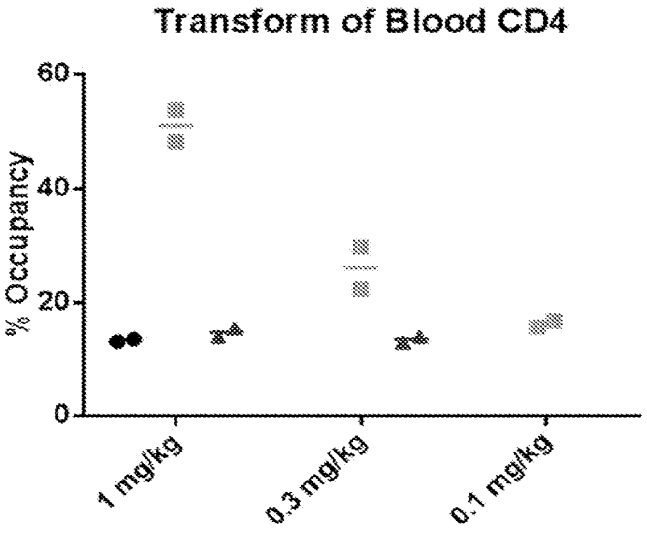


FIGURE 20B

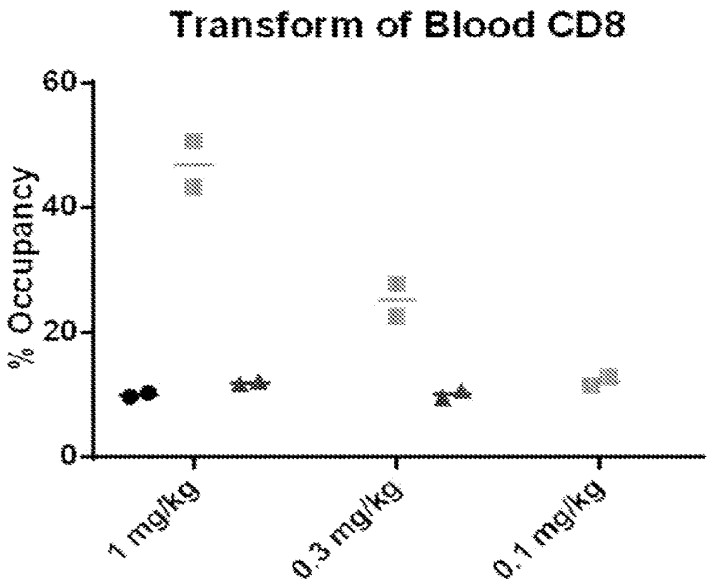


FIGURE 20C

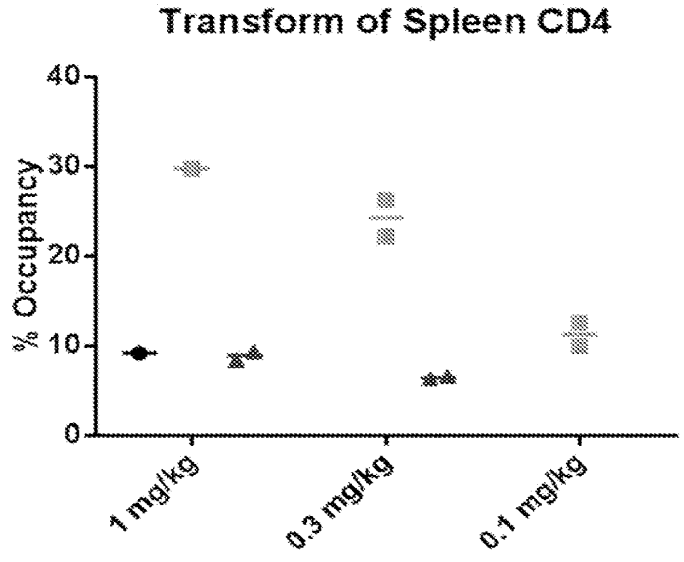


FIGURE 20D

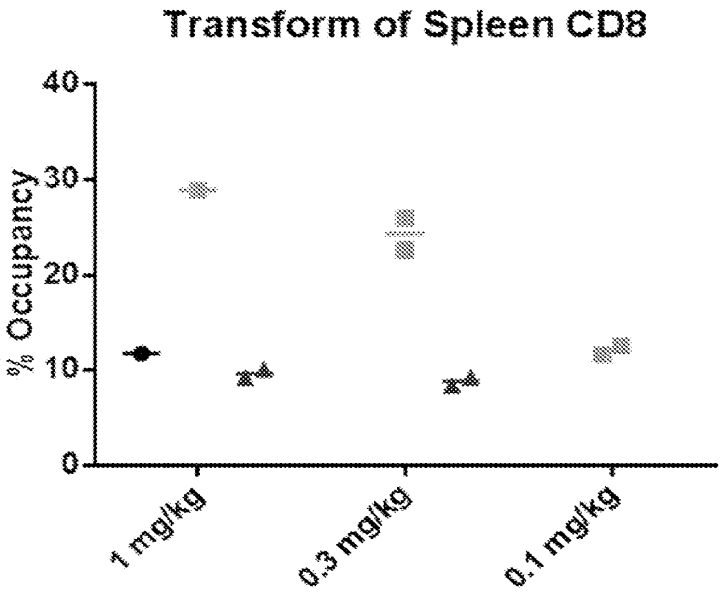


FIGURE 21A

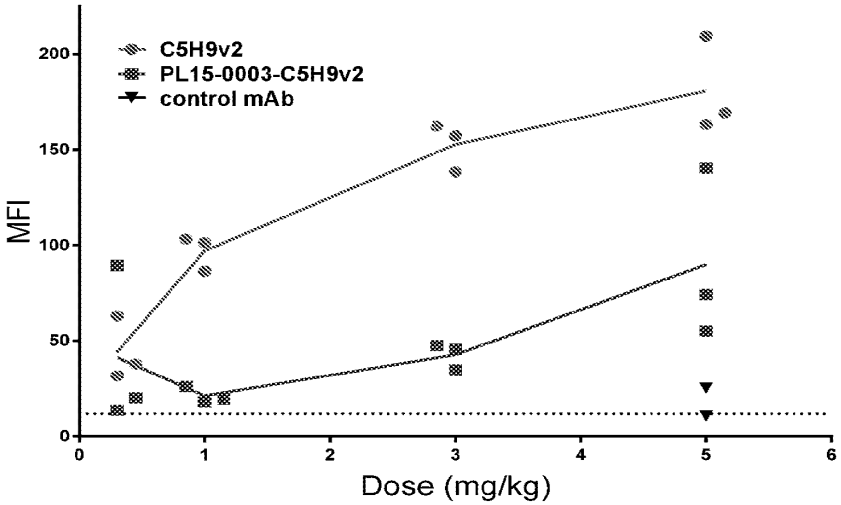


FIGURE 21B

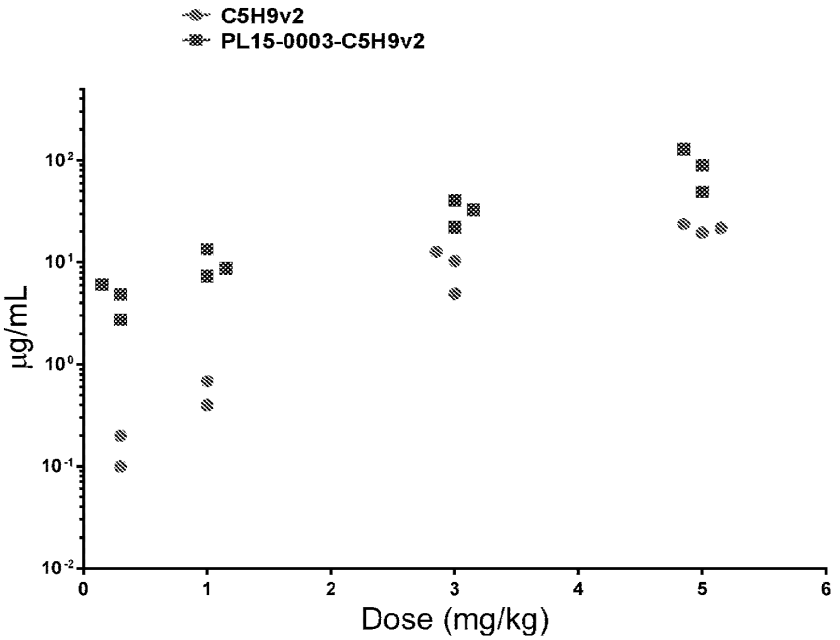


FIGURE 22

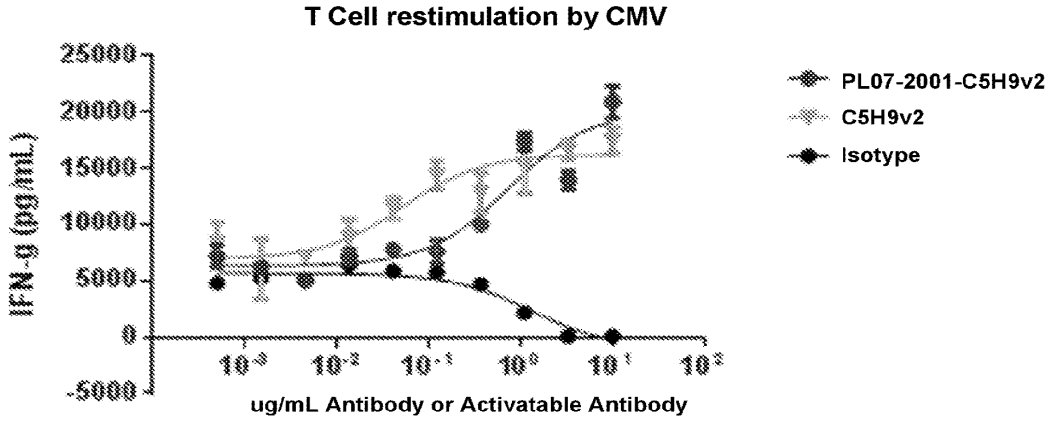


FIGURE 23A

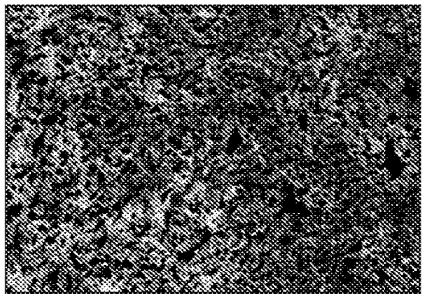


FIGURE 23B

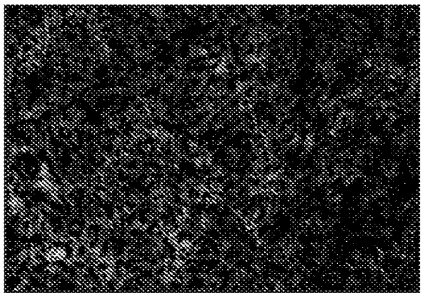


FIGURE 23C

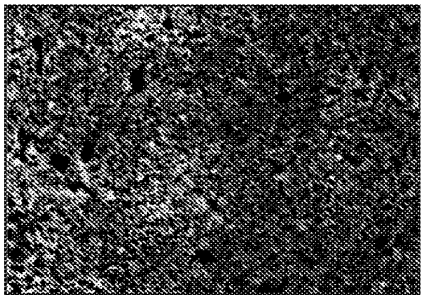


FIGURE 23D

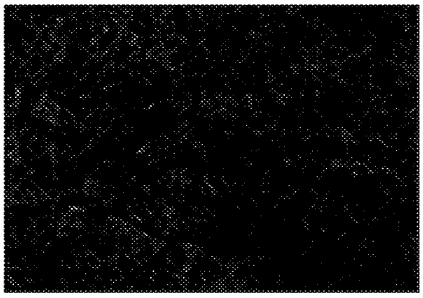


FIGURE 24

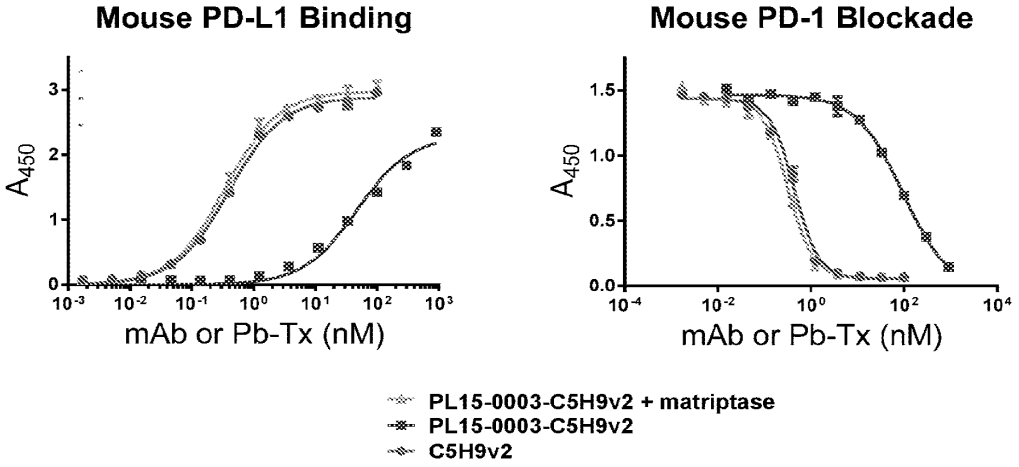
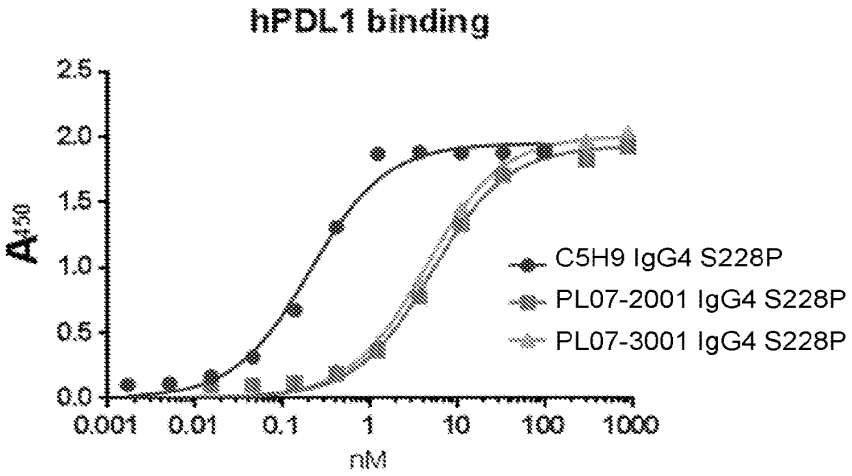
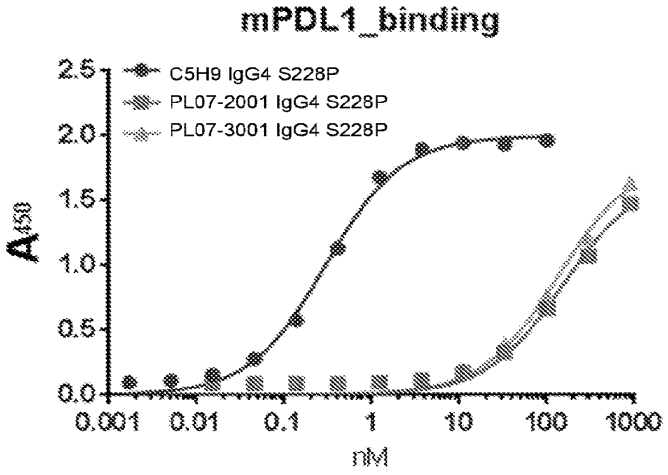


FIGURE 25A



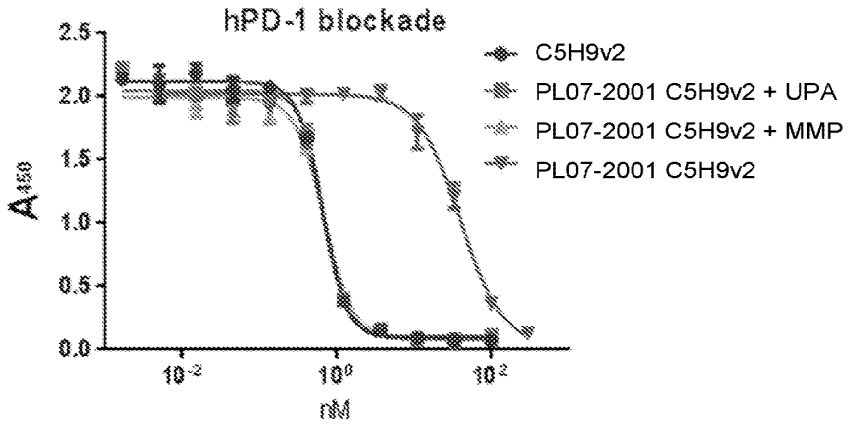
hPDL1	PL-2001	PL-3001
ME	25	21

FIGURE 25B



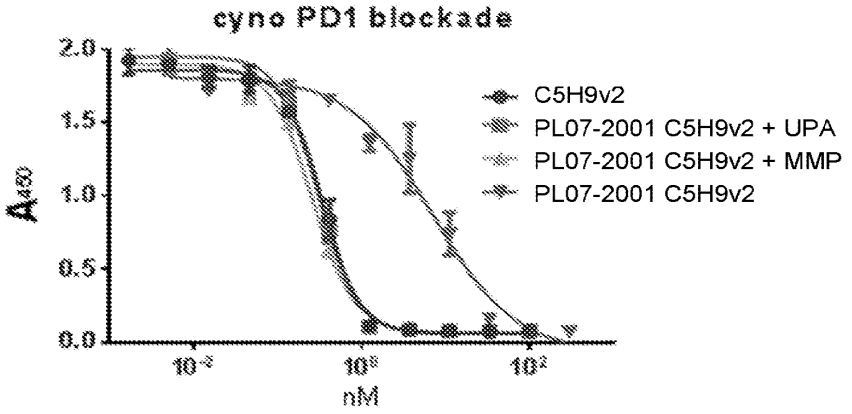
mPDL1	PL-2001	PL-3001
ME	510	450

FIGURE 26A



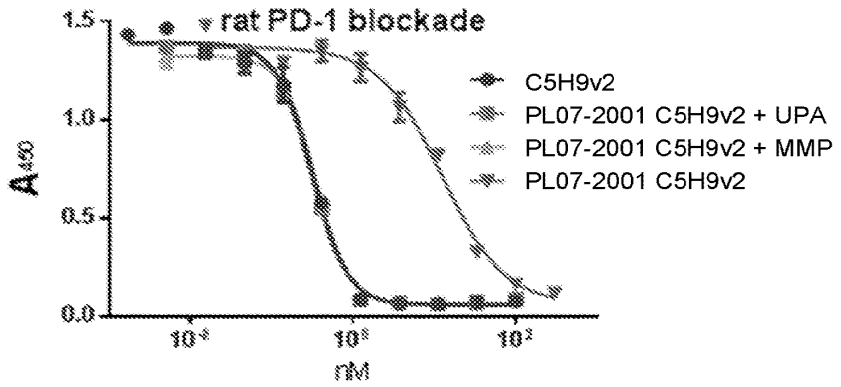
	C5H9v2	PL07-2001 C5H9v2 + UPA	PL07-2001 C5H9v2 + MMP	PL07-2001 C5H9v2
IC50 (nM)	0.7	0.7	0.7	39.8
ME	1	1	1	57

FIGURE 26B



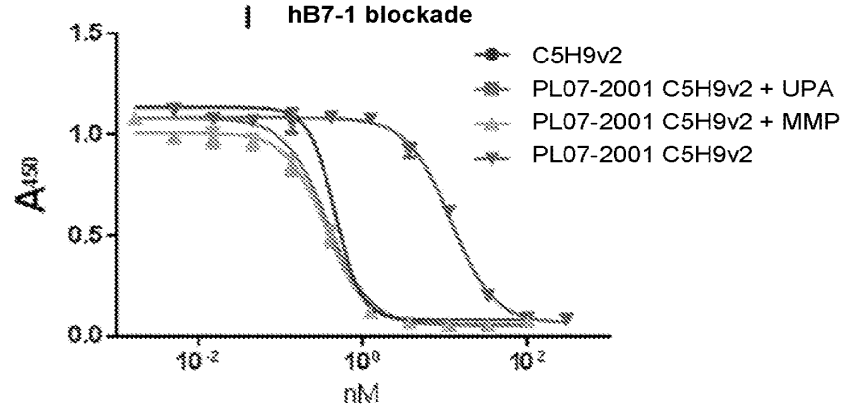
	C5H9v2	PL07-2001 C5H9v2 + UPA	PL07-2001 C5H9v2 + MMP	PL07-2001 C5H9v2
IC50 (nM)	0.3	0.3	0.3	7
ME	1	1	1	23

FIGURE 26C



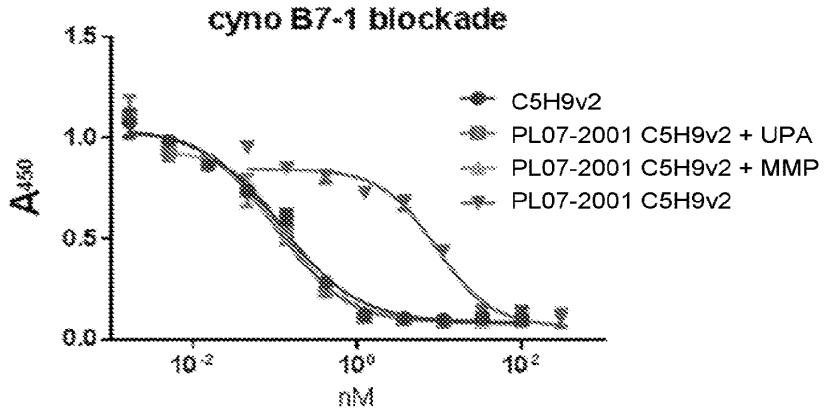
	C5H9v2	PL07-2001 C5H9v2 + UPA	PL07-2001 C5H9v2 + MMP	PL07-2001 C5H9v2
IC50 (nM)	0.3	0.3	0.3	11.9
ME	1	1	1	40

FIGURE 27A



	C5H9v2	PL07-2001 C5H9v2 + UPA	PL07-2001 C5H9v2 + MMP	PL07-2001 C5H9v2
IC50 (nM)	0.5	0.5	0.4	11.7
ME	1	1	1	29

FIGURE 27B



	C5H9v2	PL07-2001 C5H9v2 + UPA	PL07-2001 C5H9v2 + MMP	PL07-2001 C5H9v2
IC50 (nM)	0.1	0.1	0.1	9.6
ME	1	1	1	96

FIGURE 28

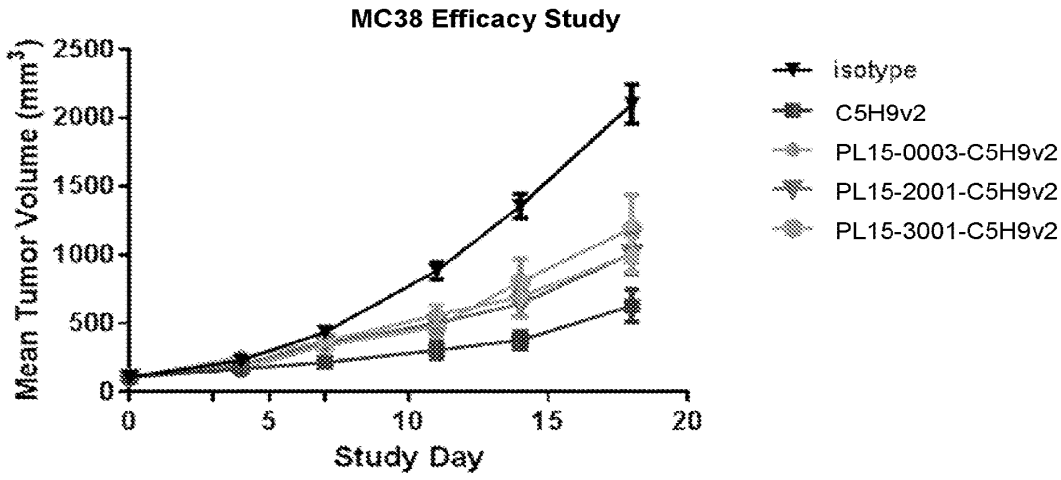


FIGURE 29A

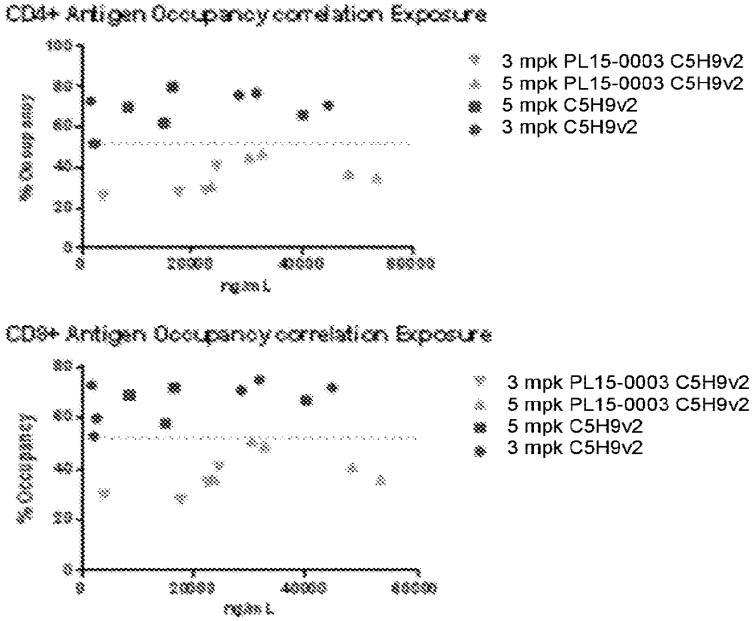


FIGURE 29B

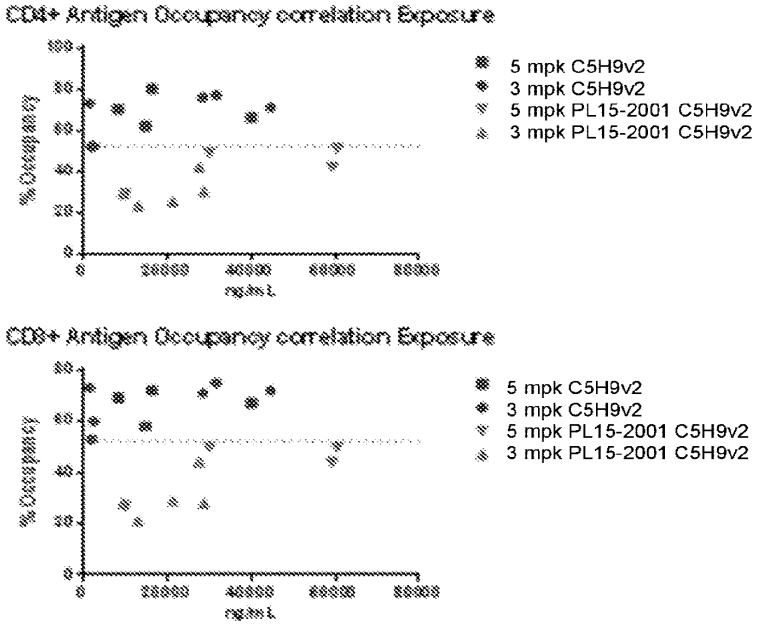


FIGURE 29C

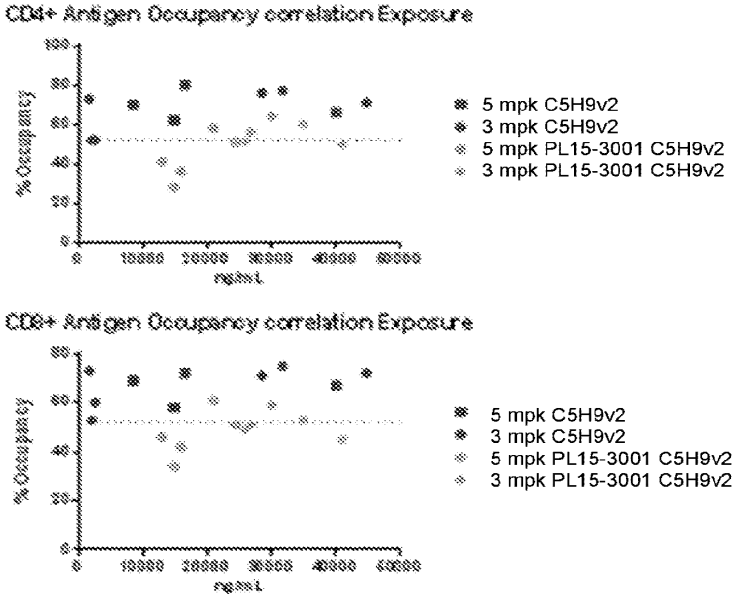
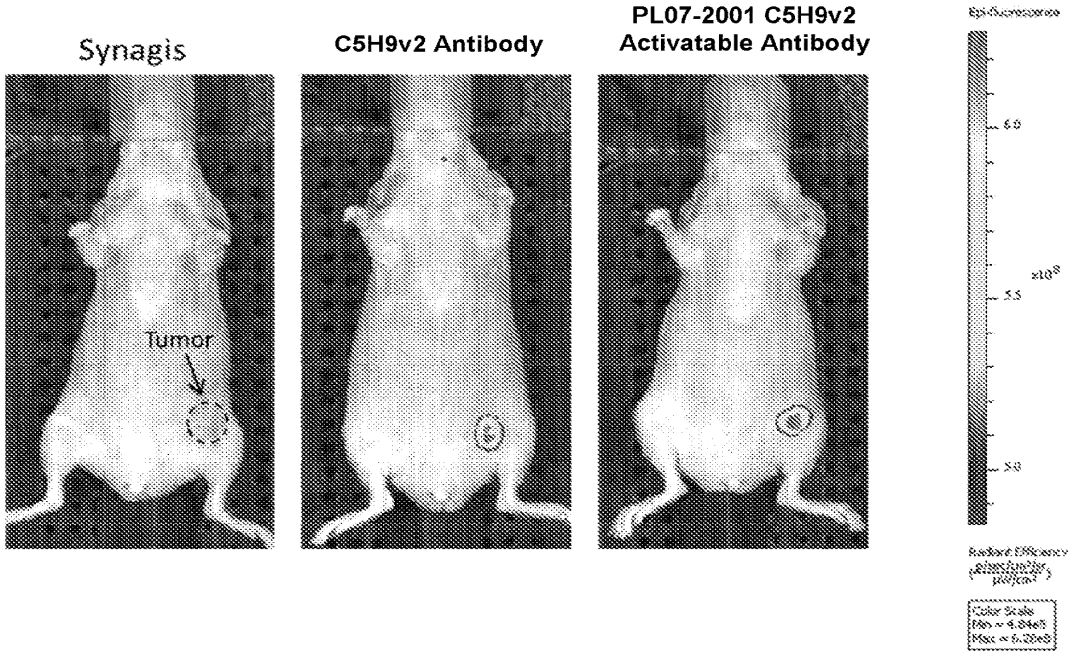


FIGURE 30



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(54) **Title**: GM-CSF/CD40L VACCINE AND CHECKPOINT INHIBITOR COMBINATION THERAPY

(57) **Abstract**: A method is disclosed for treating a cancer in a subject. The method comprises administering to the subject a composition comprising a therapeutically effective amount of a checkpoint inhibitor and a therapeutically effective amount of a tumor vaccine. In some embodiments, the tumor vaccine comprises radiated autologous tumor cells and a cell line engineered to express granulocyte- macrophage colony-stimulating factor (GM-CSF) and cluster of differentiation 40 (CD40) ligand. In some embodiments, the checkpoint inhibitor comprises an anti-programmed death-1 (anti-PD-1) antibody (e.g., BMS 936558), anti-programmed death ligand-1 (anti-PD-L1) antibody (e.g., cloneMIHI), anti-cytotoxic T lymphocyte antigen-4 (anti-CTLA-4) antibody (e.g., Ipilimumab, BMS), or any combination thereof.

GM-CSF/CD40L VACCINE AND CHECKPOINT INHIBITOR COMBINATION THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application No. 62/171,829, filed June 5, 2015, which is hereby incorporated herein by reference in its entirety.

BACKGROUND

It has been shown that cancers have multiple mechanisms of preventing the activation of and evading an immune response. Therefore, a single manipulation such as immunization with a vaccine will not likely be adequate for clinical efficacy.

SUMMARY

A method is disclosed for treating a cancer in a subject. The method comprises administering to the subject a composition comprising a therapeutically effective amount of a checkpoint inhibitor and a therapeutically effective amount of a tumor vaccine. In some embodiments, the tumor vaccine comprises radiated autologous tumor cells and a cell line engineered to express GM-CSF and CD40 ligand.

In some cases, the vaccine comprises a mixture of one, two, or more human lung adenocarcinoma cell lines and a bystander cell line engineered to secrete GM-CSF and express CD40 ligand (GM.CD40L). GM-CSF secretion at the vaccine site is expected to recruit and differentiate dendritic cells (DCs), which will be activated by their encounter with CD40 ligand on the surface of the bystander cells. These activated and antigen-loaded DCs will then migrate to the draining lymph nodes and activate tumor antigen-specific T cells (CD4 and CD8). These activated T cells will then recirculate to metastatic sites and kill tumor cells.

The cell line can be further engineered to express one more factors selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-15, erythropoietin, GCSF, M-CSF, platelet derived growth factor (PDGF), MSF, FLT-3 ligand, EGF, fibroblast growth factor (FGF), bFGF (FGF-2), FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, insulin-like growth factorl (IGF-1), insulin-like growth factor 2 (IGF-2); vascular endothelial growth factor (VEGF), IFN-

gamma, IFN-alpha, IFN-beta, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M, stem cell factor (SCF), TGF-α, and TGF-β1. The cell line is preferably MHC negative, and can be a tumor cell line.

The combination of the disclosed vaccine with an immune checkpoint can amplify the T-cell response and enhance the efficacy of tumor infiltrating T cells, thus resulting in a more potent antitumor effect compared to a single agent. In some embodiments, the checkpoint inhibitor comprises an anti-PD-1 antibody (e.g., BMS 936558), anti-PD-L1 antibody (e.g., cloneM1H1), anti-CTLA-4 antibody (e.g., Ipilimumab, BMS), or any combination thereof.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DETAILED DESCRIPTION

Tumor vaccines in general have very little single agent activity because even if they are effective in expanding tumor-specific T cells in the lymphoid compartment, these T cells are rendered inactive in the tumor microenvironment due to exposure to PD-L1. Therefore it is necessary to combine tumor vaccines with agents that are directed at the tumor microenvironment.

In order to actively drive an antitumor immune response, therapeutic cancer vaccines have been developed. Unlike the prophylactic vaccines that are used preventatively to treat infectious diseases, therapeutic vaccines are designed to treat established cancer by stimulating an immune response against a specific tumor-associated antigen. A number of tumor-associated antigens are known in the art, and methods for detecting them arc well known.

In some embodiments the tumor vaccine comprises "gene-modified universal producer cells" that are a source of expressed GM-CSF and CD40 ligand. In some embodiments, the cell line is MHC molecule negative cell line, such as the K562 cell line (available from the American Type Culture Collection, Manassas, VA) - the use of such cells should reduce the incidence of allogeneic responses to the cells upon introduction into individuals. In some cases, transformed cells express exogenous GM-CSF and CD40 ligand transgenes.

In some embodiments, the "gene-modified universal producer cells" can also contain and express transgenes encoding one or more compounds selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-15, erythropoietin, G-CSF, M-CSF, platelet derived growth factor (PDGF), MSF, FLT-3 ligand, EGF, fibroblast growth factor (FGF; e.g., aFGF (FGF-1), bFGF (FGF-2), FGF-3, FGF-4, FGF-5, FGF-6, or FGF-7), insulin-like growth factors (e.g., IGF-1, IGF-2); vascular endothelial growth factor (VEGF); interferons (e.g., IFN-gamma, IFN-alpha, IFN-beta); leukemia inhibitory factor (LIF); ciliary neurotrophic factor (CNTF); oncostatin M; stem cell factor (SCF); transforming growth factors (e.g., TGF-a, TGF-~1, TGF-~1, TGF-~1). DNA molecules can encode mammalian, including human or murine, versions of the aforementioned compounds.

In some embodiments, peripheral blood lymphocytes of a individual afflicted with a tumor, cancer, or malignancy are cultured with the antigen presenting cells (e.g., dendritic cells), GM-CSF/CD40L expressing cells (or cells derived from a GM-CSF/CD40L cell line), and optinally apoptotic autologous tumor, cancer, or malignant cells (or antigens derived therefrom). The cultured cells are then re-administered to the patient as a form of autologous therapy (see, for example, U.S. Patent No. 5,192,537 [hereby incorporated by reference in its entirety]). The methodology is also applicable to patients experiencing a recurrence of a tumor, cancer, or malignancy. In a further embodiment, suppressor T-cells are removed from the individual's peripheral blood lymphocytes and the remaining cells are suspended in a tissue culture medium containing GM-CSF /CD40L expressing cells and irradiated autologous tumor or cancer cells (or the antigens derived therefrom). The cells then are incubated, at temperatures of about 37°C to about 40°C, for a period of time so that they are activated. Those cells specific for the tumor antigen are clonally expanded and, optionally, treated to re-deplete various kinds of suppressor cells and/or boost their activity. Activated cells may be co-administered with cells expressing GM-CSF/CD40L and/or with one or more cytokines.

In some cases, the tumor vaccine comprises (1) radiated tumor cells (e.g., autologous tumor cells) providing the source of shared tumor antigens and (2) a bystander cell line (GM.CD40L) engineered to secrete GM-CSF and express CD40 ligand on its surface. GM-CSF secretion at the vaccine site can recruit and differentiate DCs, which will be activated by their encounter with CD40 ligand on the

surface of the bystander cells. Apoptotic bodies from the radiated tumor cells will then be processed by the DCs.

Tumor vaccines can be used in combination with a checkpoint inhibitor. The two known inhibitory checkpoint pathways involve signaling through the cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed-death 1 (PD-1) receptors. These proteins are members of the CD28-B7 family of cosignaling molecules that play important roles throughout all stages of T cell function. The PD-1 receptor (also known as CD279) is expressed on the surface of activated T cells. Its ligands, PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC; CD273), are expressed on the surface of APCs such as dendritic cells or macrophages. PD-L1 is the predominant ligand, while PD-L2 has a much more restricted expression pattern. When the ligands bind to PD-1, an inhibitory signal is transmitted into the T cell, which reduces cytokine production and suppresses T-cell proliferation. A list of immune-checkpoint targeting antibodies in clinical trials is provided in Table 2.

Table 2. Clinically evaluated immune-checkpoint blocking antibodies

Target	Antibody
CTLA-4	Ipilimumab (MDX-010)
	Tremelimumab (CP-675,206)
PD1	Nivolumab (BMS-936558 or MDX1106)
	CT-011
	MK-3475
PDL1	MDX-1105 (BMS-936559)
	MPDL3280A
	MSB0010718C
PDL2	rHIgM12B7
B7-H3	MGA271
LAG3	BMS-986016

Human monoclonal antibodies to programmed death 1 (PD-1) and methods for treating cancer using anti-PD-1 antibodies alone or in combination with other immunotherapeutics are described in U.S. Patent No. 8,008,449, which is incorporated by reference for these antibodies. Anti-PD-L1 antibodies and uses therefor are described in U.S. Patent No. 8,552,154, which is incorporated by reference for these antibodies. Anticancer agent comprising anti-PD-1 antibody or

anti-PD-L1 antibody are described in U.S. Patent No. 8,617,546, which is incorporated by reference for these antibodies.

In some embodiments, the PDL1 inhibitor comprises an antibody that specifically binds PDL1, such as BMS-936559 (Bristol-Myers Squibb) or MPDL3280A (Roche). In some embodiments, the PD1 inhibitor comprises an antibody that specifically binds PD1, such as lambrolizumab (Merck), nivolumab (Bristol-Myers Squibb), or MEDI4736 (AstraZeneca). Human monoclonal antibodies to PD-1 and methods for treating cancer using anti-PD-1 antibodies alone or in combination with other immunotherapeutics are described in U.S. Patent No. 8,008,449, which is incorporated by reference for these antibodies. Anti-PD-L1 antibodies and uses therefor are described in U.S. Patent No. 8,552,154, which is incorporated by reference for these antibodies. Anticancer agent comprising anti-PD-1 antibody or anti-PD-L1 antibody are described in U.S. Patent No. 8,617,546, which is incorporated by reference for these antibodies.

The disclosed compositions and methods can be used in combination with other cancer immunotherapies. There are two distinct types of immunotherapy: passive immunotherapy uses components of the immune system to direct targeted cytotoxic activity against cancer cells, without necessarily initiating an immune response in the patient, while active immunotherapy actively triggers an endogenous immune response. Passive strategies include the use of the monoclonal antibodies (mAbs) produced by B cells in response to a specific antigen. The development of hybridoma technology in the 1970s and the identification of tumor-specific antigens permitted the pharmaceutical development of mAbs that could specifically target tumor cells for destruction by the immune system. Thus far, mAbs have been the biggest success story for immunotherapy; the top three best-selling anticancer drugs in 2012 were mAbs. Among them is rituximab (Rituxan, Genentech), which binds to the CD20 protein that is highly expressed on the surface of B cell malignancies such as non-Hodgkin's lymphoma (NHL). Rituximab is approved by the FDA for the treatment of NHL and chronic lymphocytic leukemia (CLL) in combination with chemotherapy. Another important mAb is trastuzumab (Herceptin; Genentech), which revolutionized the treatment of HER2 (human epidermal growth factor receptor 2)-positive breast cancer by targeting the expression of HER2.

Generating optimal "killer" CD8 T cell responses also requires T cell receptor activation plus co-stimulation, which can be provided through ligation of tumor necrosis factor receptor family members, including OX40 (CD134) and 4-1BB (CD137). OX40 is of particular interest as treatment with an activating (agonist) anti-OX40 mAb augments T cell differentiation and cytolytic function leading to enhanced anti-tumor immunity against a variety of tumors.

Numerous anti-cancer drugs are available for combination with the present method and compositions. The following is a non-exhaustive lists of anti-cancer (anti-neoplastic) drugs that can be used in conjunction with irradiation: Acivicin; Aclarubicin; Acodazole Hydrochloride; AcrQnine; Adozelesin; Aldesleukin; Altretamine; Ambomycin; Ametantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine; Busulfan; Cactinomycin; Calusterone; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; Dactinomycin; Daunorubicin Hydrochloride; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflomithine Hydrochloride; Elsamitrucin; Enloplatin; Enpromate; Epipropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Ethiodized Oil I 131; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate; Fluorouracil; Flurocitabine; Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Gold Au 198; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofosine; Iproplatin; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; Leuprolide Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Metoprine; Meturedepa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper;

Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; Peplomycin Sulfate; Perfosfamide; Pipobroman; Piposulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Riboprine; Rogletimide; Safmgol; Safingol Hydrochloride; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Strontium Chloride Sr 89; Sulofenur; Talisomycin; Taxane; Taxoid; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Tiazofurin; Tirapazamine; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole Hydrochloride; Uracil Mustard; Uredepa; Vapreotide; Verteporfin; Vinblastine Sulfate; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride.

The cancer of the disclosed methods can be any cell in a subject undergoing unregulated growth, invasion, or metastasis. In some aspects, the cancer can be any neoplasm or tumor for which radiotherapy is currently used. Alternatively, the cancer can be a neoplasm or tumor that is not sufficiently sensitive to radiotherapy using standard methods. Thus, the cancer can be a sarcoma, lymphoma, leukemia, carcinoma, blastoma, or germ cell tumor. A representative but non-limiting list of cancers that the disclosed compositions can be used to treat include lymphoma, B cell lymphoma, T cell lymphoma, mycosis fungoides, Hodgkin's Disease, myeloid leukemia, bladder cancer, brain cancer, nervous system cancer, head and neck cancer, squamous cell carcinoma of head and neck, kidney cancer, lung cancers such as small cell lung cancer and non-small cell lung cancer (NSCLC), neuroblastoma/glioblastoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, liver cancer, melanoma, squamous cell carcinomas of the mouth, throat, larynx, and lung, colon cancer, cervical cancer, cervical carcinoma, breast cancer, epithelial cancer, renal cancer, genitourinary cancer, pulmonary cancer, esophageal

carcinoma, head and neck carcinoma, large bowel cancer, hematopoietic cancers; testicular cancer; colon and rectal cancers, prostatic cancer, and pancreatic cancer. For example, Table 3 provides a list of the categories and types of cancers.

Table 3. Cancer Cases
Oral cavity & pharynx
Tongue
Mouth
Pharynx
Other oral cavity
Digestive system
Esophagus
Stomach
Small intestine
Colon
Rectum
Anus, anal canal, & anorectum
Liver & intrahepatic bile duct
Gallbladder & other biliary
Pancreas
Other digestive organs
Respiratory system
Larynx
Lung & bronchus
Other respiratory organs
Bones & joints
Soft tissue (including heart)
Skin (excluding basal & squamous)
Melanoma-skin
Other nonepithelial skin
Breast
Genital system
Uterine cervix
Uterine corpus
Ovary
Vulva
Vagina
Prostate
Testis
Penis
Urinary system
Urinary bladder
Kidney & renal pelvis
Ureter & other urinary organs
Eye & orbit
Brain & other nervous system
Endocrine system

Thyroid
Other endocrine
Lymphoma
Hodgkin lymphoma
Non-Hodgkin lymphoma
Myeloma
Leukemia
Acute lymphocytic leukemia
Chronic lymphocytic leukemia
Acute myeloid leukemia
Chronic myeloid leukemia
Other leukemia‡
Other & unspecified primary sites

Compositions, Formulations and Methods of Administration

In vivo application of the disclosed compounds, and compositions containing them, can be accomplished by any suitable method and technique presently or prospectively known to those skilled in the art. For example, the disclosed compounds can be formulated in a physiologically- or pharmaceutically-acceptable form and administered by any suitable route known in the art including, for example, oral, nasal, rectal, topical, and parenteral routes of administration. As used herein, the term parenteral includes subcutaneous, intradermal, intravenous, intramuscular, intraperitoneal, and intrasternal administration, such as by injection. Administration of the disclosed compounds or compositions can be a single administration, or at continuous or distinct intervals as can be readily determined by a person skilled in the art.

The compounds disclosed herein, and compositions comprising them, can also be administered utilizing liposome technology, slow release capsules, implantable pumps, and biodegradable containers. These delivery methods can, advantageously, provide a uniform dosage over an extended period of time. The compounds can also be administered in their salt derivative forms or crystalline forms.

The compounds disclosed herein can be formulated according to known methods for preparing pharmaceutically acceptable compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E.W. Martin (1995) describes formulations that can be used in connection with the disclosed methods. In general, the compounds disclosed herein can be formulated

such that an effective amount of the compound is combined with a suitable carrier in order to facilitate effective administration of the compound. The compositions used can also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspension, suppositories, injectable and infusible solutions, and sprays. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically-acceptable carriers and diluents which are known to those skilled in the art. Examples of carriers or diluents for use with the compounds include ethanol, dimethyl sulfoxide, glycerol, alumina, starch, saline, and equivalent carriers and diluents. To provide for the administration of such dosages for the desired therapeutic treatment, compositions disclosed herein can advantageously comprise between about 0.1% and 99%, and especially, 1 and 15% by weight of the total of one or more of the subject compounds based on the weight of the total composition including carrier or diluent.

Formulations suitable for administration include, for example, aqueous sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions, which can include suspending agents and thickening agents. The formulations can be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and can be stored in a freeze dried (lyophilized) condition requiring only the condition of the sterile liquid carrier, for example, water for injections, prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powder, granules, tablets, *etc.* It should be understood that in addition to the ingredients particularly mentioned above, the compositions disclosed herein can include other agents conventional in the art having regard to the type of formulation in question.

Compounds disclosed herein, and compositions comprising them, can be delivered to a cell either through direct contact with the cell or via a carrier means. Carrier means for delivering compounds and compositions to cells are known in the art and include, for example, encapsulating the composition in a liposome moiety. Another means for delivery of compounds and compositions disclosed herein to a cell comprises attaching the compounds to a protein or nucleic acid that is targeted for delivery to the target cell. U.S. Patent No. 6,960,648 and U.S. Application

Publication Nos. 20030032594 and 20020120100 disclose amino acid sequences that can be coupled to another composition and that allows the composition to be translocated across biological membranes. U.S. Application Publiation No. 20020035243 also describes compositions for transporting biological moieties across cell membranes for intracellular delivery. Compounds can also be incorporated into polymers, examples of which include poly (D-L lactide-co-glycolide) polymer for intracranial tumors; poly[bis(p-carboxyphenoxy) propane:sebacic acid] in a 20:80 molar ratio (as used in GLIADEL); chondroitin; chitin; and chitosan.

For the treatment of oncological disorders, the compounds disclosed herein can be administered to a patient in need of treatment in combination with other antitumor or anticancer substances and/or with radiation and/or photodynamic therapy and/or with surgical treatment to remove a tumor. These other substances or treatments can be given at the same as or at different times from the compounds disclosed herein. For example, the compounds disclosed herein can be used in combination with mitotic inhibitors such as taxol or vinblastine, alkylating agents such as cyclophosamide or ifosfamide, antimetabolites such as 5-fluorouracil or hydroxyurea, DNA intercalators such as adriamycin or bleomycin, topoisomerase inhibitors such as etoposide or camptothecin, antiangiogenic agents such as angiostatin, antiestrogens such as tamoxifen, and/or other anti-cancer drugs or antibodies, such as, for example, GLEEVEC (Novartis Pharmaceuticals Corporation) and HERCEPTIN (Genentech, Inc.), respectively, or an immunotherapeutic such as ipilimumab and bortezomib.

In certain examples, compounds and compositions disclosed herein can be locally administered at one or more anatomical sites, such as sites of unwanted cell growth (such as a tumor site or benign skin growth, *e.g.*, injected or topically applied to the tumor or skin growth), optionally in combination with a pharmaceutically acceptable carrier such as an inert diluent. Compounds and compositions disclosed herein can be systemically administered, such as intravenously or orally, optionally in combination with a pharmaceutically acceptable carrier such as an inert diluent, or an assimilable edible carrier for oral delivery. They can be enclosed in hard or soft shell gelatin capsules, can be compressed into tablets, or can be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound can be combined with one or more excipients and used in the form of

ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, aerosol sprays, and the like.

The tablets, troches, pills, capsules, and the like can also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring can be added. When the unit dosage form is a capsule, it can contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials can be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules can be coated with gelatin, wax, shellac, or sugar and the like. A syrup or elixir can contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound can be incorporated into sustained-release preparations and devices.

Compounds and compositions disclosed herein, including pharmaceutically acceptable salts, or hydrates thereof, can be administered intravenously, intramuscularly, or intraperitoneally by infusion or injection. Solutions of the active agent or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient, which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. The ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example,

glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. Optionally, the prevention of the action of microorganisms can be brought about by various other antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the inclusion of agents that delay absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating a compound and/or agent disclosed herein in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, compounds and agents disclosed herein can be applied in as a liquid or solid. However, it will generally be desirable to administer them topically to the skin as compositions, in combination with a dermatologically acceptable carrier, which can be a solid or a liquid. Compounds and agents and compositions disclosed herein can be applied topically to a subject's skin to reduce the size (and can include complete removal) of malignant or benign growths, or to treat an infection site. Compounds and agents disclosed herein can be applied directly to the growth or infection site. Preferably, the compounds and agents are applied to the growth or infection site in a formulation such as an ointment, cream, lotion, solution, tincture, or the like.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be

added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers, for example.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Useful dosages of the compounds and agents and pharmaceutical compositions disclosed herein can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art.

In one embodiment, the disclosed compositions are administered in a dose equivalent to parenteral administration of about 0.1 ng to about 100 g per kg of body weight, about 10 ng to about 50 g per kg of body weight, about 100 ng to about 1 g per kg of body weight, from about 1μg to about 100 mg per kg of body weight, from about 1 μg to about 50 mg per kg of body weight, from about 1 mg to about 500 mg per kg of body weight; and from about 1 mg to about 50 mg per kg of body weight. Alternatively, the amount of compositions are administered to achieve a therapeutic effective dose is about 0.1 ng, 1 ng, 10 ng, 100 ng, 1 μg, 10 μg, 100 μg, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 500 mg per kg of body weight or greater.

Although the compositions may be administered once or several times a day, and the duration of the treatment may be once per day for a period of about 1, 2, 3, 4, 5, 6, 7 days or more, it is more preferably to administer either a single dose in the form of an individual dosage unit or several smaller dosage units or by multiple administration of subdivided dosages at certain intervals. For instance, a dosage unit can be administered from about 0 hours to about 1 hr, about 1 hr to about 24 hr, about 1 to about 72 hours, about 1 to about 120 hours, or about 24 hours to at least about 120 hours. Alternatively, the dosage unit can be administered from about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 30, 40, 48, 72, 96, 120 hours. Subsequent dosage units can be administered any time following the initial administration such that a therapeutic effect is achieved. Therapy with the

disclosed compositions can instead include a multi-level dosing regimen wherein the composition is administered during two or more time periods, preferably having a combined duration of about 12 hours to about 7 days, including, 1, 2, 3, 4, or 5 days or about 15, 15, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, or 144 hours or about 1 to 24 hours, about 12 to 36 hours, about 24 to 48 hours, about 36 to 60 hours, about 48 to 72 hours, about 60 to 96 hours, about 72 to 108 hours, about 96 to 120 hours, or about 108 to 136 hours. In one embodiment, the two-level dosing regimen has a combined duration of about 1 day to about 5 days; in other embodiments, the two-level dosing regimen has a combined duration of about 1 day to about 3 days.

Also disclosed are pharmaceutical compositions that comprise a compound disclosed herein in combination with a pharmaceutically acceptable carrier. Pharmaceutical compositions adapted for oral, topical or parenteral administration, comprising an amount of a compound constitute a preferred aspect. The dose administered to a patient, particularly a human, should be sufficient to achieve a therapeutic response in the patient over a reasonable time frame, without lethal toxicity, and preferably causing no more than an acceptable level of side effects or morbidity. One skilled in the art will recognize that dosage will depend upon a variety of factors including the condition (health) of the subject, the body weight of the subject, kind of concurrent treatment, if any, frequency of treatment, therapeutic ratio, as well as the severity and stage of the pathological condition.

Definitions

The term "subject" refers to any individual who is the target of administration or treatment. The subject can be a vertebrate, for example, a mammal. Thus, the subject can be a human or veterinary patient. The term "patient" refers to a subject under the treatment of a clinician, e.g., physician.

The term "therapeutically effective" refers to the amount of the composition used is of sufficient quantity to ameliorate one or more causes or symptoms of a disease or disorder. Such amelioration only requires a reduction or alteration, not necessarily elimination.

The term "treatment" refers to the medical management of a patient with the intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder. This term includes active treatment, that is, treatment directed specifically

toward the improvement of a disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, or disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, or disorder.

The term "inhibit" refers to a decrease in an activity, response, condition, disease, or other biological parameter. This can include but is not limited to the complete ablation of the activity, response, condition, or disease. This may also include, for example, a 10% reduction in the activity, response, condition, or disease as compared to the native or control level. Thus, the reduction can be a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between as compared to native or control levels.

The term "hyperplastic cell" or "hyperplasm" refers to a cell undergoing physiological (normal) cell proliferation ("hyperplasia").

The term "neoplastic cell" or "neoplasm" refers to a cell undergoing abnormal cell proliferation ("neoplasia"). The growth of neoplastic cells exceeds and is not coordinated with that of the normal tissues around it. The growth typically persists in the same excessive manner even after cessation of the stimuli, and typically causes formation of a tumor. Neoplasms may be benign, premalignant, or malignant.

The term "cancer" or "malignant neoplasm" refers to a cell that displays uncontrolled growth, invasion upon adjacent tissues, and often metastasis to other locations of the body.

The term "tumor" refers to an abnormal mass of tissue containing neoplastic cells.

The term "metastasis" refers to the spread of malignant tumor cells from one organ or part to another non-adjacent organ or part. Cancer cells can "break away," "leak," or "spill" from a primary tumor, enter lymphatic and blood vessels, circulate through the bloodstream, and settle down to grow within normal tissues elsewhere in

the body. When tumor cells metastasize, the new tumor is called a secondary or metastatic cancer or tumor.

The term "antibody" refers to natural or synthetic antibodies that selectively bind a target antigen. The term includes polyclonal and monoclonal antibodies. In addition to intact immunoglobulin molecules, also included in the term "antibodies" are fragments or polymers of those immunoglobulin molecules, and human or humanized versions of immunoglobulin molecules that selectively bind the target antigen.

The terms "peptide," "protein," and "polypeptide" are used interchangeably to refer to a natural or synthetic molecule comprising two or more amino acids linked by the carboxyl group of one amino acid to the alpha amino group of another.

As used herein, "peptidomimetic" means a mimetic of a peptide which includes some alteration of the normal peptide chemistry. Peptidomimetics typically enhance some property of the original peptide, such as increase stability, increased efficacy, enhanced delivery, increased half life, etc. Use of peptidomimetics can involve the incorporation of a non-amino acid residue with non-amide linkages at a given position. One embodiment of the present invention is a peptidomimetic wherein the compound has a bond, a peptide backbone or an amino acid component replaced with a suitable mimic. Some non-limiting examples of unnatural amino acids which may be suitable amino acid mimics include β-alanine, L-α-amino butyric acid, L-γ-amino butyric acid, L-α-amino isobutyric acid, L-ε-amino caproic acid, 7-amino heptanoic acid, L-aspartic acid, L-glutamic acid, N-ε-Boc-N-α-CBZ-L-lysine, N-ε-Boc-N-α-Fmoc-L-lysine, L-methionine sulfone, L-norleucine, L-norvaline, N-α-Boc-N-δCBZ-L-ornithine, N-δ-Boc-N-α-CBZ-L-ornithine, Boc-p-nitro-L-phenylalanine, Boc-hydroxyproline, and Boc-L-thioproline.

The term "tumor infiltrating lymphocyte" or "TIL" refers to white blood cells that have left the bloodstream and migrated into a tumor.

The term "regression" does not necessarily imply 100% or complete regression. Rather, there are varying degrees of regression of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. The term also encompasses delaying the onset of the disease, or a symptom or condition thereof.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Publications cited herein and the materials for which they are cited are specifically incorporated by reference.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

EXAMPLES

Example 1: A randomized Phase I/II study of nivolumab in combination with GM.CD40L vaccine in adenocarcinoma of the lung.

The following is a description of phase II trial to establish the efficacy of combining anti-PD-1 therapy with a GM.CD40L tumor vaccine.

WHAT IS CLAIMED IS:

1. A method for treating a cancer in a subject, comprising administering to the subject a composition comprising a therapeutically effective amount of an immune checkpoint inhibitor and a therapeutically effective amount of a tumor vaccine.
2. The method of claim 1, wherein the checkpoint inhibitor comprises an anti-PD-1 antibody, anti-PD-L1 antibody, anti-CTLA-4 antibody, or a combination thereof.
3. The method of claim 2, wherein the anti-PD-1 antibody comprises nivolumab.
4. The method of any preceding claim, wherein the cancer comprises a non-small cell lung cancer.
5. The method of any preceding claim, wherein the tumor vaccine comprises radiated autologous tumor cells and a cell line engineered to express GM-CSF and CD40 ligand.
6. The method of claim 5, wherein the cell is further engineered to express one more factors selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-15, erythropoietin, GCSF, M-CSF, platelet derived growth factor (PDGF), MSF, FLT-3 ligand, EGF, fibroblast growth factor (FGF), bFGF (FGF-2), FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, insulin-like growth factorl (IGF-1), insulin-like growth factor 2 (IGF-2); vascular endothelial growth factor (VEGF), IFN-gamma, IFN-alpha, IFN-beta, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M, stem cell factor (SCF), TGF-α, and TGF-β1.
7. The method of claim 5 or 6, wherein the cell is MHC negative.
8. The method of any one of claims 5 to 7, wherein the cell is a tumor cell.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/35922

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395; A61K 39/00 (2016.01)
CPC - A61K 2039/505; C07K 2317/21; C07K 2316/96; C07K 16/2866; A61K 39/0011
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 39/395; A61K 39/00 (2016.01); USPC - 424/143.1; 424/142.1; 424/133.1; 424/277.1; 424/184.1
CPC - A61K 2039/505; C07K 2317/21; C07K 2316/96; C07K 16/2866; A61K 39/0011

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - A61K 39/395; A61K 39/00 (2016.01); USPC - 424/143.1; 424/142.1; 424/133.1; 424/277.1; 424/184.1
CPC - A61K 2039/505; C07K 2317/21; C07K 2316/96; C07K 16/2866; A61K 39/0011 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); PatBase; Medline, Google: anti-programmed death-1, anti-PD-1, nivolumab, ligand, anti-PD-L1, antibody, Cytotoxic T lymphocyte antigen-4, anti-CTLA-4, immune Checkpoint, inhibitor, treating, cancer, non-small cell lung cancer, NSCLC, administer, vaccine, combination, BMS 936558, clone M1H1, ipilimumab, tumor, effective

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/0125463 A1 (BRISTOL-MYERS SQUIBB COMPANY) 07 May 2015 (07.05.2015), Abstract, para [0005], [0006], [0008], [0014], [0026], [0039], [0114], [0126], [0133], [0152], [0153], [0154], [0186], [0198], [0214], and [0268]	1-4
X	US 2013/0309250 A1 (BRISTOL-MYERS SQUIBB COMPANY) 21 November 2013 (21.11.2013), Abstract, para [0003], [0004], [0006], [0007], [0012], [0024], [0028], [0119], [0128], [0129], [0134], [0151], [0159], and [0225]	1-4
A	POSTOW et al., Immune Checkpoint Blockade in Cancer Therapy. J Clin Oncol. 2015 Jun, Vol. 33(17), p. 1974-82. Epub 2015 Jan 20. PDF File: pg 1-10. Entire documentation, especially Abstract; pg 2, col 2, last para, col 2, middle and last para, and Fig 1; pg 3, Fig 2, and Table 1; and pg 6, col 1, para 1 and 2, and col 2, para 3	1-4
A	DURAISWAMY et al., Dual Blockade of PD-1 and CTLA-4 Combined with Tumor Vaccine Effectively Restores T Cell Rejection Function in Tumors. Cancer Res. 2013, Vol. 73(12), p. 3591-603. Especially Abstract; pg 3600, col 1 and 2, Fig 7; and pg 3601, col 1, para 2 and 3	1-4
A	Chow, Exploring Novel Immune-Related Toxicities and Endpoints with Immune-Checkpoint Inhibitors in Non-Small Cell Lung Cancer. Am Soc Clin Oncol Educ Book. 2013. pg e280-e286. Entire documentation, especially Abstract; pg e281, col 1, last para - Key Points, and col 2, para 1-3; and pg e282, col 1, para 1	1-4
A,P	KLEPONIS et al., Fueling the engine and releasing the break: combinational therapy of cancer vaccines and immune checkpoint inhibitors. Cancer Biol Med. 2015 Sep, Vol. 12(3), p. 201-8. Entire documentation, especially Abstract; pg 202, col 1, para 1-2, and col 2, middle para; pg 203, col 2, middle and last para; and pg 205, col 1, last para, and col 2, middle para	1-4

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 July 2016	19 SEP 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/35922

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

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

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

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

3. ☒ Claims Nos.: 5-8
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

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60/748,919	8 December 2005 (08.12.2005)	US

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(54) **Title:** HUMAN MONOCLONAL ANTIBODIES TO PROGRAMMED DEATH 1(PD-1) AND METHODS FOR TREATING CANCER USING ANTI-PD-1 ANTIBODIES ALONE OR IN COMBINATION WITH OTHER IMMUNOTHERAPEUTICS

(57) **Abstract:** The present invention provides isolated monoclonal antibodies, particularly human monoclonal antibodies, that specifically bind to PD-1 with high affinity. Nucleic acid molecules encoding the antibodies of the invention, expression vectors, host cells and methods for expressing the antibodies of the invention are also provided. Immunoconjugates, bispecific molecules and pharmaceutical compositions comprising the antibodies of the invention are also provided. The invention also provides methods for detecting PD-1, as well as methods for treating various diseases, including cancer and infectious diseases, using anti-PD-1 antibodies. The present invention further provides methods for using a combination immunotherapy, such as the combination of anti-CTLA-4 and anti-PD-1 antibodies, to treat hyperproliferative disease, such as cancer. The invention also provides methods for altering adverse events related to treatment with such antibodies individually.

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DESCRIPTION

HUMAN MONOCLONAL ANTIBODIES TO PROGRAMMED DEATH 1 (PD-1) AND METHODS FOR TREATING CANCER USING ANTI-PD-1 ANTIBODIES ALONE OR IN COMBINATION WITH OTHER IMMUNOTHERAPEUTICS

Technical Field

The present invention relates generally to immunotherapy in the treatment of human disease and reduction of adverse events related thereto. More specifically, the present invention relates to the use of anti-PD-1 antibodies and the use of combination immunotherapy, including the combination of anti-CTLA-4 and anti-PD-1 antibodies, to treat cancer and/or to decrease the incidence or magnitude of adverse events related to treatment with such antibodies individually.

Background of the Invention

The protein Programmed Death 1 (PD-1) is an inhibitory member of the CD28 family of receptors, that also includes CD28, CTLA-4, ICOS and BTLA. PD-1 is expressed on activated B cells, T cells, and myeloid cells (Agata *et al.*, *supra*; Okazaki *et al.* (2002) *Curr. Opin. Immunol.* 14: 391779-82; Bennett *et al.* (2003) *J Immunol* 170:711-8). The initial members of the family, CD28 and ICOS, were discovered by functional effects on augmenting T cell proliferation following the addition of monoclonal antibodies (Hutloff *et al.* (1999) *Nature* 397:263-266; Hansen *et al.* (1980) *Immunogenics* 10:247-260). PD-1 was discovered through screening for differential expression in apototic cells (Ishida *et al.* (1992) *EMBO J* 11:3887-95). The other members of the family, CTLA-4, and BTLA were discovered through screening for differential expression in cytotoxic T lymphocytes and TH1 cells, respectively. CD28, ICOS and CTLA-4 all have an unpaired cysteine residue allowing for homodimerization. In contrast, PD-1 is suggested to exist as a monomer, lacking the unpaired cysteine residue characteristic in other CD28 family members.

The PD-1 gene is a 55 kDa type I transmembrane protein that is part of the Ig gene superfamily (Agata *et al.* (1996) *Int Immunol* 8:765-72). PD-1 contains a membrane proximal immunoreceptor tyrosine inhibitory motif (ITIM) and a membrane distal tyrosine-based switch motif (ITSM) (Thomas, M.L. (1995) *J Exp Med* 181:1953-6; Vivier, E and Daeron, M (1997) *Immunol Today* 18:286-91). Although structurally similar to CTLA-4, PD-1 lacks the MYPPPY motif that is critical for B7-1 and B7-2 binding. Two ligands for PD-1 have been identified, PD-L1 and PD-L2, that have been shown to downregulate T cell activation upon binding to PD-1 (Freeman *et al.* (2000) *J Exp Med* 192:1027-34; Latchman *et al.* (2001) *Nat Immunol* 2:261-8; Carter *et al.* (2002) *Eur J Immunol* 32:634-43). Both PD-L1 and PD-L2 are B7 homologs that bind to PD-1, but do not bind to other CD28 family members. One ligand for PD-1, PD-L1 is abundant in a variety of human cancers (Dong *et al.*

(2002) *Nat. Med.* 8:787-9). The interaction between PD-1 and PD-L1 results in a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation, and immune evasion by the cancerous cells (Dong *et al.* (2003) *J. Mol. Med.* 81:281-7; Blank *et al.* (2005) *Cancer Immunol. Immunother.* 54:307-314; Konishi *et al.* (2004) *Clin. Cancer Res.* 10:5094-100). Immune suppression can be reversed by inhibiting the local interaction of PD-1 with PD-L1, and the effect is additive when the interaction of PD-1 with PD-L2 is blocked as well (Iwai *et al.* (2002) *Proc. Nat'l. Acad. Sci. USA* 99:12293-7; Brown *et al.* (2003) *J. Immunol.* 170:1257-66).

PD-1 is an inhibitory member of the CD28 family expressed on activated B cells, T cells, and myeloid cells (Agata *et al.*, *supra*; Okazaki *et al.* (2002) *Curr Opin Immunol* 14: 391779-82; Bennett *et al.* (2003) *J Immunol* 170:711-8). PD-1 deficient animals develop various autoimmune phenotypes, including autoimmune cardiomyopathy and a lupus-like syndrome with arthritis and nephritis (Nishimura *et al.* (1999) *Immunity* 11:141-51; Nishimura *et al.* (2001) *Science* 291:319-22). Additionally, PD-1 has been found to play a role in autoimmune encephalomyelitis, systemic lupus erythematosus, graft-versus-host disease (GVHD), type I diabetes, and rheumatoid arthritis (Salama *et al.* (2003) *J Exp Med* 198:71-78; Prokunina and Alarcon-Riquelme (2004) *Hum Mol Genet* 13:R143; Nielsen *et al.* (2004) *Lupus* 13:510). In a murine B cell tumor line, the ITSM of PD-1 was shown to be essential to block BCR-mediated Ca^{2+}-flux and tyrosine phosphorylation of downstream effector molecules (Okazaki *et al.* (2001) *PNAS* 98:13866-71).

Accordingly, agents that recognize PD-1, and methods of using such agents, are desired.

Disclosure of the Invention

The present invention provides isolated monoclonal antibodies, in particular human monoclonal antibodies, that bind to PD-1 and that exhibit numerous desirable properties. These properties include, for example, high affinity binding to human PD-1, but lacking substantial cross-reactivity with either human CD28, CTLA-4 or ICOS. Still further, antibodies of the invention have been shown to modulate immune responses. Accordingly, another aspect of the invention pertains to methods of modulating immune responses using anti-PD-1 antibodies. In particular, the invention provides a method of inhibiting growth of tumor cells *in vivo* using anti-PD-1 antibodies.

In one aspect, the invention pertains to an isolated monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody exhibits at least one of the following properties:

(a) binds to human PD-1 with a K_D of 1×10^{-7} M or less;

(b) does not substantially bind to human CD28, CTLA-4 or ICOS;

(c) increases T-cell proliferation in an Mixed Lymphocyte Reaction (MLR) assay;

(d) increases interferon-gamma production in an MLR assay;

(e) increases IL-2 secretion in an MLR assay;

(f) binds to human PD-1 and cynomolgus monkey PD-1;

(g) inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(h) stimulates antigen-specific memory responses;

(i) stimulates antibody responses;

(j) inhibits tumor cell growth *in vivo*.

Preferably the antibody is a human antibody, although in alternative embodiments the antibody can be, for example, a murine antibody, a chimeric antibody or humanized antibody.

In more preferred embodiments, the antibody binds to human PD-1 with a K_D of 5 x 10^{-8} M or less, binds to human PD-1 with a K_D of 1 x 10^{-8} M or less, binds to human PD-1 with a K_D of 5 x 10^{-9} M or less, or binds to human PD-1 with a K_D of between 1 x10^{-8} M and 1 x 10^{-10} M.

In another embodiment, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, wherein the antibody cross-competes for binding to PD-1 with a reference antibody comprising:

(a) a human heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7; and

(b) a human light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14.

In various embodiments, the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8; or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9; or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 3; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 10; or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 11; or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 12; or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 13; or the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14.

In another aspect, the invention pertains to an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene, wherein the antibody specifically binds PD-1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 4-39 gene, wherein the antibody specifically binds PD-1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L6 gene, wherein the antibody specifically binds PD-1. The invention further provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L15 gene, wherein the antibody specifically binds PD-1.

In a preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 3-33 gene; and

(b) a light chain variable region of a human V_K L6 gene;

wherein the antibody specifically binds to PD-1.

In another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region of a human V_H 4-39 gene; and

(b) a light chain variable region of a human V_K L15 gene;

wherein the antibody specifically binds to PD-1.

In another aspect, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising:

a heavy chain variable region that comprises CDR1, CDR2, and CDR3 sequences; and

a light chain variable region that comprises CDR1, CDR2, and CDR3 sequences, wherein:

(a) the heavy chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35, and conservative modifications thereof;

(b) the light chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56, and conservative modifications thereof; and

(c) the antibody specifically binds to human PD-1.

Preferably, the heavy chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, and conservative modifications thereof; and the light chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and conservative modifications thereof. Preferably, the heavy chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, and conservative modifications thereof; and the light chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, and conservative modifications thereof.

In yet another aspect, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region and a light chain variable region, wherein:

(a) the heavy chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7;

(b) the light chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14;

(c) the antibody binds to human PD-1 with a K_D of 1×10^{-7} M or less; and

(d) the antibody does not substantially bind to human CD28, CTLA-4 or ICOS.

In a preferred embodiment, the antibodies additionally comprise at least one of the following properties:

(a) the antibody increases T-cell proliferation in an MLR assay;

(b) the antibody increases interferon-gamma production in an MLR assay; or

(c) the antibody increases IL-2 secretion in an MLR assay.

Additionally or alternatively, the antibody may comprise one or more of the other features listed above.

In preferred embodiments, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21;

(b) a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28;

(c) a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35;

(d) a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42;

(e) a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49; and

(f) a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56;

wherein the antibody specifically binds PD-1.

A preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 15;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 22;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 29;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 36;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 43; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 50.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 16;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 23;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 30;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 37;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 44; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 51.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 17;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 24;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 31;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 38;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 45; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 52.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 18;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 25;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 32;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 39;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 46; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 53.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 19;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 26;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 33;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 40;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 47; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 54.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 20;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 27;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 34;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 41;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 48; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 55.

Another preferred combination comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 21;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 28;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 35;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 42;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 49; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 56.

Other preferred antibodies of the invention, or antigen-binding portions thereof, comprise:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7; and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14;

wherein the antibody specifically binds PD-1.

A preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 3; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 10.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 11.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 12.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 13.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14.

The antibodies of the invention can be, for example, full-length antibodies, for example of an IgG1 or IgG4 isotype. Alternatively, the antibodies can be antibody fragments, such as Fab or Fab'2 fragments, or single chain antibodies.

The invention also provides an immunoconjugate comprising an antibody of the invention, or antigen-binding portion thereof, linked to a therapeutic agent, such as a cytotoxin or a radioactive isotope. The invention also provides a bispecific molecule comprising an antibody, or antigen-binding portion thereof, of the invention, linked to a second functional moiety having a different binding specificity than said antibody, or antigen-binding portion thereof.

Compositions comprising an antibody, or antigen-binding portion thereof, or immunoconjugate or bispecific molecule of the invention, and a pharmaceutically acceptable carrier, are also provided.

Nucleic acid molecules encoding the antibodies, or antigen-binding portions thereof, of the invention are also encompassed by the invention, as well as expression vectors comprising such nucleic acids and host cells comprising such expression vectors. Moreover, the invention provides a transgenic mouse comprising human immunoglobulin heavy and light chain transgenes, wherein the mouse expresses an antibody of the invention, as well as hybridomas prepared from such a mouse, wherein the hybridoma produces the antibody of the invention.

In yet another aspect, the invention provides a method of modulating an immune response in a subject comprising administering to the subject the antibody, or antigen-binding

portion thereof, of the invention such that the immune response in the subject is modulated. Preferably, the antibody of the invention enhances, stimulates or increases the immune response in the subject.

In a further aspect, the invention provides a method of inhibiting growth of tumor cells in a subject, comprising administering to a subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof. The antibodies of the invention are preferred for use in the method although other anti-PD-1 antibodies can be used instead (or in combination with an anti-PD-1 antibody of the invention). For example, a chimeric, humanized or fully human anti-PD-1 antibody can be used in the method of inhibiting tumor growth.

In a further aspect, the invention provides a method of treating an infectious disease in a subject, comprising administering to a subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof. The antibodies of the invention are preferred for use in the method although other anti-PD-1 antibodies can be used instead (or in combination with an anti-PD-1 antibody of the invention). For example, a chimeric, humanized or fully human anti-PD-1 antibody can be used in the method of treating an infectious disease.

Still further, the invention provides a method of enhancing an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) an anti-PD-1 antibody, or antigen-binding portion thereof, such that an immune response to the antigen in the subject is enhanced. The antigen can be, for example, a tumor antigen, a viral antigen, a bacterial antigen or an antigen from a pathogen. The antibodies of the invention are preferred for use in the method although other anti-PD-1 antibodies can be used instead (or in combination with an anti-PD-1 antibody of the invention). For example, a chimeric, humanized or fully human anti-PD-1 antibody can be used in the method of enhancing an immune response to an antigen in a subject.

The invention also provides methods for making "second generation" anti-PD-1 antibodies based on the sequences of the anti-PD-1 antibodies provided herein. For example, the invention provides a method for preparing an anti-PD-1 antibody comprising:

(a) providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, and/or a CDR2 sequence that is selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28; and/or a CDR3 sequence that is selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35; or (ii) a light chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, and/or a CDR2 sequence that is selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and/or a CDR3 sequence that is selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56;

(b) altering at least one amino acid residue within at least one variable region antibody sequence, said sequence being selected from the heavy chain variable region antibody sequence and the light chain variable region antibody sequence, to create at least one altered antibody sequence; and

(c) expressing the altered antibody sequence as a protein.

Other features and advantages of the instant invention will be apparent from the following detailed description and examples which should not be construed as limiting. The contents of all references, GenBank entries, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

Brief Description of the Drawings

Figure 1A shows the nucleotide sequence (SEQ ID NO: 57) and amino acid sequence (SEQ ID NO: 1) of the heavy chain variable region of the 17D8 human monoclonal antibody. The CDR1 (SEQ ID NO: 15), CDR2 (SEQ ID NO: 22) and CDR3 (SEQ ID NO: 29) regions are delineated and the V, D and J germline derivations are indicated.

Figure 1B shows the nucleotide sequence (SEQ ID NO: 64) and amino acid sequence (SEQ ID NO: 8) of the light chain variable region of the 17D8 human monoclonal antibody. The CDR1 (SEQ ID NO: 36), CDR2 (SEQ ID NO: 43) and CDR3 (SEQ ID NO: 50) regions are delineated and the V and J germline derivations are indicated.

Figure 2A shows the nucleotide sequence (SEQ ID NO: 58) and amino acid sequence (SEQ ID NO: 2) of the heavy chain variable region of the 2D3 human monoclonal antibody. The CDR1 (SEQ ID NO: 16), CDR2 (SEQ ID NO: 23) and CDR3 (SEQ ID NO: 30) regions are delineated and the V and J germline derivations are indicated.

Figure 2B shows the nucleotide sequence (SEQ ID NO: 65) and amino acid sequence (SEQ ID NO: 9) of the light chain variable region of the 2D3 human monoclonal antibody. The CDR1 (SEQ ID NO: 37), CDR2 (SEQ ID NO: 44) and CDR3 (SEQ ID NO: 51) regions are delineated and the V and J germline derivations are indicated.

Figure 3A shows the nucleotide sequence (SEQ ID NO: 59) and amino acid sequence (SEQ ID NO: 3) of the heavy chain variable region of the 4H1 human monoclonal antibody. The CDR1 (SEQ ID NO: 17), CDR2 (SEQ ID NO: 24) and CDR3 (SEQ ID NO: 31) regions are delineated and the V and J germline derivations are indicated.

Figure 3B shows the nucleotide sequence (SEQ ID NO: 66) and amino acid sequence (SEQ ID NO: 10) of the light chain variable region of the 4H1 human monoclonal antibody. The CDR1 (SEQ ID NO: 38), CDR2 (SEQ ID NO: 45) and CDR3 (SEQ ID NO: 52) regions are delineated and the V and J germline derivations are indicated.

Figure 4A shows the nucleotide sequence (SEQ ID NO: 60) and amino acid sequence (SEQ ID NO: 4) of the heavy chain variable region of the 5C4 human monoclonal antibody. The CDR1 (SEQ ID NO: 18), CDR2 (SEQ ID NO: 25) and CDR3 (SEQ ID NO: 32) regions are delineated and the V and J germline derivations are indicated.

Figure 4B shows the nucleotide sequence (SEQ ID NO: 67) and amino acid sequence (SEQ ID NO: 11) of the light chain variable region of the 5C4 human monoclonal antibody. The CDR1 (SEQ ID NO: 39), CDR2 (SEQ ID NO: 46) and CDR3 (SEQ ID NO: 53) regions are delineated and the V and J germline derivations are indicated.

Figure 5A shows the nucleotide sequence (SEQ ID NO: 61) and amino acid sequence (SEQ ID NO: 5) of the heavy chain variable region of the 4A11 human monoclonal antibody. The CDR1 (SEQ ID NO: 19), CDR2 (SEQ ID NO: 26) and CDR3 (SEQ ID NO: 33) regions are delineated and the V and J germline derivations are indicated.

Figure 5B shows the nucleotide sequence (SEQ ID NO: 68) and amino acid sequence (SEQ ID NO: 12) of the light chain variable region of the 4A11 human monoclonal antibody. The CDR1 (SEQ ID NO: 40), CDR2 (SEQ ID NO: 47) and CDR3 (SEQ ID NO: 54) regions are delineated and the V and J germline derivations are indicated.

Figure 6A shows the nucleotide sequence (SEQ ID NO: 62) and amino acid sequence (SEQ ID NO: 6) of the heavy chain variable region of the 7D3 human monoclonal antibody. The CDR1 (SEQ ID NO: 20), CDR2 (SEQ ID NO: 27) and CDR3 (SEQ ID NO: 34) regions are delineated and the V and J germline derivations are indicated.

Figure 6B shows the nucleotide sequence (SEQ ID NO: 69) and amino acid sequence (SEQ ID NO: 13) of the light chain variable region of the 7D3 human monoclonal antibody. The CDR1 (SEQ ID NO: 41), CDR2 (SEQ ID NO: 48) and CDR3 (SEQ ID NO: 55) regions are delineated and the V and J germline derivations are indicated.

Figure 7A shows the nucleotide sequence (SEQ ID NO: 63) and amino acid sequence (SEQ ID NO: 7) of the heavy chain variable region of the 5F4 human monoclonal antibody. The CDR1 (SEQ ID NO: 21), CDR2 (SEQ ID NO: 28) and CDR3 (SEQ ID NO: 35) regions are delineated and the V and J germline derivations are indicated.

Figure 7B shows the nucleotide sequence (SEQ ID NO: 70) and amino acid sequence (SEQ ID NO: 14) of the light chain variable region of the 5F4 human monoclonal antibody. The CDR1 (SEQ ID NO: 42), CDR2 (SEQ ID NO: 49) and CDR3 (SEQ ID NO: 56) regions are delineated and the V and J germline derivations are indicated.

Figure 8 shows the alignment of the amino acid sequence of the heavy chain variable region of 17D8, 2D3, 4H1, 5C4 and 7D3 with the human germline V_H 3-33 amino acid sequence (SEQ ID NO: 71).

Figure 9 shows the alignment of the amino acid sequence of the light chain variable region of 17D8, 2D3 and 7D3 with the human germline V_k L6 amino acid sequence (SEQ ID NO: 73).

Figure 10 shows the alignment of the amino acid sequence of the light chain variable region of 4H1 and 5C4 with the human germline V_k L6 amino acid sequence (SEQ ID NO: 73).

Figure 11 shows the alignment of the amino acid sequence of the heavy chain variable region of 4A11 and 5F4 with the human germline V_H 4-39 amino acid sequence (SEQ ID NO: 72).

Figure 12 shows the alignment of the amino acid sequence of the light chain variable region of 4A11 and 5F4 with the human germline V_k L15 amino acid sequence (SEQ ID NO: 74).

Figures 13A-13B show the results of flow cytometry experiments demonstrating that the human monoclonal antibodies 5C4 and 4H1, directed against human PD-1, binds the cell surface of CHO cells transfected with full-length human PD-1. Figure 13A shows to the flow cytometry plot for 5C4. Figure 13B shows the flow cytometry plot for 4H1. Thin line represents the binding to CHO cells and solid line represents the binding to CHO hPD-1 cells.

Figure 14 shows a graph demonstrating that the human monoclonal antibodies 17D8, 2D3, 4H1, 5C4, and 4A11, directed against human PD-1, bind specifically to PD-1, and not to other members of the CD28 family.

Figures 15A-15C show the results of flow cytometry experiments demonstrating that the human monoclonal antibodies 4H1 and 5C4, directed against human PD-1, binds to PD-1 on the cell surface. Figure 15A shows binding to activated human T-cells. Figure 15B shows the binding to cynomolgous monkey T-cells. Figure 15C shows the binding to CHO transfected cells expressing PD-1.

Figures 16A-16C show the results of experiments demonstrating that human monoclonal antibodies against human PD-1 promote T-cell proliferation, IFN-gamma secretion and IL-2 secretion in a mixed lymphocyte reaction assay. Figure 16A is a bar graph showing concentration dependent T-cell proliferation; Figure 16B is a bar graph showing concentration dependent IFN-gamma secretion; Figure 16C is a bar graph showing concentration dependent IL-2 secretion.

Figures 17A-17B show the results of flow cytometry experiments demonstrating that human monoclonal antibodies against human PD-1 block the binding of PD-L1 and PD-L2 to CHO transfected cells expressing PD-1. Figure 17A is a graph showing inhibition of binding of PD-L1; Figure 17B is a graph showing inhibition of binding of PD-L2.

Figure 18 shows the results of flow cytometry experiments demonstrating that human monoclonal antibodies against human PD-1 do not promote T-cell apoptosis.

Figure 19 shows the results of experiments demonstrating that anti-PD-1 HuMabs have a concentration dependent effect on IFN gamma secretion by PBMCs from CMV-positive donors when PBMCs were stimulated with a CMV lysate and anti-PD-1.

Figure 20 shows the results of tumor growth experiments in a mouse model system demonstrating that treatment *in vivo* of mouse tumors with anti-PD-1 antibodies inhibits the growth of tumors.

Figures 21A to 21D show the tumor volume over time in individual mice that were implanted with MC38 colon tumor cells (PD-L1$^-$) and on the same day treated with one of the

following therapies: (A) mouse IgG (control), (B) anti-CTLA-4 antibody, (C) anti-PD-1 antibody, and (D) anti-CTLA-4 antibody and anti-PD-1 antibody. The mice received subsequent antibody treatments on days 3, 6 and 10 as described in Example 13 and tumor volume was monitored over 60 days.

Figure 22 shows the mean tumor volume of the mice shown in Figure 21.

Figure 23 shows the median tumor volume of the mice shown in Figure 21.

Figures 24A to 24D show the tumor volume over time in individual mice that were implanted with MC38 colon tumor cells (PD-L1$^-$) and one week later treated with one of the following therapies: (A) mouse IgG (control), (B) anti-CTLA-4 antibody, (C) anti-PD-1 antibody, and (D) anti-CTLA-4 antibody and anti-PD-1 antibody. The tumor volume on the first day of treatment was about 315 mm^3. The mice received subsequent antibody treatments on days 3, 6 and 10 as described in Example 14.

Figure 25 shows the mean tumor volume of the mice shown in Figure 24.

Figure 26 shows the median tumor volume of the mice shown in Figure 24.

Figure 27 shows the mean tumor volume over time in individual mice that were implanted with MC38 colon tumor cells (PD-L1$^-$) (day -7) and then treated on days 0, 3, 6 and 10 post-implantation (as described in Example 15) with one of the following therapies: (A) mouse IgG as a control (20 mg/kg, X_{20}) (B) anti-PD-1 antibody (10 mg/kg) and mouse IgG (10 mg/kg) ($P_{10}X_{10}$), (C) anti-CTLA-4 antibody (10 mg/kg) and mouse IgG (10 mg/kg) ($C_{10}X_{10}$), (D) anti-CTLA-4 antibody and anti-PD-1 antibody (10 mg/kg each) ($C_{10}P_{10}$), (E) anti-CTLA-4 antibody and anti-PD-1 antibody (3 mg/kg each) (C_3P_3), and (F) anti-CTLA-4 antibody and anti-PD-1 antibody (1 mg/kg each) (C_1P_1). Two groups of mice were treated with each antibody sequentially as follows: (G) anti-CTLA-4 antibody (10 mg/kg, day 0), anti-CTLA-4 antibody (10 mg/kg, day 3), anti-PD-1 antibody (10 mg/kg, day 6), and anti-PD-1 antibody (10 mg/kg, day 10) ($C_{10}C_{10}P_{10}P_{10}$); and (H) anti-PD-1 antibody (10 mg/kg, day 0), anti-PD-1 antibody (10 mg/kg, day 3), anti-CTLA-4 antibody (10 mg/kg, day 6), and anti-CTLA-4 antibody (10 mg/kg, day 10) (10 mg/kg, day 10) ($P_{10}P_{10}C_{10}C_{10}$).

Figure 28 shows the mean tumor volume of the mice shown in Figure 27.

Figure 29 shows the median tumor volume of the mice shown in Figure 27.

Figures 30A to 30F show the tumor volume over time in individual mice that were implanted with SA1/N fibrosarcoma cells (PD-L1$^-$) and one day later treated with one of the following therapies: (A) PBS (vehicle control), (B) mouse IgG (antibody control, 10 mg/kg), (C) anti-PD-1 antibody (10 mg/kg), (D) anti-CTLA-4 antibody (10 mg/kg), (E) anti-CTLA-4 antibody (0.2 mg/kg), and (F) anti-PD-1 antibody (10 mg/kg) and anti-CTLA-4 antibody (0.2 mg/kg). The mice received subsequent antibody treatments on days 4, 7 and 11 as described in Example 16 and tumor volume was monitored over 41 days.

Figure 31 shows the mean tumor volume of the mice shown in Figure 29.

Figure 32 shows the median tumor volume of the mice shown in Figure 29.

Figures 33A to 33J show the tumor volume over time in individual mice that were implanted with SA1/N fibrosarcoma cells (PD-L1$^-$) and then treated on days 7, 10, 13 and 17 post-implantation (as described in Example 17) with one of the following therapies: (A) PBS (vehicle control), (B) mouse IgG (antibody control, 10 mg/kg), (C) anti-CTLA-4 antibody (0.25 mg/kg), (D) anti-CTLA-4 antibody (0.5 mg/kg), (E) anti-CTLA-4 antibody (5 mg/kg), (F) anti-PD-1 antibody (3 mg/kg), (G) anti-PD-1 antibody (10 mg/kg), (H) anti-PD-1 antibody (10 mg/kg) and anti-CTLA-4 antibody (0.25 mg/kg), (I) anti-PD-1 antibody (10 mg/kg) and anti-CTLA-4 antibody (0.5 mg/kg), and (F) anti-PD-1 antibody (3 mg/kg) and anti-CTLA-4 antibody (0.5 mg/kg). The tumor volume on the first day of treatment was about 110 mm^3.

Figure 34 shows the mean tumor volume of the mice shown in Figure 33.

Figure 35 shows the median tumor volume of the mice shown in Figure 33.

Figures 36A and 36B show the tumor volume over time in individual mice that were implanted with SA1/N fibrosarcoma cells (PD-L1$^-$) and then treated on days 10, 13, 16 and 19 post-implantation (as described in Example 17) with one of the following therapies: (A) mouse IgG (antibody control, 10 mg/kg) or (B) anti-PD-1 antibody (10 mg/kg) and anti-CTLA-4 antibody (1 mg/kg). The tumor volume on the first day of treatment was about 250 mm^3.

Figure 37 shows the mean tumor volume of the mice shown in Figure 36.

Figure 38 shows the median tumor volume of the mice shown in Figure 36.

Figure 39 shows the mean and median percent tumor inhibition calculated from the tumor volumes shown in Figures 33 and 36.

Figures 40A to 40D show the tumor volume in BALB/c mice that were implanted subcutaneously with RENCA renal adenocarcinoma cells (PD-L1$^+$) (Murphy and Hrushesky (1973) *J. Nat'l. Cancer Res. 50*:1013-1025) (day -12) and then treated intraperitoneally on days 0, 3, 6 and 9 post-implantation with one of the following therapies: (A) mouse IgG (antibody control, 20 mg/kg), (B) anti-PD-1 antibody (10 mg/kg), (C) anti-CTLA-4 antibody (10 mg/kg), and (D) anti-PD-1 antibody (10 mg/kg) in combination with anti-CTLA-4 antibody (10 mg/kg). The tumor volume on the first day of treatment was about 115 mm^3.

Figure 41 shows binding of mouse PD-L2-Fc fusion protein to mouse PD-1 (mPD-1) is blocked by anti-mPD-1 antibody 4H2 in a dose dependent manner. The binding is detected by measuring fluorescence of FITC-labeled donkey-anti-rat IgG by ELISA. The greater the MFI (mean fluorescence intensity) the greater the binding.

Figure 42 shows binding curves of anti-mPD-1 antibodies to immobilized mPD-1-Fc fusion protein by ELISA.

Figure 43 shows the binding curve of rat anti-mPD-1 antibody 4H2.B3 to mPD-1-expressing CHO cells. Binding was detected with donkey-anti-rat IgG, FITC conjugated and measured by FACS (MFI).

Figure 44 shows the binding curve of mPD-L1-hFc fusion protein to mPD-1-expressing CHO cells in the presence of increasing concentrations of anti-mPD-1 antibody

4H2.B3. Binding was detected with goat–anti–human IgG, FITC conjugated and measured by FACS (MFI).

Figure 45 shows the binding curves of rat anti-mPD-1 antibody 4H2.B3 to mPD-1-expressing CHO cells as compared to chimeric rat:mouse anti-mPD-1 antibody 4H2.

Figure 46 shows the binding curves of mPD-L1-hFc fusion protein to mPD-1-expressing CHO cells in the presence of increasing concentrations of either rat anti-mPD-1 antibody 4H2.B3 or chimeric rat:mouse anti-mPD-1 antibody 4H2.

Figure 47 shows the mean tumor volume of tumor-free mice previously treated with anti-PD1 antibody and re-challenged with SA1/N fibrosarcoma cells (PD-L1$^-$). Also shown is the mean tumor volume of naïve mice (control, not previously challenged or treated) implanted with SA1/N fibrosarcoma cells.

Figure 48 shows the tumor volume over time in individual mice, which survived tumor-free following implantation of MC38 colon tumor cells (PD-L1$^-$) and treatment with anti-PD1 antibody or a combination of anti-PD1 antibody with anti-CTLA-4 antibody), re-challenged with 10x more MC38 colon tumor cells than the initial treatment. Also shown is the mean tumor volume of naïve mice (control, not previously challenged or treated) implanted with MC38 colon tumor cells.

Figure 49 shows the mean tumor volume of the mice shown in Figure 48.

Figure 50 shows the mean tumor volume over time in individual mice that were implanted with CT26 colon tumor cells.

Figures 51A-B shows the results of experiments demonstrating that human monoclonal antibodies against human PD-1 promote T-cell proliferation and IFN-gamma secretion in cultures containing T regulatory cells. Figure 50A is a bar graph showing concentration dependent T-cell proliferation using HuMAb 5C4; Figure 50B is a bar graph showing concentration dependent IFN-gamma secretion using HuMAb 5C4.

Figures 52A-B shows the results of experiments demonstrating that human monoclonal antibodies against human PD-1 promote T-cell proliferation and IFN-gamma secretion in cultures containing activated T cells. Figure 51A is a bar graph showing concentration dependent T-cell proliferation using HuMAb 5C4; Figure 51B is a bar graph showing concentration dependent IFN-gamma secretion using HuMAb 5C4.

Figure 53 shows the results of an antibody dependent cellular cytotoxicity (ADCC) assay demonstrating that human monoclonal anti-PD-1 antibodies kill human activated T cells in an ADCC concentration-dependent manner in relation to the Fc region of the anti-PD-1 antibody.

Figure 54 shows the results of a complement dependent cytotoxicity (CDC) assay demonstrating that human monoclonal anti-PD-1 antibodies do not kill human activated T cells in a CDC concentration-dependent manner.

Best Mode for Carrying Out the Invention

In one aspect, the present invention relates to isolated monoclonal antibodies, particularly human monoclonal antibodies, that bind specifically to PD-1. In certain embodiments, the antibodies of the invention exhibit one or more desirable functional properties, such as high affinity binding to PD-1, lack of cross-reactivity to other CD28 family members, the ability to stimulate T cell proliferation, IFN-γ and/or IL-2 secretion in mixed lymphocyte reactions, the ability to inhibit binding of one or more PD-1 ligands (*e.g.*, PD-L1 and/or PD-L2), the ability to cross-react with cynomolgus monkey PD-1, the ability to stimulate antigen-specific memory responses, the ability to stimulate antibody responses and/or the ability to inhibit growth of tumor cells *in vivo*. Additionally or alternatively, the antibodies of the invention are derived from particular heavy and light chain germline sequences and/or comprise particular structural features such as CDR regions comprising particular amino acid sequences. In another aspect, the invention relates to the combined use of monoclonal antibodies that bind specifically to PD-1 and monoclonal antibodies that bind specifically to CTLA-4.

The invention provides, for example, isolated antibodies, methods of making such antibodies, immunoconjugates and bispecific molecules comprising such antibodies and pharmaceutical compositions containing the antibodies, immunconjugates or bispecific molecules of the invention.

In another aspect, the invention pertains to methods of inhibiting growth of tumor cells in a subject using anti-PD-1 antibodies. As demonstrated herein, anti-PD-1 antibodies are capable of inhibiting tumor cell growth *in vivo*. The invention also relates to methods of using the antibodies to modify an immune response, as well as to treat diseases such as cancer or infectious disease, or to stimulate a protective autoimmune response or to stimulate antigen-specific immune responses (e.g., by coadministration of anti-PD-1 with an antigen of interest).

In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

The terms "Programmed Death 1," "Programmed Cell Death 1," "Protein PD-1," "PD-1," PD1," "PDCD1," "hPD-1" and "hPD-I" are used interchangeably, and include variants, isoforms, species homologs of human PD-1, and analogs having at least one common epitope with PD-1. The complete PD-1 sequence can be found under GenBank Accession No. U64863.

The terms "cytotoxic T lymphocyte-associated antigen-4," "CTLA-4," "CTLA4," "CTLA-4 antigen" and "CD152" (*see, e.g.*, Murata, *Am. J. Pathol.* (1999) 155:453-460) are used interchangeably, and include variants, isoforms, species homologs of human CTLA-4, and analogs having at least one common epitope with CTLA-4 (*see, e.g.*, Balzano (1992) *Int. J. Cancer Suppl.* 7:28-32). The complete CTLA-4 nucleic acid sequence can be found under GenBank Accession No. L15006.

The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

A "signal transduction pathway" refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. As used herein, the phrase "cell surface receptor" includes, for example, molecules and complexes of molecules capable of receiving a signal and the transmission of such a signal across the plasma membrane of a cell. An example of a "cell surface receptor" of the present invention is the PD-1 receptor.

The term "antibody" as referred to herein includes whole antibodies and any antigen-binding fragment (*i.e.*, "antigen-binding portion") or single chains thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen-binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_{H1}, C_{H2} and C_{H3}. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, PD-1). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a

V_H domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); *see e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds PD-1 is substantially free of antibodies that specifically bind antigens other than PD-1). An isolated antibody that specifically binds PD-1 may, however, have cross-reactivity to other antigens, such as PD-1 molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies

isolated from an animal (*e.g.*, a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further below), (b) antibodies isolated from a host cell transformed to express the human antibody, *e.g.*, from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

As used herein, "isotype" refers to the antibody class (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes.

The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen."

The term "human antibody derivatives" refers to any modified form of the human antibody, *e.g.*, a conjugate of the antibody and another agent or antibody.

The term "humanized antibody" is intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

The term "chimeric antibody" is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

As used herein, an antibody that "specifically binds to human PD-1" is intended to refer to an antibody that binds to human PD-1 with a K_D of 1×10^{-7} M or less, more preferably 5×10^{-8} M or less, more preferably 1×10^{-8} M or less, more preferably 5×10^{-9} M or less.

The term "K_{assoc}" or "K_a", as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "K_{dis}" or "K_d," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (*i.e.*, K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A preferred method for determining the K_D of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a Biacore® system.

As used herein, the term "high affinity" for an IgG antibody refers to an antibody having a K_D of 10^{-8} M or less, more preferably 10^{-9} M or less and even more preferably 10^{-10} M or less for a target antigen. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to an antibody having a K_D of 10^{-7} M or less, more preferably 10^{-8} M or less, even more preferably 10^{-9} M or less.

The term "treatment" or "therapy" refers to administering an active agent with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect a condition (*e.g.*, a disease), the symptoms of the condition, or to prevent or delay the onset of the symptoms, complications, biochemical indicia of a disease, or otherwise arrest or inhibit further development of the disease, condition, or disorder in a statistically significant manner.

An "adverse event" (AE) as used herein is any unfavorable and generally unintended, even undesirable, sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a medical treatment. For example, an adverse event may be associated with activation of the immune system or expansion of immune system cells (*e.g.*, T cells) in response to a treatment. A medical treatment may have one or more associated AEs and each AE may have the same or different level of severity. Reference to methods capable of "altering adverse events" means a treatment regime that decreases the incidence and/or severity of one or more AEs associated with the use of a different treatment regime.

As used herein, "hyperproliferative disease" refers to conditions wherein cell growth is increased over normal levels. For example, hyperproliferative diseases or disorders include malignant diseases (*e.g.*, esophageal cancer, colon cancer, biliary cancer) and non-malignant diseases (*e.g.*, atherosclerosis, benign hyperplasia, benign prostatic hypertrophy).

As used herein, "subtherapeutic dose" means a dose of a therapeutic compound (*e.g.*, an antibody) that is lower than the usual or typical dose of the therapeutic compound when administered alone for the treatment of a hyperproliferative disease (*e.g.*, cancer). For example, a subtherapeutic dose of CTLA-4 antibody is a single dose of the antibody at less than about 3 mg/kg, *i.e.*, the known dose of anti-CTLA-4 antibody.

The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles "a" or "an" should be understood to refer to "one or more" of any recited or enumerated component.

As used herein, "about" or "comprising essentially of" mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" or "comprising essentially of" can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 20%. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values are provided in the application and claims,

unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value.

As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated.

As used herein, the term "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows chickens, amphibians, reptiles, *etc.* Except when noted, the terms "patient" or "subject" are used interchangeably.

Various aspects of the invention are described in further detail in the following subsections.

Anti-PD-1 Antibodies

The antibodies of the invention are characterized by particular functional features or properties of the antibodies. For example, the antibodies bind specifically to PD-1 (e.g., bind to human PD-1 and may cross-react with PD-1 from other species, such as cynomolgus monkey). Preferably, an antibody of the invention binds to PD-1 with high affinity, for example with a K_D of 1×10^{-7} M or less. The anti-PD-1 antibodies of the invention preferably exhibit one or more of the following characteristics:

(a) binds to human PD-1 with a K_D of 1×10^{-7} M or less;

(b) does not substantially bind to human CD28, CTLA-4 or ICOS;

(c) increases T-cell proliferation in an Mixed Lymphocyte Reaction (MLR) assay;

(d) increases interferon-gamma production in an MLR assay;

(e) increases IL-2 secretion in an MLR assay;

(f) binds to human PD-1 and cynomolgus monkey PD-1;

(g) inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(h) stimulates antigen-specific memory responses;

(i) stimulates antibody responses;

(j) inhibits tumor cell growth *in vivo*.

Preferably, the antibody binds to human PD-1 with a K_D of 5×10^{-8} M or less, binds to human PD-1 with a K_D of 1×10^{-8} M or less, binds to human PD-1 with a K_D of 5×10^{-9} M or less, or binds to human PD-1 with a K_D of between 1×10^{-8} M and 1×10^{-10} M or less.

An antibody of the invention may exhibit any combination of the above-listed features, such as two, three, four, five or more of the above-listed features.

Standard assays to evaluate the binding ability of the antibodies toward PD-1 are known in the art, including for example, ELISAs, Western blots and RIAs. The binding kinetics (*e.g.*, binding affinity) of the antibodies also can be assessed by standard assays known in the art, such as by Biacore analysis. Suitable assays for evaluating any of the above-described characteristics are described in detail in the Examples.

Monoclonal Antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4

Preferred antibodies of the invention are the human monoclonal antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4isolated and structurally characterized as described in Examples 1 and 2. The V_H amino acid sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7, respectively. The V_L amino acid sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14, respectively.

Given that each of these antibodies can bind to PD-1, the V_H and V_L sequences can be "mixed and matched" to create other anti-PD-1 binding molecules of the invention. PD-1 binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (*e.g.*, ELISAs). Preferably, when V_H and V_L chains are mixed and matched, a V_H sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_H sequence. Likewise, preferably a V_L sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_L sequence.

Accordingly, in one aspect, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof comprising:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7; and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14;

wherein the antibody specifically binds PD-1, preferably human PD-1.

Preferred heavy and light chain combinations include:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 3; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 10; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 11; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 12; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 13; or

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14.

In another aspect, the invention provides antibodies that comprise the heavy chain and light chain CDR1s, CDR2s and CDR3s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4, or combinations thereof. The amino acid sequences of the V_H CDR1s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, respectively. The amino acid sequences of the V_H CDR2s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, respectively. The amino acid sequences of the V_H CDR3s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35, respectively. The amino acid sequences of the V_k CDR1s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, respectively. The amino acid sequences of the V_k CDR2s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, respectively. The amino acid sequences of the V_k CDR3s of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56, respectively. The CDR regions are delineated using the Kabat system (Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

Given that each of these antibodies can bind to PD-1 and that antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3 regions, the V_H CDR1, CDR2, and CDR3 sequences and V_k CDR1, CDR2, and CDR3 sequences can be "mixed and matched" (*i.e.*, CDRs from different antibodies can be mixed and match, although each antibody must contain a V_H CDR1, CDR2, and CDR3 and a V_k CDR1, CDR2, and CDR3) to create other anti-PD-1 binding molecules of the invention. PD-1 binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (*e.g.*, ELISAs, Biacore analysis). Preferably, when V_H CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular V_H sequence is replaced with a structurally similar CDR sequence(s). Likewise, when V_k CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular V_k sequence preferably is replaced with a structurally similar CDR sequence(s). It will be readily apparent to the ordinarily skilled artisan that novel V_H and V_L sequences can be created by substituting one or more V_H and/or V_L CDR region sequences with structurally similar sequences from the CDR sequences disclosed herein for monoclonal antibodies antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4.

Accordingly, in another aspect, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21;

(b) a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28;

(c) a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35;

(d) a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42;

(e) a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49; and

(f) a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56;

wherein the antibody specifically binds PD-1, preferably human PD-1.

In a preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 15;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 22;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 29;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 36;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 43; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 50.

In another preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 16;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 23;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 30;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 37;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 44; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 51.

In another preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 17;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 24;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 31;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 38;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 45; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 52.

In another preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 18;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 25;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 32;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 39;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 46; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 53.

In another preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 19;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 26;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 33;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 40;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 47; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 54.

Antibodies Having Particular Germline Sequences

In certain embodiments, an antibody of the invention comprises a heavy chain variable region from a particular germline heavy chain immunoglobulin gene and/or a light chain variable region from a particular germline light chain immunoglobulin gene.

For example, in a preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene, wherein the antibody specifically binds PD-1, preferably human PD-1. In another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 4-39 gene, wherein the antibody specifically binds PD-1, preferably human PD-1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L6 gene, wherein the antibody specifically binds PD-1, preferably human PD-1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L15 gene, wherein the antibody specifically binds PD-1, preferably human PD-1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, wherein the antibody:

(a) comprises a heavy chain variable region that is the product of or derived from a human V_H 3-33 or 4-39 gene (which gene encodes the amino acid sequence set forth in SEQ ID NO: 71 or 73, respectively);

(b) comprises a light chain variable region that is the product of or derived from a human V_K L6 or L15 gene (which gene encodes the amino acid sequence set forth in SEQ ID NO: 72 or 74, respectively); and

(c) specifically binds to PD-1.

Examples of antibodies having V_H and V_K of V_H 3-33 and V_K L6, respectively, are 17D8, 2D3, 4H1, 5C4, and 7D3. Examples of antibodies having V_H and V_K of V_H 4-39 and V_K L15, respectively are 4A11 and 5F4.

As used herein, a human antibody comprises heavy or light chain variable regions that is "the product of" or "derived from" a particular germline sequence if the variable regions of the antibody are obtained from a system that uses human germline immunoglobulin genes. Such systems include immunizing a transgenic mouse carrying human immunoglobulin genes with the antigen of interest or screening a human immunoglobulin gene library displayed on phage with the antigen of interest. A human antibody that is "the product of" or "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (*i.e.*, greatest % identity) to the sequence of the human antibody. A human antibody that is "the product of" or "derived from" a particular human germline immunoglobulin sequence may contain amino acid differences as compared to the germline sequence, due to, for example, naturally-occurring somatic mutations or intentional introduction of site-directed mutation. However, a selected human antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (*e.g.*, murine germline sequences). In certain cases, a human antibody may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the human antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

Homologous Antibodies

In yet another embodiment, an antibody of the invention comprises heavy and light chain variable regions comprising amino acid sequences that are homologous to the amino acid sequences of the preferred antibodies described herein, and wherein the antibodies retain the desired functional properties of the anti-PD-1 antibodies of the invention.

For example, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region and a light chain variable region, wherein:

(a) the heavy chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7;

(b) the light chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14; and

the antibody exhibits one or more of the following properties:

(c) the antibody binds to human PD-1 with a K_D of 1 x 10^{-7} M or less;

(d) the antibody does not substantially bind to human CD28, CTLA-4 or ICOS;

(e) the antibody increases T-cell proliferation in an MLR assay;

(f) the antibody increases interferon-gamma production in an MLR assay;

(g) the antibody increases Il-2 secretion in an MLR assay;

(h) the antibody binds to human PD-1 and cynomolgus monkey PD-1;

(i) the antibody inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(j) the antibody stimulates antigen-specific memory responses;

(k) the antibody stimulates antibody responses;

(l) the antibody inhibits tumor cell growth *in vivo*.

In other embodiments, the V_H and/or V_L amino acid sequences may be 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the sequences set forth above. An antibody having V_H and V_L regions having high (*i.e.*, 80% or greater) homology to the V_H and V_L regions of the sequences set forth above, can be obtained by mutagenesis (*e.g.*, site-directed or PCR-mediated mutagenesis) of nucleic acid molecules encoding SEQ ID NOs: 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67 , 68, 69 and 70, followed by testing of the encoded altered antibody for retained function (*i.e.*, the functions set forth in (c) through (l) above) using the functional assays described herein.

As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Additionally or alternatively, the protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST protein searches

can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the antibody molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. (See www.ncbi.nlm.nih.gov).

Antibodies with Conservative Modifications

In certain embodiments, an antibody of the invention comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences and a light chain variable region comprising CDR1, CDR2 and CDR3 sequences, wherein one or more of these CDR sequences comprise specified amino acid sequences based on the preferred antibodies described herein (*e.g.*, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4), or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the anti-PD-1 antibodies of the invention. Accordingly, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences, wherein:

(a) the heavy chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35, and conservative modifications thereof;

(b) the light chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequence of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56, and conservative modifications thereof; and

the antibody exhibits one or more of the following properties:

(c) the antibody binds to human PD-1 with a K_D of 1 x 10^{-7} M or less;

(d) the antibody does not substantially bind to human CD28, CTLA-4 or ICOS;

(e) the antibody increases T-cell proliferation in an MLR assay;

(f) the antibody increases interferon-gamma production in an MLR assay;

(g) the antibody increases Il-2 secretion in an MLR assay;

(h) the antibody binds to human PD-1 and cynomolgus monkey PD-1;

(i) the antibody inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(j) the antibody stimulates antigen-specific memory responses;

(k) the antibody stimulates antibody responses;

(l) the antibody inhibits tumor cell growth *in vivo*.

In a preferred embodiment, the heavy chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, and conservative modifications thereof; and the light chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and

conservative modifications thereof. In another preferred embodiment, the heavy chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, and conservative modifications thereof; and the light chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, and conservative modifications thereof.

As used herein, the term "conservative sequence modifications" is intended to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the CDR regions of an antibody of the invention can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested for retained function (*i.e.*, the functions set forth in (c) through (l) above) using the functional assays described herein.

Antibodies that Bind to the Same Epitope as Anti-PD-1 Antibodies of the Invention

In another embodiment, the invention provides antibodies that bind to the same epitope on human PD-1 as any of the PD-1 monoclonal antibodies of the invention (*i.e.*, antibodies that have the ability to cross-compete for binding to PD-1 with any of the monoclonal antibodies of the invention). In preferred embodiments, the reference antibody for cross-competition studies can be the monoclonal antibody 17D8 (having V_H and V_L sequences as shown in SEQ ID NOs: 1 and 8, respectively), or the monoclonal antibody 2D3 (having V_H and V_L sequences as shown in SEQ ID NOs: 2 and 9, respectively), or the monoclonal antibody 4H1 (having V_H and V_L sequences as shown in SEQ ID NOs: 3 and 10, respectively), or the monoclonal antibody 5C4 (having V_H and V_L sequences as shown in SEQ ID NOs: 4 and 11, respectively), or the monoclonal antibody 4A11 (having V_H and V_L sequences as shown in SEQ ID NOs: 5 and 12, or the monoclonal antibody 7D3 (having V_H and V_L sequences as shown in SEQ ID NOs: 6 and 13, or the monoclonal antibody 5F4 (having V_H and V_L sequences as shown in SEQ ID NOs: 7 and 14, respectively). Such cross-competing antibodies can be identified based on their ability to cross-compete with 17D8, 2D3, 4H1, 5C4,

4A11, 7D3 or 5F4 in standard PD-1 binding assays. For example, BIAcore analysis, ELISA assays or flow cytometry may be used to demonstrate cross-competition with the antibodies of the current invention. The ability of a test antibody to inhibit the binding of, for example, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, to human PD-1 demonstrates that the test antibody can compete with 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 for binding to human PD-1 and thus binds to the same epitope on human PD-1 as 17D8, 2D3, 4H1, 5C4, or 4A11. In a preferred embodiment, the antibody that binds to the same epitope on human PD-1 as 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 is a human monoclonal antibody. Such human monoclonal antibodies can be prepared and isolated as described in the Examples.

Engineered and Modified Antibodies

An antibody of the invention further can be prepared using an antibody having one or more of the V_H and/or V_L sequences disclosed herein as starting material to engineer a modified antibody, which modified antibody may have altered properties from the starting antibody. An antibody can be engineered by modifying one or more residues within one or both variable regions (*i.e.*, V_H and/or V_L), for example within one or more CDR regions and/or within one or more framework regions. Additionally or alternatively, an antibody can be engineered by modifying residues within the constant region(s), for example to alter the effector function(s) of the antibody.

One type of variable region engineering that can be performed is CDR grafting. Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, *e.g.*, Riechmann, L. *et al.* (1998) *Nature* 332:323-327; Jones, P. *et al.* (1986) *Nature* 321:522-525; Queen, C. *et al.* (1989) *Proc. Natl. Acad. See. U.S.A.* 86:10029-10033; U.S. Patent No. 5,225,539 to Winter, and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al.*)

Accordingly, another embodiment of the invention pertains to an isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, and SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35, respectively, and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56, respectively. Thus, such antibodies contain the V_H and V_L CDR sequences of

monoclonal antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 yet may contain different framework sequences from these antibodies.

Such framework sequences can be obtained from public DNA databases or published references that include germline antibody gene sequences. For example, germline DNA sequences for human heavy and light chain variable region genes can be found in the "VBase" human germline sequence database (available on the Internet at www.mrc-cpe.cam.ac.uk/vbase), as well as in Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson, I. M., *et al.* (1992) "The Repertoire of Human Germline V_H Sequences Reveals about Fifty Groups of V_H Segments with Different Hypervariable Loops" *J. Mol. Biol.* 227:776-798; and Cox, J. P. L. *et al.* (1994) "A Directory of Human Germ-line V_H Segments Reveals a Strong Bias in their Usage" *Eur. J. Immunol.* 24:827-836; the contents of each of which are expressly incorporated herein by reference. As another example, the germline DNA sequences for human heavy and light chain variable region genes can be found in the GenBank database. For example, the following heavy chain germline sequences found in the HCo7 HuMAb mouse are available in the accompanying GenBank accession numbers: 1-69 (NG_0010109, NT_024637 and BC070333), 3-33 (NG_0010109 and NT_024637) and 3-7 (NG_0010109 and NT_024637). As another example, the following heavy chain germline sequences found in the HCo12 HuMAb mouse are available in the accompanying GenBank accession numbers: 1-69 (NG_0010109, NT_024637 and BC070333), 5-51 (NG_0010109 and NT_024637), 4-34 (NG_0010109 and NT_024637), 3-30.3 (AJ556644) and 3-23 (AJ406678).

Preferred framework sequences for use in the antibodies of the invention are those that are structurally similar to the framework sequences used by selected antibodies of the invention, *e.g.*, similar to the V_H 3-33 framework sequences (SEQ ID NO: 71) and/or the V_H 4-39 framework sequences (SEQ ID NO: 73) and/or the V_K L6 framework sequences (SEQ ID NO: 72) and/or the V_K L15 framework sequences (SEQ ID NO: 74) used by preferred monoclonal antibodies of the invention. The V_H CDR1, CDR2, and CDR3 sequences, and the V_K CDR1, CDR2, and CDR3 sequences, can be grafted onto framework regions that have the identical sequence as that found in the germline immunoglobulin gene from which the framework sequence derive, or the CDR sequences can be grafted onto framework regions that contain one or more mutations as compared to the germline sequences. For example, it has been found that in certain instances it is beneficial to mutate residues within the framework regions to maintain or enhance the antigen binding ability of the antibody (see *e.g.*, U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al*).

Another type of variable region modification is to mutate amino acid residues within the V_H and/or V_K CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (*e.g.*, affinity) of the antibody of interest. Site-directed mutagenesis or PCR-mediated mutagenesis can be performed to introduce the mutation(s) and the effect on

antibody binding, or other functional property of interest, can be evaluated in *in vitro* or *in vivo* assays as described herein and provided in the Examples. Preferably conservative modifications (as discussed above) are introduced. The mutations may be amino acid substitutions, additions or deletions, but are preferably substitutions. Moreover, typically no more than one, two, three, four or five residues within a CDR region are altered.

Accordingly, in another embodiment, the invention provides isolated anti-PD-1 monoclonal antibodies, or antigen-binding portions thereof, comprising a heavy chain variable region comprising: (a) a V_H CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21; (b) a V_H CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28; (c) a V_H CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35; (d) a V_K CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42; (e) a V_K CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49; and (f) a V_K CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56.

Engineered antibodies of the invention include those in which modifications have been made to framework residues within V_H and/or V_K, *e.g.* to improve the properties of the antibody. Typically such framework modifications are made to decrease the immunogenicity of the antibody. For example, one approach is to "backmutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived.

For example, Table 1 below shows a number of amino acid changes in the framework regions of the anti-PD-1 antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 that differ from

the heavy chain parent germline sequence. To return one or more of the amino acid residues in the framework region sequences to their germline configuration, the somatic mutations can be "backmutated" to the germline sequence by, for example, site-directed mutagenesis or PCR-mediated mutagenesis.

Amino acid changes may occur in the framework regions of anti-PD-1 antibodies that differ from the light chain parent germline sequence. For example, for 17D8, amino acid residue #47 (within FR2) of V_K is an isoleucine whereas this residue in the corresponding V_K L6 germline sequence is a leucine. To return the framework region sequences to their germline configuration, the somatic mutations can be "backmutated" to the germline sequence by, for example, site-directed mutagenesis or PCR-mediated mutagenesis (*e.g.*, residue #47 (residue #13 of FR2) of the V_K of 17D8 can be "backmutated" from isoleucine to leucine).

As another example, for 4A11, amino acid residue #20 (within FR1) of V_K is a serine whereas this residue in the corresponding V_K L15 germline sequence is a threonine. To return the framework region sequences to their germline configuration, for example, residue #20 of the V_K of 4A11 can be "backmutated" from serine to threonine. Such "backmutated" antibodies are also intended to be encompassed by the invention.

The alignment of V_H regions for 17D8, 2D3, 4H1, 5C4 and 7D3, against the parent germline V_H 3-33 sequence is shown in Figure 8. The alignment of V_H regions for 4A11 and 5F4 against the parent germline V_H 4-39 sequence is shown in Figure 11.

Table 1. Modifications to antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 from the heavy chain germline configuration.

Anti-PD-1 Ab	Amino acid position	Amino acid of antibody	Original amino acid of germline configuration
17D8	10	D	G
	16	G	R
	27	V	F
	28	A	T
	78	M	T
	93	M	V
2D3	10	D	G
	27	L	F
	30	T	S
	85	N	S
	98	T	R
4H1	3	Y	Q
	84	T	N
	88	V	A
	98	S	R
5C4	21	D	S
	23	K	A
	27	I	F
	80	F	Y
	98	T	R
4A11	29	L	I
	79	Q	H
	98	V	A
7D3	23	T	A
	24	T	A
	27	I	F
	70	L	I
	74	D	N
	97	V	A
	98	T	R
5F4	23	S	T
	29	L	I
	51	A	G
	77	R	K

Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Patent Publication No. 20030153043 by Carr *et al.*

In addition or alternative to modifications made within the framework or CDR regions, antibodies of the invention may be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life,

complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody of the invention may be chemically modified (*e.g.*, one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. Each of these embodiments is described in further detail below. The numbering of residues in the Fc region is that of the EU index of Kabat.

In one embodiment, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, *e.g.*, increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer *et al.* The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

In another embodiment, the Fc hinge region of an antibody is mutated to decrease the biological half life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired Staphylococcyl protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Patent No. 6,165,745 by Ward *et al.*

In another embodiment, the antibody is modified to increase its biological half life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent No. 6,277,375 to Ward. Alternatively, to increase the biological half life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta *et al.*

In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector function(s) of the antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322 can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter *et al.*

In another example, one or more amino acids selected from amino acid residues 329, 331 and 322 can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent Nos. 6,194,551 by Idusogie *et al.*

In another example, one or more amino acid residues within amino acid positions 231 and 239 are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in PCT Publication WO 94/29351 by Bodmer *et al.*

In yet another example, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 309, 312, 315, 320, 322, 324, 326, 327, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439. This approach is described further in PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγR1, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields, R.L. *et al.* (2001) *J. Biol. Chem.* 276:6591-6604). Specific mutations at positions 256, 290, 298, 333, 334 and 339 were shown to improve binding to FcγRIII. Additionally, the following combination mutants were shown to improve FcγRIII binding: T256A/S298A, S298A/E333A, S298A/K224A and S298A/E333A/K334A.

In still another embodiment, the glycosylation of an antibody is modified. For example, an aglycoslated antibody can be made (*i.e.*, the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in U.S. Patent Nos. 5,714,350 and 6,350,861 by Co *et al.*

Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. For example, the cell lines Ms704, Ms705, and Ms709 lack the fucosyltransferase gene, FUT8 (alpha (1,6) fucosyltransferase), such that antibodies expressed in the Ms704, Ms705, and Ms709 cell lines lack fucose on their carbohydrates. The Ms704, Ms705, and Ms709 $FUT8^{-/-}$ cell lines were created by the targeted disruption of the FUT8 gene in CHO/DG44 cells using two replacement vectors (see U.S. Patent Publication No. 20040110704 by Yamane *et al.* and Yamane-Ohnuki *et al.* (2004) *Biotechnol Bioeng* 87:614-22). As another example, EP 1,176,195 by Hanai *et al.* describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit

hypofucosylation by reducing or eliminating the alpha 1,6 bond-related enzyme. Hanai *et al.* also describe cell lines which have a low enzyme activity for adding fucose to the N-acetylglucosamine that binds to the Fc region of the antibody or does not have the enzyme activity, for example the rat myeloma cell line YB2/0 (ATCC CRL 1662). PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R.L. *et al.* (2002) *J. Biol. Chem.* 277:26733-26740). PCT Publication WO 99/54342 by Umana *et al.* describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (*e.g.*, beta(1,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana *et al.* (1999) *Nat. Biotech.* 17:176-180). Alternatively, the fucose residues of the antibody may be cleaved off using a fucosidase enzyme. For example, the fucosidase alpha-L-fucosidase removes fucosyl residues from antibodies (Tarentino, A.L. *et al.* (1975) *Biochem.* 14:5516-23).

Another modification of the antibodies herein that is contemplated by the invention is pegylation. An antibody can be pegylated to, for example, increase the biological (*e.g.*, serum) half life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies of the invention. See for example, EP 0 154 316 by Nishimura *et al.* and EP 0 401 384 by Ishikawa *et al.*

Methods of Engineering Antibodies

As discussed above, the anti-PD-1 antibodies having V_H and V_K sequences disclosed herein can be used to create new anti-PD-1 antibodies by modifying the VH and/or V_K sequences, or the constant region(s) attached thereto. Thus, in another aspect of the invention, the structural features of an anti-PD-1 antibody of the invention, *e.g.* 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, are used to create structurally related anti-PD-1 antibodies that retain at least one functional property of the antibodies of the invention, such as binding to human PD-1. For example, one or more CDR regions of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, or mutations thereof, can be combined recombinantly with known framework regions and/or other CDRs to create additional, recombinantly-engineered, anti-PD-1 antibodies of the

invention, as discussed above. Other types of modifications include those described in the previous section. The starting material for the engineering method is one or more of the V_H and/or V_K sequences provided herein, or one or more CDR regions thereof. To create the engineered antibody, it is not necessary to actually prepare (*i.e.*, express as a protein) an antibody having one or more of the V_H and/or V_K sequences provided herein, or one or more CDR regions thereof. Rather, the information contained in the sequence(s) is used as the starting material to create a "second generation" sequence(s) derived from the original sequence(s) and then the "second generation" sequence(s) is prepared and expressed as a protein.

Accordingly, in another embodiment, the invention provides a method for preparing an anti-PD-1 antibody comprising:

(a) providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, a CDR2 sequence selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35; and/or (ii) a light chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, a CDR2 sequence selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56;

(b) altering at least one amino acid residue within the heavy chain variable region antibody sequence and/or the light chain variable region antibody sequence to create at least one altered antibody sequence; and

(c) expressing the altered antibody sequence as a protein.

Standard molecular biology techniques can be used to prepare and express the altered antibody sequence.

Preferably, the antibody encoded by the altered antibody sequence(s) is one that retains one, some or all of the functional properties of the anti-PD-1 antibodies described herein, which functional properties include, but are not limited to:

(a) the antibody binds to human PD-1 with a K_D of 1×10^{-7} M or less;

(b) the antibody does not substantially bind to human CD28, CTLA-4 or ICOS;

(c) the antibody increases T-cell proliferation in an MLR assay;

(d) the antibody increases interferon-gamma production in an MLR assay;

(e) the antibody increases Il-2 secretion in an MLR assay;

(f) the antibody binds to human PD-1 and cynomolgus monkey PD-1;

(g) the antibody inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(h) the antibody stimulates antigen-specific memory responses;

(i) the antibody stimulates antibody responses;

(j) the antibody inhibits tumor cell growth *in vivo*.

The functional properties of the altered antibodies can be assessed using standard assays available in the art and/or described herein, such as those set forth in the Examples (*e.g.*, flow cytometry, binding assays).

In certain embodiments of the methods of engineering antibodies of the invention, mutations can be introduced randomly or selectively along all or part of an anti-PD-1 antibody coding sequence and the resulting modified anti-PD-1 antibodies can be screened for binding activity and/or other functional properties as described herein. Mutational methods have been described in the art. For example, PCT Publication WO 02/092780 by Short describes methods for creating and screening antibody mutations using saturation mutagenesis, synthetic ligation assembly, or a combination thereof. Alternatively, PCT Publication WO 03/074679 by Lazar *et al.* describes methods of using computational screening methods to optimize physiochemical properties of antibodies.

Nucleic Acid Molecules Encoding Antibodies of the Invention

Another aspect of the invention pertains to nucleic acid molecules that encode the antibodies of the invention. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. *See*, F. Ausubel, *et al.*, ed. (1987) Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York. A nucleic acid of the invention can be, for example, DNA or RNA and may or may not contain intronic sequences. In a preferred embodiment, the nucleic acid is a cDNA molecule.

Nucleic acids of the invention can be obtained using standard molecular biology techniques. For antibodies expressed by hybridomas (*e.g.*, hybridomas prepared from transgenic mice carrying human immunoglobulin genes as described further below), cDNAs encoding the light and heavy chains of the antibody made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. For antibodies obtained from an immunoglobulin gene library (*e.g.*, using phage display techniques), nucleic acid encoding the antibody can be recovered from the library.

Preferred nucleic acids molecules of the invention are those encoding the VH and VL sequences of the 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 monoclonal antibodies. DNA sequences encoding the VH sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 57, 58, 59, 60, 61, 62 and 63, respectively. DNA sequences encoding the VL sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are shown in SEQ ID NOs: 64, 65, 66, 67, 68, 69 and 70, respectively.

Once DNA fragments encoding VH and VL segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes

or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

The isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see *e.g.*, Kabat, E. A., el al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG1 or IgG4 constant region. For a Fab fragment heavy chain gene, the VH-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see *e.g.*, Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but most preferably is a kappa constant region.

To create a scFv gene, the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence $(Gly_4\text{-}Ser)_3$, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, (1990) *Nature* 348:552-554).

Production of Monoclonal Antibodies of the Invention

Monoclonal antibodies (mAbs) of the present invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology *e.g.*, the standard somatic cell hybridization technique of Kohler and Milstein (1975) *Nature* 256: 495. Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibody can be employed *e.g.*, viral or oncogenic transformation of B lymphocytes.

The preferred animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are known in the art. Fusion partners (*e.g.*, murine myeloma cells) and fusion procedures are also known.

Chimeric or humanized antibodies of the present invention can be prepared based on the sequence of a murine monoclonal antibody prepared as described above. DNA encoding the heavy and light chain immunoglobulins can be obtained from the murine hybridoma of interest and engineered to contain non-murine (*e.g.*, human) immunoglobulin sequences using standard molecular biology techniques. For example, to create a chimeric antibody, the murine variable regions can be linked to human constant regions using methods known in the art (see *e.g.*, U.S. Patent No. 4,816,567 to Cabilly *et al.*). To create a humanized antibody, the murine CDR regions can be inserted into a human framework using methods known in the art (see *e.g.*, U.S. Patent No. 5,225,539 to Winter, and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al.*).

In a preferred embodiment, the antibodies of the invention are human monoclonal antibodies. Such human monoclonal antibodies directed against PD-1 can be generated using transgenic or transchromosomic mice carrying parts of the human immune system rather than the mouse system. These transgenic and transchromosomic mice include mice referred to herein as HuMAb mice and KM mice™, respectively, and are collectively referred to herein as "human Ig mice."

The HuMAb mouse® (Medarex, Inc.) contains human immunoglobulin gene miniloci that encode unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (see *e.g.*, Lonberg, *et al.* (1994) Nature 368(6474): 856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal (Lonberg, N. *et al.* (1994), *supra*; reviewed in Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* 13: 65-93, and Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci.* 764:536-546). The preparation and use of HuMab mice, and the genomic modifications carried by such mice, is further described in Taylor, L. *et al.* (1992) *Nucleic Acids Research* 20:6287-6295; Chen, J. *et al.* (1993) *International Immunology* 5: 647-656; Tuaillon *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:3720-3724; Choi *et al.* (1993) *Nature Genetics* 4:117-123; Chen, J. *et al.* (1993) *EMBO J.* 12: 821-830; Tuaillon *et al.* (1994) *J. Immunol.* 152:2912-2920; Taylor, L. *et al.* (1994) *International Immunology* 6: 579-591; and Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851, the contents of all of which are hereby specifically incorporated by reference in their entirety. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay; U.S. Patent No. 5,545,807 to Surani *et al.*;

PCT Publication Nos. WO 92/03918, WO 93/12227, WO 94/25585, WO 97/13852, WO 98/24884 and WO 99/45962, all to Lonberg and Kay; and PCT Publication No. WO 01/14424 to Korman *et al.*

In another embodiment, human antibodies of the invention can be raised using a mouse that carries human immunoglobulin sequences on transgenes and transchomosomes, such as a mouse that carries a human heavy chain transgene and a human light chain transchromosome. Such mice, referred to herein as "KM mice™", are described in detail in PCT Publication WO 02/43478 to Ishida *et al.*

Still further, alternative transgenic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-PD-1 antibodies of the invention. For example, an alternative transgenic system referred to as the Xenomouse (Abgenix, Inc.) can be used; such mice are described in, for example, U.S. Patent Nos. 5,939,598; 6,075,181; 6,114,598; 6, 150,584 and 6,162,963 to Kucherlapati *et al.*

Moreover, alternative transchromosomic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-PD-1 antibodies of the invention. For example, mice carrying both a human heavy chain transchromosome and a human light chain transchromosome, referred to as "TC mice" can be used; such mice are described in Tomizuka *et al.* (2000) *Proc. Natl. Acad. Sci. USA* 97:722-727. Furthermore, cows carrying human heavy and light chain transchromosomes have been described in the art (Kuroiwa *et al.* (2002) *Nature Biotechnology* 20:889-894) and can be used to raise anti-PD-1 antibodies of the invention.

Human monoclonal antibodies of the invention can also be prepared using phage display methods for screening libraries of human immunoglobulin genes. Such phage display methods for isolating human antibodies are established in the art. See for example: U.S. Patent Nos. 5,223,409; 5,403,484; and 5,571,698 to Ladner *et al.*; U.S. Patent Nos. 5,427,908 and 5,580,717 to Dower *et al.*; U.S. Patent Nos. 5,969,108 and 6,172,197 to McCafferty *et al.*; and U.S. Patent Nos. 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915 and 6,593,081 to Griffiths *et al.*

Human monoclonal antibodies of the invention can also be prepared using SCID mice into which human immune cells have been reconstituted such that a human antibody response can be generated upon immunization. Such mice are described in, for example, U.S. Patent Nos. 5,476,996 and 5,698,767 to Wilson *et al.*

Immunization of Human Ig Mice

When human Ig mice are used to raise human antibodies of the invention, such mice can be immunized with a purified or enriched preparation of PD-1 antigen and/or recombinant PD-1, or an PD-1 fusion protein, as described by Lonberg, N. *et al.* (1994) *Nature* 368(6474): 856-859; Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851; and PCT Publication WO 98/24884 and WO 01/14424. Preferably, the mice will be 6-16 weeks of age upon the

first infusion. For example, a purified or recombinant preparation (5-50 μg) of PD-1 antigen can be used to immunize the human Ig mice intraperitoneally.

Detailed procedures to generate fully human monoclonal antibodies to PD-1 are described in Example 1 below. Cumulative experience with various antigens has shown that the transgenic mice respond when initially immunized intraperitoneally (IP) with antigen in complete Freund's adjuvant, followed by every other week IP immunizations (up to a total of 6) with antigen in incomplete Freund's adjuvant. However, adjuvants other than Freund's are also found to be effective. In addition, whole cells in the absence of adjuvant are found to be highly immunogenic. The immune response can be monitored over the course of the immunization protocol with plasma samples being obtained by retroorbital bleeds. The plasma can be screened by ELISA (as described below), and mice with sufficient titers of anti-PD-1 human immunoglobulin can be used for fusions. Mice can be boosted intravenously with antigen 3 days before sacrifice and removal of the spleen. It is expected that 2-3 fusions for each immunization may need to be performed. Between 6 and 24 mice are typically immunized for each antigen. Usually both HCo7 and HCo12 strains are used. In addition, both HCo7 and HCo12 transgene can be bred together into a single mouse having two different human heavy chain transgenes (HCo7/HCo12). Alternatively or additionally, the KM mouse™ strain can be used, as described in Example 1.

Generation of Hybridomas Producing Human Monoclonal Antibodies of the Invention

To generate hybridomas producing human monoclonal antibodies of the invention, splenocytes and/or lymph node cells from immunized mice can be isolated and fused to an appropriate immortalized cell line, such as a mouse myeloma cell line. The resulting hybridomas can be screened for the production of antigen-specific antibodies. For example, single cell suspensions of splenic lymphocytes from immunized mice can be fused to one-sixth the number of P3X63-Ag8.653 nonsecreting mouse myeloma cells (ATCC, CRL 1580) with 50% PEG. Alternatively, the single cell suspensions of splenic lymphocytes from immunized mice can be fused using an electric field based electrofusion method, using a Cyto Pulse large chamber cell fusion electroporator (Cyto Pulse Sciences, Inc., Glen Burnie, MD). Cells are plated at approximately 2 x 10^5 in flat bottom microtiter plate, followed by a two week incubation in selective medium containing 20% fetal Clone Serum, 18% "653" conditioned media, 5% origen (IGEN), 4 mM L-glutamine, 1 mM sodium pyruvate, 5mM HEPES, 0.055 mM 2-mercaptoethanol, 50 units/ml penicillin, 50 mg/ml streptomycin, 50 mg/ml gentamycin and 1X HAT (Sigma; the HAT is added 24 hours after the fusion). After approximately two weeks, cells can be cultured in medium in which the HAT is replaced with HT. Individual wells can then be screened by ELISA for human monoclonal IgM and IgG antibodies. Once extensive hybridoma growth occurs, medium can be observed usually after 10-14 days. The antibody secreting hybridomas can be replated, screened again, and if still positive for human IgG, the monoclonal antibodies can be subcloned at least twice by limiting

dilution. The stable subclones can then be cultured *in vitro* to generate small amounts of antibody in tissue culture medium for characterization.

To purify human monoclonal antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, N.J.). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD_{280} using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at -80 °C.

Generation of Transfectomas Producing Monoclonal Antibodies of the Invention

Antibodies of the invention also can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as is well known in the art (*e.g.*, Morrison, S. (1985) *Science* 229:1202).

For example, to express the antibodies, or antibody fragments thereof, DNAs encoding partial or full-length light and heavy chains, can be obtained by standard molecular biology techniques (*e.g.*, PCR amplification or cDNA cloning using a hybridoma that expresses the antibody of interest) and the DNAs can be inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). The light and heavy chain variable regions of the antibodies described herein can be used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the V_H segment is operatively linked to the C_H segment(s) within the vector and the V_K segment is operatively linked to the C_L segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in

a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel (Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, CA (1990)). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences, may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.* Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP) and polyoma. Alternatively, nonviral regulatory sequences may be used, such as the ubiquitin promoter or β-globin promoter. Still further, regulatory elements composed of sequences from different sources, such as the SRα promoter system, which contains sequences from the SV40 early promoter and the long terminal repeat of human T cell leukemia virus type 1 (Takebe, Y. *et al.* (1988) *Mol. Cell. Biol.* 8:466-472).

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, *e.g.*, U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Prokaryotic expression of antibody genes has been reported to be ineffective for production of high yields of active antibody (Boss, M. A. and Wood, C. R. (1985) *Immunology Today* 6:12-13).

Preferred mammalian host cells for expressing the recombinant antibodies of the

invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R. J. Kaufman and P. A. Sharp (1982) *Mol. Biol. 159*:601-621), NSO myeloma cells, COS cells and SP2 cells. In particular, for use with NSO myeloma cells, another preferred expression system is the GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338,841. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Characterization of Antibody Binding to Antigen

Antibodies of the invention can be tested for binding to PD-1 by, for example, standard ELISA. Briefly, microtiter plates are coated with purified PD-1 at 0.25 μg/ml in PBS, and then blocked with 5% bovine serum albumin in PBS. Dilutions of antibody (*e.g.*, dilutions of plasma from PD-1-immunized mice) are added to each well and incubated for 1-2 hours at 37°C. The plates are washed with PBS/Tween and then incubated with secondary reagent (*e.g.*, for human antibodies, a goat-anti-human IgG Fc-specific polyclonal reagent) conjugated to alkaline phosphatase for 1 hour at 37°C. After washing, the plates are developed with pNPP substrate (1 mg/ml), and analyzed at OD of 405-650. Preferably, mice which develop the highest titers will be used for fusions.

An ELISA assay as described above can also be used to screen for hybridomas that show positive reactivity with PD-1 immunogen. Hybridomas that bind with high avidity to PD-1 are subcloned and further characterized. One clone from each hybridoma, which retains the reactivity of the parent cells (by ELISA), can be chosen for making a 5-10 vial cell bank stored at -140 °C, and for antibody purification.

To purify anti-PD-1 antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, NJ). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD_{280} using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at -80 °C.

To determine if the selected anti-PD-1 monoclonal antibodies bind to unique epitopes, each antibody can be biotinylated using commercially available reagents (Pierce, Rockford, IL). Competition studies using unlabeled monoclonal antibodies and biotinylated monoclonal antibodies can be performed using PD-1 coated-ELISA plates as described above. Biotinylated mAb binding can be detected with a strep-avidin-alkaline phosphatase probe.

To determine the isotype of purified antibodies, isotype ELISAs can be performed using reagents specific for antibodies of a particular isotype. For example, to determine the isotype of a human monoclonal antibody, wells of microtiter plates can be coated with 1 μg/ml of anti-human immunoglobulin overnight at 4 °C. After blocking with 1% BSA, the plates are reacted with 1 μg /ml or less of test monoclonal antibodies or purified isotype controls, at ambient temperature for one to two hours. The wells can then be reacted with either human IgG1 or human IgM-specific alkaline phosphatase-conjugated probes. Plates are developed and analyzed as described above.

Anti-PD-1 human IgGs can be further tested for reactivity with PD-1 antigen by Western blotting. Briefly, PD-1 can be prepared and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the separated antigens are transferred to nitrocellulose membranes, blocked with 10% fetal calf serum, and probed with the monoclonal antibodies to be tested. Human IgG binding can be detected using anti-human IgG alkaline phosphatase and developed with BCIP/NBT substrate tablets (Sigma Chem. Co., St. Louis, Mo.).

Immunoconjugates

In another aspect, the present invention features an anti-PD-1 antibody, or a fragment thereof, conjugated to a therapeutic moiety, such as a cytotoxin, a drug (*e.g.*, an immunosuppressant) or a radiotoxin. Such conjugates are referred to herein as "immunoconjugates". Immunoconjugates that include one or more cytotoxins are referred to as "immunotoxins." A cytotoxin or cytotoxic agent includes any agent that is detrimental to (*e.g.*, kills) cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents also include, for example, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

Other preferred examples of therapeutic cytotoxins that can be conjugated to an antibody of the invention include duocarmycins, calicheamicins, maytansines and auristatins, and derivatives thereof. An example of a calicheamicin antibody conjugate is commercially available (Mylotarg™; Wyeth-Ayerst).

Cytoxins can be conjugated to antibodies of the invention using linker technology available in the art. Examples of linker types that have been used to conjugate a cytotoxin to

an antibody include, but are not limited to, hydrazones, thioethers, esters, disulfides and peptide-containing linkers. A linker can be chosen that is, for example, susceptible to cleavage by low pH within the lysosomal compartment or susceptible to cleavage by proteases, such as proteases preferentially expressed in tumor tissue such as cathepsins (*e.g.*, cathepsins B, C, D).

For further discussion of types of cytotoxins, linkers and methods for conjugating therapeutic agents to antibodies, see also Saito, G. *et al.* (2003) *Adv. Drug Deliv. Rev.* 55:199-215; Trail, P.A. *et al.* (2003) *Cancer Immunol. Immunother.* 52:328-337; Payne, G. (2003) *Cancer Cell* 3:207-212; Allen, T.M. (2002) *Nat. Rev. Cancer* 2:750-763; Pastan, I. and Kreitman, R. J. (2002) *Curr. Opin. Investig. Drugs* 3:1089-1091; Senter, P.D. and Springer, C.J. (2001) *Adv. Drug Deliv. Rev.* 53:247-264.

Antibodies of the present invention also can be conjugated to a radioactive isotope to generate cytotoxic radiopharmaceuticals, also referred to as radioimmunoconjugates. Examples of radioactive isotopes that can be conjugated to antibodies for use diagnostically or therapeutically include, but are not limited to, iodine^{131}, indium^{111}, yttrium^{90} and lutetium^{177}. Method for preparing radioimmunconjugates are established in the art. Examples of radioimmunoconjugates are commercially available, including Zevalin™ (IDEC Pharmaceuticals) and Bexxar™ (Corixa Pharmaceuticals), and similar methods can be used to prepare radioimmunoconjugates using the antibodies of the invention.

The antibody conjugates of the invention can be used to modify a given biological response, and the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, an enzymatically active toxin, or active fragment thereof, such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor or interferon-γ; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*,

"The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982).

Bispecific Molecules

In another aspect, the present invention features bispecific molecules comprising an anti-PD-1 antibody, or a fragment thereof, of the invention. An antibody of the invention, or antigen-binding portions thereof, can be derivatized or linked to another functional molecule, *e.g.*, another peptide or protein (*e.g.*, another antibody or ligand for a receptor) to generate a bispecific molecule that binds to at least two different binding sites or target molecules. The antibody of the invention may in fact be derivatized or linkd to more than one other functional molecule to generate multispecific molecules that bind to more than two different binding sites and/or target molecules; such multispecific molecules are also intended to be encompassed by the term "bispecific molecule" as used herein. To create a bispecific molecule of the invention, an antibody of the invention can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic, such that a bispecific molecule results.

Accordingly, the present invention includes bispecific molecules comprising at least one first binding specificity for PD-1 and a second binding specificity for a second target epitope. In a particular embodiment of the invention, the second target epitope is an Fc receptor, *e.g.*, human FcγRI (CD64) or a human Fcα receptor (CD89). Therefore, the invention includes bispecific molecules capable of binding both to FcγR or FcαR expressing effector cells (*e.g.*, monocytes, macrophages or polymorphonuclear cells (PMNs)), and to target cells expressing PD-1. These bispecific molecules target PD-1 expressing cells to effector cell and trigger Fc receptor-mediated effector cell activities, such as phagocytosis of an PD-1 expressing cells, antibody dependent cell-mediated cytotoxicity (ADCC), cytokine release, or generation of superoxide anion.

In an embodiment of the invention in which the bispecific molecule is multispecific, the molecule can further include a third binding specificity, in addition to an anti-Fc binding specificity and an anti-PD-1 binding specificity. In one embodiment, the third binding specificity is an anti-enhancement factor (EF) portion, *e.g.*, a molecule which binds to a surface protein involved in cytotoxic activity and thereby increases the immune response against the target cell. The "anti-enhancement factor portion" can be an antibody, functional antibody fragment or a ligand that binds to a given molecule, *e.g.*, an antigen or a receptor, and thereby results in an enhancement of the effect of the binding determinants for the F_c receptor or target cell antigen. The "anti-enhancement factor portion" can bind an F_c receptor or a target cell antigen. Alternatively, the anti-enhancement factor portion can bind to an entity that is different from the entity to which the first and second binding specificities bind. For example, the anti-enhancement factor portion can bind a cytotoxic T-cell (*e.g.* via CD2, CD3,

CD8, CD28, CD4, CD40, ICAM-1 or other immune cell that results in an increased immune response against the target cell).

In one embodiment, the bispecific molecules of the invention comprise as a binding specificity at least one antibody, or an antibody fragment thereof, including, *e.g.*, an Fab, Fab', $F(ab')_2$, Fv, or a single chain Fv. The antibody may also be a light chain or heavy chain dimer, or any minimal fragment thereof such as a Fv or a single chain construct as described in Ladner *et al.* U.S. Patent No. 4,946,778, the contents of which is expressly incorporated by reference.

In one embodiment, the binding specificity for an Fcγ receptor is provided by a monoclonal antibody, the binding of which is not blocked by human immunoglobulin G (IgG). As used herein, the term "IgG receptor" refers to any of the eight γ-chain genes located on chromosome 1. These genes encode a total of twelve transmembrane or soluble receptor isoforms which are grouped into three Fcγ receptor classes: FcγRI (CD64), FcγRII(CD32), and FcγRIII (CD16). In one preferred embodiment, the Fcγ receptor a human high affinity FcγRI. The human FcγRI is a 72 kDa molecule, which shows high affinity for monomeric IgG (10^8 - $10^9 M^{-1}$).

The production and characterization of certain preferred anti-Fcγ monoclonal antibodies are described by Fanger *et al.* in PCT Publication WO 88/00052 and in U.S. Patent No. 4,954,617, the teachings of which are fully incorporated by reference herein. These antibodies bind to an epitope of FcγRI, FcγRII or FcγRIII at a site which is distinct from the Fc γ binding site of the receptor and, thus, their binding is not blocked substantially by physiological levels of IgG. Specific anti-FcγRI antibodies useful in this invention are mAb 22, mAb 32, mAb 44, mAb 62 and mAb 197. The hybridoma producing mAb 32 is available from the American Type Culture Collection, ATCC Accession No. HB9469. In other embodiments, the anti-Fcγ receptor antibody is a humanized form of monoclonal antibody 22 (H22). The production and characterization of the H22 antibody is described in Graziano, R.F. *et al.* (1995) *J. Immunol* 155 (10): 4996-5002 and PCT Publication WO 94/10332. The H22 antibody producing cell line was deposited at the American Type Culture Collection under the designation HA022CL1 and has the accession no. CRL 11177.

In still other preferred embodiments, the binding specificity for an Fc receptor is provided by an antibody that binds to a human IgA receptor, *e.g.*, an Fc-alpha receptor (FcαRI (CD89)), the binding of which is preferably not blocked by human immunoglobulin A (IgA). The term "IgA receptor" is intended to include the gene product of one α-gene (FcαRI) located on chromosome 19. This gene is known to encode several alternatively spliced transmembrane isoforms of 55 to 110 kDa. FcαRI (CD89) is constitutively expressed on monocytes/macrophages, eosinophilic and neutrophilic granulocytes, but not on non-effector cell populations. FcαRI has medium affinity ($\approx 5 \times 10^7 M^{-1}$) for both IgA1 and IgA2, which is increased upon exposure to cytokines such as G-CSF or GM-CSF (Morton, H.C. *et al.* (1996) *Critical Reviews in Immunology* 16:423-440). Four FcαRI-specific monoclonal

antibodies, identified as A3, A59, A62 and A77, which bind FcαRI outside the IgA ligand binding domain, have been described (Monteiro, R.C. *et al.* (1992) *J. Immunol.* 148:1764).

FcαRI and FcγRI are preferred trigger receptors for use in the bispecific molecules of the invention because they are (1) expressed primarily on immune effector cells, *e.g.*, monocytes, PMNs, macrophages and dendritic cells; (2) expressed at high levels (*e.g.*, 5,000-100,000 per cell); (3) mediators of cytotoxic activities (*e.g.*, ADCC, phagocytosis); (4) mediate enhanced antigen presentation of antigens, including self-antigens, targeted to them.

While human monoclonal antibodies are preferred, other antibodies which can be employed in the bispecific molecules of the invention are murine, chimeric and humanized monoclonal antibodies.

The bispecific molecules of the present invention can be prepared by conjugating the constituent binding specificities, *e.g.*, the anti-FcR and anti-PD-1 binding specificities, using methods known in the art. For example, each binding specificity of the bispecific molecule can be generated separately and then conjugated to one another. When the binding specificities are proteins or peptides, a variety of coupling or cross-linking agents can be used for covalent conjugation. Examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-S-acetyl-thioacetate (SATA), 5, 5'-dithiobis(2-nitrobenzoic acid) (DTNB), o-phenylenedimaleimide (oPDM), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohaxane-1-carboxylate (sulfo-SMCC) (see *e.g.*, Karpovsky *et al.* (1984) *J. Exp. Med.* 160:1686; Liu, MA *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82:8648). Other methods include those described in Paulus (1985) Behring Ins. Mitt. No. 78, 118-132; Brennan *et al.* (1985) *Science* 229:81-83), and Glennie *et al.* (1987) *J. Immunol.* 139: 2367-2375). Preferred conjugating agents are SATA and sulfo-SMCC, both available from Pierce Chemical Co. (Rockford, IL).

When the binding specificities are antibodies, they can be conjugated via sulfhydryl bonding of the C-terminus hinge regions of the two heavy chains. In a particularly preferred embodiment, the hinge region is modified to contain an odd number of sulfhydryl residues, preferably one, prior to conjugation.

Alternatively, both binding specificities can be encoded in the same vector and expressed and assembled in the same host cell. This method is particularly useful where the bispecific molecule is a mAb x mAb, mAb x Fab, Fab x $F(ab')_2$ or ligand x Fab fusion protein. A bispecific molecule of the invention can be a single chain molecule comprising one single chain antibody and a binding determinant, or a single chain bispecific molecule comprising two binding determinants. Bispecific molecules may comprise at least two single chain molecules. Methods for preparing bispecific molecules are described for example in U.S. Patent Number 5,260,203; U.S. Patent Number 5,455,030; U.S. Patent Number 4,881,175; U.S. Patent Number 5,132,405; U.S. Patent Number 5,091,513; U.S. Patent Number 5,476,786; U.S. Patent Number 5,013,653; U.S. Patent Number 5,258,498; and U.S. Patent Number 5,482,858.

Binding of the bispecific molecules to their specific targets can be confirmed by, for example, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), FACS analysis, bioassay (*e.g.*, growth inhibition), or Western Blot assay. Each of these assays generally detects the presence of protein-antibody complexes of particular interest by employing a labeled reagent (*e.g.*, an antibody) specific for the complex of interest. For example, the FcR-antibody complexes can be detected using *e.g.*, an enzyme-linked antibody or antibody fragment which recognizes and specifically binds to the antibody-FcR complexes. Alternatively, the complexes can be detected using any of a variety of other immunoassays. For example, the antibody can be radioactively labeled and used in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a γ counter or a scintillation counter or by autoradiography.

Pharmaceutical Compositions

In another aspect, the present invention provides a composition, *e.g.*, a pharmaceutical composition, containing one or a combination of monoclonal antibodies, or antigen-binding portion(s) thereof, of the present invention, formulated together with a pharmaceutically acceptable carrier. Such compositions may include one or a combination of (*e.g.*, two or more different) antibodies, or immunoconjugates or bispecific molecules of the invention. For example, a pharmaceutical composition of the invention can comprise a combination of antibodies (or immunoconjugates or bispecifics) that bind to different epitopes on the target antigen or that have complementary activities.

Pharmaceutical compositions of the invention also can be administered in combination therapy, *i.e.*, combined with other agents. For example, the combination therapy can include an anti-PD-1 antibody of the present invention combined with at least one other anti-inflammatory or immunosuppressant agent. Examples of therapeutic agents that can be used in combination therapy are described in greater detail below in the section on uses of the antibodies of the invention.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active compound, *i.e.*, antibody, immunoconjuage, or bispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

The pharmaceutical compounds of the invention may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any

undesired toxicological effects (see *e.g.*, Berge, S.M., *et al.* (1977) *J. Pharm. Sci.* 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N, N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

A pharmaceutical composition of the invention also may include a pharmaceutically acceptable anti-oxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, *supra*, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.01 per cent to about ninety-nine percent of active ingredient, preferably from about 0.1 per cent to about 70 per cent, most preferably from about 1 per cent to about 30 per cent of active ingredient in combination with a pharmaceutically acceptable carrier.

Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active

compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

For administration of the antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Preferred dosage regimens for an anti-PD-1 antibody of the invention include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, with the antibody being given using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks.

In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 μg /ml and in some methods about 25-300 μg /ml.

Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show the longest half life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular

compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A "therapeutically effective dosage" of an anti-PD-1 antibody of the invention preferably results in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. For example, for the treatment of tumors, a "therapeutically effective dosage" preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit tumor growth can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition *in vitro* by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

In another aspect, the instant disclosure provides a pharmaceutical kit of parts comprising an anti-PD-1 antibody and an anti-CTLA-4 antibody, as described herein. The kit may also further comprise instructions for use in the treatment of a hyperproliferative disease (such as cancer as described herein). In another embodiment, the anti-PD-1 and anti-CTLA-4 antibodies may be co-packaged in unit dosage form.

In certain embodiments, two or more monoclonal antibodies with different binding specificities (*e.g.*, anti-PD-1 and anti-CTLA-4) are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibody can be administered as a single dose or more commonly can be administered on multiple occasions. Intervals between single dosages can be, for example, weekly, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 μg /ml and in some methods about 25-300 μg/ml.

A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Preferred routes of administration for antibodies of the invention include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other

parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Alternatively, an antibody of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g., Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

Therapeutic compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. These patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

In certain embodiments, the human monoclonal antibodies of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (*see, e.g.*, V.V.

Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.*, U.S. Patent 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P.G. Bloeman *et al.* (1995) *FEBS Lett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe *et al.* (1995) *Am. J. Physiol.* 1233:134); p120 (Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090); see also K. Keinanen; M.L. Laukkanen (1994) *FEBS Lett.* 346:123; J.J. Killion; I.J. Fidler (1994) *Immunomethods* 4:273.

Uses and Methods of the Invention

The antibodies, antibody compositions and methods of the present invention have numerous *in vitro* and *in vivo* utilities involving, for example, detection of PD-1 or enhancement of immune response by blockade of PD-1. In a preferred embodiment, the antibodies of the present invention are human antibodies. For example, these molecules can be administered to cells in culture, *in vitro* or *ex vivo*, or to human subjects, *e.g.*, *in vivo*, to enhance immunity in a variety of situations. Accordingly, in one aspect, the invention provides a method of modifying an immune response in a subject comprising administering to the subject the antibody, or antigen-binding portion thereof, of the invention such that the immune response in the subject is modified. Preferably, the response is enhanced, stimulated or up-regulated.

As used herein, the term "subject" is intended to include human and non-human animals. Non-human animals includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dogs, cats, cows, horses, chickens, amphibians, and reptiles, although mammals are preferred, such as non-human primates, sheep, dogs, cats, cows and horses. Preferred subjects include human patients in need of enhancement of an immune response. The methods are particularly suitable for treating human patients having a disorder that can be treated by augmenting the T-cell mediated immune response. In a particular embodiment, the methods are particularly suitable for treatment of cancer cells *in vivo*. To achieve antigen-specific enhancement of immunity, the anti-PD-1 antibodies can be administered together with an antigen of interest. When antibodies to PD-1 are administered together with another agent, the two can be administered in either order or simultaneously.

The invention further provides methods for detecting the presence of human PD-1 antigen in a sample, or measuring the amount of human PD-1 antigen, comprising contacting the sample, and a control sample, with a human monoclonal antibody, or an antigen-binding portion thereof, which specifically binds to human PD-1, under conditions that allow for formation of a complex between the antibody or portion thereof and human PD-1. The formation of a complex is then detected, wherein a difference complex formation between the sample compared to the control sample is indicative the presence of human PD-1 antigen in the sample.

Given the specific binding of the antibodies of the invention for PD-1, compared to CD28, ICOS and CTLA-4, the antibodies of the invention can be used to specifically detect

PD-1 expression on the surface of cells and, moreover, can be used to purify PD-1 via immunoaffinity purification.

Cancer

Blockade of PD-1 by antibodies can enhance the immune response to cancerous cells in the patient. The ligand for PD-1, PD-L1, is not expressed in normal human cells, but is abundant in a variety of human cancers (Dong *et al.* (2002) *Nat Med* 8:787-9). The interaction between PD-1 and PD-L1 results in a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation, and immune evasion by the cancerous cells (Dong *et al.* (2003) *J Mol Med* 81:281-7; Blank *et al.* (2005) *Cancer Immunol. Immunother.* 54:307-314; Konishi *et al.* (2004) *Clin. Cancer Res.* 10:5094-100). Immune suppression can be reversed by inhibiting the local interaction of PD-1 to PD-L1 and the effect is additive when the interaction of PD-1 to PD-L2 is blocked as well (Iwai *et al.* (2002) *PNAS* 99:12293-7; Brown *et al.* (2003) *J. Immunol.* 170:1257-66). While previous studies have shown that T-cell proliferation can be restored by inhibiting the interaction of PD-1 to PD-L1, there have been no reports of a direct effect on cancer tumor growth *in vivo* by blocking the PD-1/PD-L1 interaction. In one aspect, the present invention relates to treatment of a subject *in vivo* using an anti-PD-1 antibody such that growth of cancerous tumors is inhibited. An anti-PD-1 antibody may be used alone to inhibit the growth of cancerous tumors. Alternatively, an anti-PD-1 antibody may be used in conjunction with other immunogenic agents, standard cancer treatments, or other antibodies, as described below.

Accordingly, in one embodiment, the invention provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof. Preferably, the antibody is a human anti-PD-1 antibody (such as any of the human anti-human PD-1 antibodies described herein). Additionally or alternatively, the antibody may be a chimeric or humanized anti-PD-1 antibody.

Preferred cancers whose growth may be inhibited using the antibodies of the invention include cancers typically responsive to immunotherapy. Non-limiting examples of preferred cancers for treatment include melanoma (*e.g.*, metastatic malignant melanoma), renal cancer (*e.g.* clear cell carcinoma), prostate cancer (*e.g.* hormone refractory prostate adenocarcinoma), breast cancer, colon cancer and lung cancer (*e.g.* non-small cell lung cancer). Additionally, the invention includes refractory or recurrent malignancies whose growth may be inhibited using the antibodies of the invention.

Examples of other cancers that may be treated using the methods of the invention include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of

the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. The present invention is also useful for treatment of metastatic cancers, especially metastatic cancers that express PD-L1 (Iwai *et al.* (2005) *Int. Immunol.* 17:133-144).

Optionally, antibodies to PD-1 can be combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He et al (2004) *J. Immunol.* 173:4919-28). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MART1 and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF (discussed further below).

In humans, some tumors have been shown to be immunogenic such as melanomas. It is anticipated that by raising the threshold of T cell activation by PD-1 blockade, we may expect to activate tumor responses in the host.

PD-1 blockade is likely to be most effective when combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (see Rosenberg, S., 2000, Development of Cancer Vaccines, ASCO Educational Book Spring: 60-62; Logothetis, C., 2000, ASCO Educational Book Spring: 300-302; Khayat, D. 2000, ASCO Educational Book Spring: 414-428; Foon, K. 2000, ASCO Educational Book Spring: 730-738; see also Restifo, N. and Sznol, M., Cancer Vaccines, Ch. 61, pp. 3023-3043 in DeVita, V. *et al.* (eds.), 1997, Cancer: Principles and Practice of Oncology. Fifth Edition). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff *et al.* (1993) *Proc. Natl. Acad. Sci U.S.A.* 90: 3539-43).

The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (Rosenberg, SA (1999) Immunity 10: 281-7). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp100, MAGE antigens, and Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host. PD-1 blockade may be used in conjunction with a collection of recombinant proteins and/or peptides

expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally viewed by the immune system as self antigens and are therefore tolerant to them. The tumor antigen may also include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim, N *et al.* (1994) *Science* 266: 2011-2013). (These somatic tissues may be protected from immune attack by various means). Tumor antigen may also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences (ie. bcr-abl in the Philadelphia chromosome), or idiotype from B cell tumors.

Other tumor vaccines may include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific antigen which may be used in conjunction with PD-1 blockade is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot, R & Srivastava, P (1995) *Science* 269:1585-1588; Tamura, Y. *et al.* (1997) *Science* 278:117-120).

Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced *ex vivo* and loaded with various protein and peptide antigens as well as tumor cell extracts (Nestle, F. *et al.* (1998) *Nature Medicine* 4: 328-332). DCs may also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler, A. *et al.* (2000) *Nature Medicine* 6:332-336). As a method of vaccination, DC immunization may be effectively combined with PD-1 blockade to activate more potent anti-tumor responses.

PD-1 blockade may also be combined with standard cancer treatments. PD-1 blockade may be effectively combined with chemotherapeutic regimes. In these instances, it may be possible to reduce the dose of chemotherapeutic reagent administered (Mokyr, M. *et al.* (1998) *Cancer Research* 58: 5301-5304). An example of such a combination is an anti-PD-1 antibody in combination with decarbazine for the treatment of melanoma. Another example of such a combination is an anti-PD-1 antibody in combination with interleukin-2 (IL-2) for the treatment of melanoma. The scientific rationale behind the combined use of PD-1 blockade and chemotherapy is that cell death, that is a consequence of the cytotoxic action of most chemotherapeutic compounds, should result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with PD-1 blockade through cell death are radiation, surgery, and hormone deprivation. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors may also be combined with PD-1 blockade. Inhibition of angiogenesis leads to tumor cell death which may feed tumor antigen into host antigen presentation pathways.

PD-1 blocking antibodies can also be used in combination with bispecific antibodies that target Fc alpha or Fc gamma receptor-expressing effectors cells to tumor cells (see, *e.g.*, U.S. Pat. Nos. 5,922,845 and 5,837,243). Bispecific antibodies can be used to target two separate antigens. For example anti-Fc receptor/anti tumor antigen (*e.g.*, Her-2/neu) bispecific antibodies have been used to target macrophages to sites of tumor. This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would by augmented by the use of PD-1 blockade. Alternatively, antigen may be delivered directly to DCs by the use of bispecific antibodies which bind to tumor antigen and a dendritic cell specific cell surface marker.

Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of proteins which are expressed by the tumors and which are immunosuppressive. These include among others TGF-beta (Kehrl, J. *et al.* (1986) *J. Exp. Med.* 163: 1037-1050), IL-10 (Howard, M. & O'Garra, A. (1992) *Immunology Today* 13: 198-200), and Fas ligand (Hahne, M. *et al.* (1996) *Science* 274: 1363-1365). Antibodies to each of these entities may be used in combination with anti-PD-1 to counteract the effects of the immunosuppressive agent and favor tumor immune responses by the host.

Other antibodies which may be used to activate host immune responsiveness can be used in combination with anti-PD-1. These include molecules on the surface of dendritic cells which activate DC function and antigen presentation. Anti-CD40 antibodies are able to substitute effectively for T cell helper activity (Ridge, J. *et al.* (1998) Nature 393: 474-478) and can be used in conjuction with PD-1 antibodies (Ito, N. *et al.* (2000) *Immunobiology* 201 (5) 527-40). Activating antibodies to T cell costimulatory molecules such as CTLA-4 (e.g., US Patent No. 5,811,097), OX-40 (Weinberg, A. *et al.* (2000) *Immunol* 164: 2160-2169), 4-1BB (Melero, I. *et al.* (1997) *Nature Medicine* 3: 682-685 (1997), and ICOS (Hutloff, A. *et al.* (1999) *Nature* 397: 262-266) may also provide for increased levels of T cell activation.

Bone marrow transplantation is currently being used to treat a variety of tumors of hematopoietic origin. While graft versus host disease is a consequence of this treatment, therapeutic benefit may be obtained from graft vs. tumor responses. PD-1 blockade can be used to increase the effectiveness of the donor engrafted tumor specific T cells.

There are also several experimental treatment protocols that involve *ex vivo* activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to antigen-specific T cells against tumor (Greenberg, R. & Riddell, S. (1999) *Science* 285: 546-51). These methods may also be used to activate T cell responses to infectious agents such as CMV. *Ex vivo* activation in the presence of anti-PD-1 antibodies may be expected to increase the frequency and activity of the adoptively transferred T cells.

Infectious Diseases

Other methods of the invention are used to treat patients that have been exposed to particular toxins or pathogens. Accordingly, another aspect of the invention provides a

method of treating an infectious disease in a subject comprising administering to the subject an anti-PD-1 antibody, or antigen-binding portion thereof, such that the subject is treated for the infectious disease. Preferably, the antibody is a human anti-human PD-1 antibody (such as any of the human anti-PD-1 antibodies described herein). Additionally or alternatively, the antibody can be a chimeric or humanized antibody.

Similar to its application to tumors as discussed above, antibody mediated PD-1 blockade can be used alone, or as an adjuvant, in combination with vaccines, to stimulate the immune response to pathogens, toxins, and self-antigens. Examples of pathogens for which this therapeutic approach may be particularly useful, include pathogens for which there is currently no effective vaccine, or pathogens for which conventional vaccines are less than completely effective. These include, but are not limited to HIV, Hepatitis (A, B, & C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus, Pseudomonas Aeruginosa. PD-1 blockade is particularly useful against established infections by agents such as HIV that present altered antigens over the course of the infections. These novel epitopes are recognized as foreign at the time of anti-human PD-1 administration, thus provoking a strong T cell response that is not dampened by negative signals through PD-1.

Some examples of pathogenic viruses causing infections treatable by methods of the invention include HIV, hepatitis (A, B, or C), herpes virus (*e.g.*, VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

Some examples of pathogenic bacteria causing infections treatable by methods of the invention include chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lymes disease bacteria.

Some examples of pathogenic fungi causing infections treatable by methods of the invention include Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum.

Some examples of pathogenic parasites causing infections treatable by methods of the invention include Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, and Nippostrongylus brasiliensis.

In all of the above methods, PD-1 blockade can be combined with other forms of immunotherapy such as cytokine treatment (*e.g.*, interferons, GM-CSF, G-CSF, IL-2), or bispecific antibody therapy, which provides for enhanced presentation of tumor antigens (see, *e.g.*, Holliger (1993) *Proc. Natl. Acad. Sci.* USA 90:6444-6448; Poljak (1994) *Structure* 2:1121-1123).

Autoimmune reactions

Anti-PD-1 antibodies may provoke and amplify autoimmune responses. Indeed, induction of anti-tumor responses using tumor cell and peptide vaccines reveals that many anti-tumor responses involve anti-self reactivities (depigmentation observed in anti-CTLA-4 + GM-CSF-modified B16 melanoma in van Elsas *et al.* supra; depigmentation in Trp-2 vaccinated mice (Overwijk, W. *et al.* (1999) *Proc. Natl. Acad. Sci. U.S.A.* 96: 2982-2987); autoimmune prostatitis evoked by TRAMP tumor cell vaccines (Hurwitz, A. (2000) *supra*), melanoma peptide antigen vaccination and vitilago observed in human clinical trials (Rosenberg, SA and White, DE (1996) *J. Immunother Emphasis Tumor Immunol* 19 (1): 81-4).

Therefore, it is possible to consider using anti-PD-1 blockade in conjunction with various self proteins in order to devise vaccination protocols to efficiently generate immune responses against these self proteins for disease treatment. For example, Alzheimers disease involves inappropriate accumulation of Aβ peptide in amyloid deposits in the brain; antibody responses against amyloid are able to clear these amyloid deposits (Schenk *et al.*, (1999) *Nature* 400: 173-177).

Other self proteins may also be used as targets such as IgE for the treatment of allergy and asthma, and TNFα for rhematoid arthritis. Finally, antibody responses to various hormones may be induced by the use of anti-PD-1 antibody. Neutralizing antibody responses to reproductive hormones may be used for contraception. Neutralizing antibody response to hormones and other soluble factors that are required for the growth of particular tumors may also be considered as possible vaccination targets.

Analogous methods as described above for the use of anti-PD-1 antibody can be used for induction of therapeutic autoimmune responses to treat patients having an inappropriate accumulation of other self-antigens, such as amyloid deposits, including Aβ in Alzheimer's disease, cytokines such as TNFα, and IgE.

Vaccines

Anti-PD-1 antibodies may be used to stimulate antigen-specific immune responses by coadministration of an anti-PD-1 antibody with an antigen of interest (*e.g.*, a vaccine). Accordingly, in another aspect the invention provides a method of enhancing an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) an anti-PD-1 antibody, or antigen-binding portion thereof, such that an immune response to the antigen in the subject is enhanced. Preferably, the antibody is a human anti-human PD-1 antibody (such as any of the human anti-PD-1 antibodies described herein). Additionally or alternatively, the antibody can be a chimeric or humanized antibody. The

antigen can be, for example, a tumor antigen, a viral antigen, a bacterial antigen or an antigen from a pathogen. Non-limiting examples of such antigens include those discussed in the sections above, such as the tumor antigens (or tumor vaccines) discussed above, or antigens from the viruses, bacteria or other pathogens described above.

Suitable routes of administering the antibody compositions (*e.g.*, human monoclonal antibodies, multispecific and bispecific molecules and immunoconjugates) of the invention *in vivo* and *in vitro* are well known in the art and can be selected by those of ordinary skill. For example, the antibody compositions can be administered by injection (*e.g.*, intravenous or subcutaneous). Suitable dosages of the molecules used will depend on the age and weight of the subject and the concentration and/or formulation of the antibody composition.

As previously described, human anti-PD-1 antibodies of the invention can be co-administered with one or other more therapeutic agents, *e.g.*, a cytotoxic agent, a radiotoxic agent or an immunosuppressive agent. The antibody can be linked to the agent (as an immunocomplex) or can be administered separate from the agent. In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent or can be co-administered with other known therapies, *e.g.*, an anti-cancer therapy, *e.g.*, radiation. Such therapeutic agents include, among others, anti-neoplastic agents such as doxorubicin (adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, decarbazine and cyclophosphamide hydroxyurea which, by themselves, are only effective at levels which are toxic or subtoxic to a patient. Cisplatin is intravenously administered as a 100 mg/dose once every four weeks and adriamycin is intravenously administered as a 60-75 mg/ml dose once every 21 days. Co-administration of the human anti-PD-1 antibodies, or antigen binding fragments thereof, of the present invention with chemotherapeutic agents provides two anti-cancer agents which operate via different mechanisms which yield a cytotoxic effect to human tumor cells. Such co-administration can solve problems due to development of resistance to drugs or a change in the antigenicity of the tumor cells which would render them unreactive with the antibody.

Also within the scope of the present invention are kits comprising the antibody compositions of the invention (*e.g.*, human antibodies, bispecific or multispecific molecules, or immunoconjugates) and instructions for use. The kit can further contain a least one additional reagent, or one or more additional human antibodies of the invention (e.g., a human antibody having a complementary activity which binds to an epitope in PD-1 antigen distinct from the first human antibody). Kits typically include a label indicating the intended use of the contents of the kit. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

Combination therapy

The present invention is based, in part, on the following experimental data. Mouse tumor models (MC38 colon cancer and SA1/N fibrosarcoma) were used to examine the *in vivo* effect of treating a tumor by combining immunostimulatory therapeutic antibodies -

anti-CTLA-4 and anti-PD-1. The immunotherapeutic combination was provided either simultaneous with the implant of tumor cells (Examples 14 and 17) or after the tumor cells were implanted for a time sufficient to become an established tumor (Examples 15, 16 and 18). Regardless of the timing of antibody treatment, it was found that anti-CTLA-4 antibody treatment alone and anti-PD-1 antibody (chimeric antibody in which a rat anti-mouse PD-1 was modified with a mouse immunoglobulin Fc region, *see* Example 1) treatment alone had a modest effect on reducing tumor growth in the MC38 tumor model (*see, e.g.*, Figures 21, 24 and 27). The anti-CTLA-4 antibody alone was quite effective in the SA1/N tumor model (*see* Figure 30D), which required a lower anti-CTLA-4 antibody dose for the combination studies in this model. Nonetheless, the combination treatment of anti-CTLA-4 antibody and anti-PD-1 antibody showed an unexpected, significantly greater effect on reducing tumor growth as compared to treatment with either antibody alone (*see, e.g.*, Figures 21D, 24D, 30F and 33H-J). In addition, the results of Examples 14, 16 and 18 show that the combination treatment of anti-CTLA-4 antibody and anti-PD-1 antibody had a significant (synergistic) effect on tumor growth even at sub-optimal therapeutic doses as compared to treatment with either antibody alone (*i.e.*, the combination therapy was surprisingly more effective at subtherapeutic doses than either monotherapy). Without wishing to be bound by theory, it is possible that by raising the threshold of T cell activation by PD-1 and CTLA-4 blockade, anti-tumor responses may be activated in a host.

In one embodiment, the present invention provides a method for treating a hyperproliferative disease, comprising administering a PD-1 antibody and a CTLA-4 antibody to a subject. In further embodiments, the anti-PD-1 antibody is administered at a subtherapeutic dose, the anti-CTLA-4 antibody is administered at a subtherapeutic dose, or both are administered at a subtherapeutic dose. In another embodiment, the present invention provides a method for altering an adverse event associated with treatment of a hyperproliferative disease with an immunostimulatory agent, comprising administering an anti-PD-1 antibody and a subtherapeutic dose of anti-CTLA-4 antibody to a subject. In certain embodiments, the subject is human. In certain embodiments, the anti-CTLA-4 antibody is human sequence monoclonal antibody 10D1 and the anti-PD-1 antibody is human sequence monoclonal antibody, such as 17D8, 2D3, 4H1, 5C4 and 4A11. Human sequence monoclonal antibodies 17D8, 2D3, 4H1, 5C4 and 4A11 have been isolated and structurally characterized, as described in U.S. Provisional Patent No. 60/679,466.

The anti-CTLA-4 antibody and anti-PD-1 monoclonal antibodies (mAbs) and the human sequence antibodies of the invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology, *e.g.*, the standard somatic cell hybridization technique of Kohler and Milstein (1975) *Nature* 256:495. Any technique for producing monoclonal antibody can be employed, *e.g.*, viral or oncogenic transformation of B lymphocytes. One animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure. Immunization

protocols and techniques for isolation of immunized splenocytes for fusion are known in the art. Fusion partners (*e.g.*, murine myeloma cells) and fusion procedures are also known (*see, e.g.*, Harlow and Lane (1988) *Antibodies, A Laboratory Manual,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor New York).

Anti-CTLA-4 antibodies of the instant invention can bind to an epitope on human CTLA-4 so as to inhibit CTLA-4 from interacting with a human B7 counterreceptor. Because interaction of human CTLA-4 with human B7 transduces a signal leading to inactivation of T-cells bearing the human CTLA-4 receptor, antagonism of the interaction effectively induces, augments or prolongs the activation of T cells bearing the human CTLA-4 receptor, thereby prolonging or augmenting an immune response. Anti-CTLA-4 antibodies are described in U.S. Patent Nos. 5,811,097; 5,855,887; 6,051,227; in PCT Application Publication Nos. WO 01/14424 and WO 00/37504; and in U.S. Patent Publication No. 2002/0039581. Each of these references is specifically incorporated herein by reference for purposes of description of anti-CTLA-4 antibodies. An exemplary clinical anti-CTLA-4 antibody is human monoclonal antibody 10D1 as disclosed in WO 01/14424 and U.S. Patent Application No. 09/644,668. Antibody 10D1 has been administered in single and multiple doses, alone or in combination with a vaccine, chemotherapy, or interleukin-2 to more than 500 patients diagnosed with metastatic melanoma, prostate cancer, lymphoma, renal cell cancer, breast cancer, ovarian cancer, and HIV. Other anti-CTLA-4 antibodies encompassed by the methods of the present invention include, for example, those disclosed in: WO 98/42752; WO 00/37504; U.S. Patent No. 6,207,156; Hurwitz *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 95(17):10067-10071; Camacho *et al.* (2004) *J. Clin. Oncology* 22(145): Abstract No. 2505 (antibody CP-675206); and Mokyr *et al.* (1998) *Cancer Res.* 58:5301-5304. In certain embodiments, the methods of the instant invention comprise use of an anti-CTLA-4 antibody that is a human sequence antibody, preferably a monoclonal antibody and in another embodiment is monoclonal antibody 10D1.

In certain embodiments, the anti-CTLA-4 antibody binds to human CTLA-4 with a K_D of 5×10^{-8} M or less, binds to human CTLA-4 with a K_D of 1×10^{-8} M or less, binds to human CTLA-4 with a K_D of 5×10^{-9} M or less, or binds to human CTLA-4 with a K_D of between 1×10^{-8} M and 1×10^{-10} M or less.

The combination of antibodies is useful for enhancement of an immune response against a hyperproliferative disease by blockade of PD-1 and CTLA-4. In a preferred embodiment, the antibodies of the present invention are human antibodies. For example, these molecules can be administered to cells in culture, *in vitro* or *ex vivo*, or to human subjects, *e.g.*, *in vivo*, to enhance immunity in a variety of situations. Accordingly, in one aspect, the invention provides a method of modifying an immune response in a subject comprising administering to the subject an antibody combination, or a combination of antigen-binding portions thereof, of the invention such that the immune response in the subject is modified. Preferably, the response is enhanced, stimulated or up-regulated. In another

embodiment, the instant disclosure provides a method of altering adverse events associated with treatment of a hyperproliferative disease with an immunostimulatory therapeutic agent, comprising administering an anti-PD-1 antibody and a subtherapeutic dose of anti-CTLA-4 antibody to a subject.

Blockade of PD-1 and CTLA-4 by antibodies can enhance the immune response to cancerous cells in the patient. Cancers whose growth may be inhibited using the antibodies of the instant disclosure include cancers typically responsive to immunotherapy. Representative examples of cancers for treatment with the combination therapy of the instant disclosure include melanoma (*e.g.*, metastatic malignant melanoma), renal cancer, prostate cancer, breast cancer, colon cancer and lung cancer. Examples of other cancers that may be treated using the methods of the instant disclosure include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. The present invention is also useful for treatment of metastatic cancers.

In certain embodiments, the combination of therapeutic antibodies discussed herein may be administered concurrently as a single composition in a pharmaceutically acceptable carrier, or concurrently as separate compositions with each antibody in a pharmaceutically acceptable carrier. In another embodiment, the combination of therapeutic antibodies can be administered sequentially. For example, an anti-CTLA-4 antibody and an anti-PD-1 antibody can be administered sequentially, such as anti-CTLA-4 being administered first and anti-PD-1 second, or anti-PD-1 being administered first and anti-CTLA-4 second. Furthermore, if more than one dose of the combination therapy is administered sequentially, the order of the sequential administration can be reversed or kept in the same order at each time point of administration, sequential administrations may be combined with concurrent administrations, or any combination thereof. For example, the first administration of a combination anti-CTLA-4 antibody and anti-PD-1 antibody may be concurrent, the second administration may be sequential with anti-CTLA-4 first and anti-PD-1 second, and the third

administration may be sequential with anti-PD-1 first and anti-CTLA-4 second, etc. Another representative dosing scheme may involve a first administration that is sequential with anti-PD-1 first and anti-CTLA-4 second, and subsequent administrations may be concurrent.

Optionally, the combination of anti-PD-1 and anti-CTLA-4 antibodies can be further combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He *et al.* (2004) *J. Immunol.* 173:4919-28). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MART1 and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF (discussed further below).

A combined PD-1 and CTLA-4 blockade can be further combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (*see* Rosenberg, S. (2000) Development of Cancer Vaccines, ASCO Educational Book Spring: 60-62; Logothetis, C., 2000, ASCO Educational Book Spring: 300-302; Khayat, D. (2000) ASCO Educational Book Spring: 414-428; Foon, K. (2000) ASCO Educational Book Spring: 730-738; see also Restifo and Sznol, Cancer Vaccines, Ch. 61, pp. 3023-3043 in DeVita *et al.* (eds.), 1997, Cancer: Principles and Practice of Oncology. Fifth Edition). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff *et al.* (1993) *Proc. Natl. Acad. Sci U.S.A.* 90: 3539-43).

The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (Rosenberg (1999) Immunity 10:281-7). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp100, MAGE antigens, and Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host. In certain embodiments, a combined PD-1 and CTLA-4 blockade using the antibody compositions described herein may be used in conjunction with a collection of recombinant proteins and/or peptides expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally viewed by the immune system as self-antigens and are, therefore, tolerant to them. The tumor antigen may also include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim *et al.* (1994) *Science* 266: 2011-2013). (These somatic tissues may be protected from immune attack by various means). Tumor antigen may also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two

unrelated sequences (*i.e.*, bcr-abl in the Philadelphia chromosome), or idiotype from B cell tumors.

Other tumor vaccines may include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific antigen which may be used in conjunction with PD-1 blockade is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot & Srivastava (1995) *Science* 269:1585-1588; Tamura *et al.* (1997) *Science* 278:117-120).

Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced *ex vivo* and loaded with various protein and peptide antigens as well as tumor cell extracts (Nestle *et al.* (1998) *Nature Medicine* 4: 328-332). DCs may also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler *et al.* (2000) *Nature Medicine* 6:332-336). As a method of vaccination, DC immunization may be effectively further combined with a combined PD-1 and CTLA-4 blockade to activate more potent anti-tumor responses.

A combined PD-1 and CTLA-4 blockade may also be further combined with standard cancer treatments. For example, a combined PD-1 and CTLA-4 blockade may be effectively combined with chemotherapeutic regimes. In these instances, as is observed with the combination of anti-PD-1 and anti-CTLA-4 antibodies, it may be possible to reduce the dose of other chemotherapeutic reagent administered with the combination of the instant disclosure (Mokyr *et al.* (1998) *Cancer Research* 58: 5301-5304). An example of such a combination is a combination of anti-PD-1 and anti-CTLA-4 antibodies further in combination with decarbazine for the treatment of melanoma. Another example is a combination of anti-PD-1 and anti-CTLA-4 antibodies further in combination with interleukin-2 (IL-2) for the treatment of melanoma. The scientific rationale behind the combined use of PD-1 and CTLA-4 blockade with chemotherapy is that cell death, which is a consequence of the cytotoxic action of most chemotherapeutic compounds, should result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with a combined PD-1 and CTLA-4 blockade through cell death include radiation, surgery, or hormone deprivation. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors may also be combined with a combined PD-1 and CTLA-4 blockade. Inhibition of angiogenesis leads to tumor cell death, which may also be a source of tumor antigen to be fed into host antigen presentation pathways.

A combination of PD-1 and CTLA-4 blocking antibodies can also be used in combination with bispecific antibodies that target Fcα or Fcγ receptor-expressing effector cells to tumor cells (see, *e.g.*, U.S. Pat. Nos. 5,922,845 and 5,837,243). Bispecific antibodies can

be used to target two separate antigens. For example anti-Fc receptor/anti tumor antigen (*e.g.*, Her-2/neu) bispecific antibodies have been used to target macrophages to sites of tumor. This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would by augmented by the use of a combined PD-1 and CTLA-4 blockade. Alternatively, antigen may be delivered directly to DCs by the use of bispecific antibodies which bind to tumor antigen and a dendritic cell specific cell surface marker.

In another example, a combination of anti-PD-1 and anti-CTLA-4 antibodies can be used in conjunction with anti–neoplastic antibodies, such as Rituxan® (rituximab), Herceptin® (trastuzumab), Bexxar® (tositumomab), Zevalin® (ibritumomab), Campath® (alemtuzumab), Lymphocide® (eprtuzumab), Avastin® (bevacizumab), and Tarceva® (erlotinib), and the like. By way of example and not wishing to be bound by theory, treatment with an anti–cancer antibody or an anti–cancer antibody conjugated to a toxin can lead to cancer cell death (*e.g.*, tumor cells) which would potentiate an immune response mediated by CTLA-4 or PD-1. In an exemplary embodiment, a treatment of a hyperproliferative disease (*e.g.*, a cancer tumor) may include an anti-cancer antibody in combination with anti-PD-1 and anti-CTLA-4 antibodies, concurrently or sequentially or any combination thereof, which may potentiate an anti–tumor immune responses by the host.

Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of proteins, which are expressed by the tumors and which are immunosuppressive. These include, among others, TGF-β (Kehrl, J. *et al.* (1986) *J. Exp. Med.* 163: 1037-1050), IL-10 (Howard, M. & O'Garra, A. (1992) *Immunology Today* 13: 198-200), and Fas ligand (Hahne, M. *et al.* (1996) *Science* 274: 1363-1365). In another example, antibodies to each of these entities may be further combined with an anti-PD-1 and anti-CTLA-4 combination to counteract the effects of immunosuppressive agents and favor anti-tumor immune responses by the host.

Other antibodies that may be used to activate host immune responsiveness can be further used in combination with an anti-PD-1 and anti-CTLA-4 combination. These include molecules on the surface of dendritic cells that activate DC function and antigen presentation. Anti-CD40 antibodies are able to substitute effectively for T cell helper activity (Ridge, J. *et al.* (1998) Nature 393: 474-478) and can be used in conjunction with an anti-PD-1 and anti-CTLA-4 combination (Ito, N. *et al.* (2000) *Immunobiology* 201 (5) 527-40). Activating antibodies to T cell costimulatory molecules, such as OX-40 (Weinberg, A. *et al.* (2000) *Immunol* 164: 2160-2169), 4-1BB (Melero, I. *et al.* (1997) *Nature Medicine* 3: 682-685 (1997), and ICOS (Hutloff, A. *et al.* (1999) *Nature* 397: 262-266) may also provide for increased levels of T cell activation.

Bone marrow transplantation is currently being used to treat a variety of tumors of hematopoietic origin. While graft versus host disease is a consequence of this treatment, therapeutic benefit may be obtained from graft vs. tumor responses. A combined PD-1 and

CTLA-4 blockade can be used to increase the effectiveness of the donor engrafted tumor specific T cells.

There are also several experimental treatment protocols that involve *ex vivo* activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to antigen-specific T cells against tumor (Greenberg, R. & Riddell, S. (1999) *Science* 285: 546-51). These methods may also be used to activate T cell responses to infectious agents such as CMV. *Ex vivo* activation in the presence of anti-PD-1 and anti-CTLA-4 antibodies may be expected to increase the frequency and activity of the adoptively transferred T cells.

As set forth herein, organs can exhibit immune-related adverse events following immunostimulatory therapeutic antibody therapy, such as the GI tract (diarrhea and colitis) and the skin (rash and pruritis) after treatment with anti-CTLA-4 antibody. For example, non-colonic gastrointestinal immune-related adverse events have also been observed in the esophagus (esophagitis), duodenum (duodenitis), and ileum (ileitis) after anti-CTLA-4 antibody treatment.

In certain embodiments, the present invention provides a method for altering an adverse event associated with treatment of a hyperproliferative disease with an immunostimulatory agent, comprising administering a anti-PD-1 antibody and a subtherapeutic dose of anti-CTLA-4 antibody to a subject. For example, the methods of the present invention provide for a method of reducing the incidence of immunostimulatory therapeutic antibody-induced colitis or diarrhea by administering a non-absorbable steroid to the patient. Because any patient who will receive an immunostimulatory therapeutic antibody is at risk for developing colitis or diarrhea induced by such an antibody, this entire patient population is suitable for therapy according to the methods of the present invention. Although steroids have been administered to treat inflammatory bowel disease (IBD) and prevent exacerbations of IBD, they have not been used to prevent (decrease the incidence of) IBD in patients who have not been diagnosed with IBD. The significant side effects associated with steroids, even non-absorbable steroids, have discouraged prophylactic use.

In further embodiments, a combination PD-1 and CTLA-4 blockade (*i.e.*, immunostimulatory therapeutic antibodies anti-PD-1 and anti-CTLA-4) can be further combined with the use of any non-absorbable steroid. As used herein, a "non-absorbable steroid" is a glucocorticoid that exhibits extensive first pass metabolism such that, following metabolism in the liver, the bioavailability of the steroid is low, *i.e.*, less than about 20%. In one embodiment of the invention, the non-absorbable steroid is budesonide. Budesonide is a locally-acting glucocorticosteroid, which is extensively metabolized, primarily by the liver, following oral administration. ENTOCORT EC® (Astra-Zeneca) is a pH- and time-dependent oral formulation of budesonide developed to optimize drug delivery to the ileum and throughout the colon. ENTOCORT EC® is approved in the U.S. for the treatment of mild to moderate Crohn's disease involving the ileum and/or ascending colon. The usual oral

dosage of ENTOCORT EC® for the treatment of Crohn's disease is 6 to 9 mg/day. ENTOCORT EC® is released in the intestines before being absorbed and retained in the gut mucosa. Once it passes through the gut mucosa target tissue, ENTOCORT EC® is extensively metabolized by the cytochrome P450 system in the liver to metabolites with negligible glucocorticoid activity. Therefore, the bioavailability is low (about 10%). The low bioavailability of budesonide results in an improved therapeutic ratio compared to other glucocorticoids with less extensive first-pass metabolism. Budesonide results in fewer adverse effects, including less hypothalamic-pituitary suppression, than systemically-acting corticosteroids. However, chronic administration of ENTOCORT EC® can result in systemic glucocorticoid effects such as hypercorticism and adrenal suppression. *See* PDR 58th ed. 2004; 608-610.

In still further embodiments, a combination PD-1 and CTLA-4 blockade (*i.e.*, immunostimulatory therapeutic antibodies anti-PD-1 and anti-CTLA-4) in conjunction with a non-absorbable steroid can be further combined with a salicylate. Salicylates include 5-ASA agents such as, for example: sulfasalazine (AZULFIDINE®, Pharmacia & UpJohn); olsalazine (DIPENTUM®, Pharmacia & UpJohn); balsalazide (COLAZAL®, Salix Pharmaceuticals, Inc.); and mesalamine (ASACOL®, Procter & Gamble Pharmaceuticals; PENTASA®, Shire US; CANASA®, Axcan Scandipharm, Inc.; ROWASA®, Solvay).

In accordance with the methods of the present invention, a salicylate administered in combination with anti-PD-1 and anti-CTLA-4 antibodies and a non-absorbable steroid can includes any overlapping or sequential administration of the salicylate and the non-absorbable steroid for the purpose of decreasing the incidence of colitis induced by the immunostimulatory antibodies. Thus, for example, methods for reducing the incidence of colitis induced by the immunostimulatory antibodies according to the present invention encompass administering a salicylate and a non-absorbable concurrently or sequentially (*e.g.*, a salicylate is administered 6 hours after a non-absorbable steroid), or any combination thereof. Further, according to the present invention, a salicylate and a non-absorbable steroid can be administered by the same route (*e.g.*, both are administered orally) or by different routes (e.g., a salicylate is administered orally and a non-absorbable steroid is administered rectally), which may differ from the route(s) used to administer the anti-PD-1 and anti-CTLA-4 antibodies.

The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

Examples

Example 1: Generation of Human Monoclonal Antibodies Against PD-1

Antigen

Immunization protocols utilized as antigen both (i) a recombinant fusion protein comprising the extracellular portion of PD-1 and (ii) membrane bound full-length PD-1. Both antigens were generated by recombinant transfection methods in a CHO cell line.

Transgenic HuMab and KM mice™

Fully human monoclonal antibodies to PD-1 were prepared using the HCo7 strain of HuMab transgenic mice and the KM strain of transgenic transchromosomic mice, each of which express human antibody genes. In each of these mouse strains, the endogenous mouse kappa light chain gene has been homozygously disrupted as described in Chen *et al.* (1993) *EMBO J.* 12:811-820 and the endogenous mouse heavy chain gene has been homozygously disrupted as described in Example 1 of PCT Publication WO 01/09187. Each of these mouse strains carries a human kappa light chain transgene, KCo5, as described in Fishwild *et al.* (1996) *Nature Biotechnology* 14:845-851. The HCo7 strain carries the HCo7 human heavy chain transgene as described in U.S. Patent Nos. 5,545,806; 5,625,825; and 5,545,807. The KM strain contains the SC20 transchromosome as described in PCT Publication WO 02/43478.

HuMab and KM Immunizations:

To generate fully human monoclonal antibodies to PD-1, HuMab mice and KM mice™ were immunized with purified recombinant PD-1 fusion protein and PD-1-transfected CHO cells as antigen. General immunization schemes for HuMab mice are described in Lonberg, N. *et al* (1994*) Nature* 368(6474): 856-859; Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851 and PCT Publication WO 98/24884. The mice were 6-16 weeks of age upon the first infusion of antigen. A purified recombinant preparation (5-50 μg) of PD-1 fusion protein antigen and 5 -10x10^6 cells were used to immunize the HuMab mice and KM mice™ intraperitonealy, subcutaneously (Sc) or via footpad injection.

Transgenic mice were immunized twice with antigen in complete Freund's adjuvant or Ribi adjuvant IP, followed by 3-21 days IP (up to a total of 11 immunizations) with the antigen in incomplete Freund's or Ribi adjuvant. The immune response was monitored by retroorbital bleeds. The plasma was screened by ELISA (as described below), and mice with sufficient titers of anti-PD-1 human immunogolobulin were used for fusions. Mice were boosted intravenously with antigen 3 days before sacrifice and removal of the spleen. Typically, 10-35 fusions for each antigen were performed. Several dozen mice were immunized for each antigen.

Selection of HuMab or KM Mice™ Producing Anti-PD-1 Antibodies:

To select HuMab or KM mice™ producing antibodies that bound PD-1, sera from immunized mice were tested by ELISA as described by Fishwild, D. *et al.* (1996). Briefly, microtiter plates were coated with purified recombinant PD-1 fusion protein from transfected CHO cells at 1-2 μg /ml in PBS, 100 μl/wells incubated 4 °C overnight then blocked with 200

μl/well of 5% fetal bovine serum in PBS/Tween (0.05%). Dilutions of sera from PD-1-immunized mice were added to each well and incubated for 1-2 hours at ambient temperature. The plates were washed with PBS/Tween and then incubated with a goat-anti-human IgG polyclonal antibody conjugated with horseradish peroxidase (HRP) for 1 hour at room temperature. After washing, the plates were developed with ABTS substrate (Sigma, A-1888, 0.22 mg/ml) and analyzed by spectrophotometer at OD 415-495. Mice that developed the highest titers of anti-PD-1 antibodies were used for fusions. Fusions were performed as described below and hybridoma supernatants were tested for anti-PD-1 activity by ELISA.

Generation of Hybridomas Producing Human Monoclonal Antibodies to PD-1:

The mouse splenocytes, isolated from the HuMab or KM mice, were fused to a mouse myeloma cell line either using PEG based upon standard protocols or electric field based electrofusion using a Cyto Pulse large chamber cell fusion electroporator (Cyto Pulse Sciences, Inc., Glen Burnie, MD). The resulting hybridomas were then screened for the production of antigen-specific antibodies. Single cell suspensions of splenocytes from immunized mice were fused to one-fourth the number of SP2/0 nonsecreting mouse myeloma cells (ATCC, CRL 1581) with 50% PEG (Sigma). Cells were plated at approximately 1x10 ^{5}/well in flat bottom microtiter plate, followed by about two week incubation in selective medium containing 10% fetal bovine serum, 10% P388D1 (ATCC, CRL TIB-63) conditioned medium, 3-5% origen (IGEN) in DMEM (Mediatech, CRL 10013, with high glucose, L-glutamine and sodium pyruvate) plus 5 mM HEPES, 0.055 mM 2-mercaptoethanol, 50 mg/ml gentamycin and 1x HAT (Sigma, CRL P-7185). After 1-2 weeks, cells were cultured in medium in which the HAT was replaced with HT. Individual wells were then screened by ELISA (described above) for human anti-PD-1 monoclonal IgG antibodies. Once extensive hybridoma growth occurred, medium was monitored usually after 10-14 days. The antibody-secreting hybridomas were replated, screened again and, if still positive for human IgG, anti-PD-1 monoclonal antibodies were subcloned at least twice by limiting dilution. The stable subclones were then cultured *in vitro* to generate small amounts of antibody in tissue culture medium for further characterization.

Hybridoma clones 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 were selected for further analysis.

Example 2: Structural Characterization of Human Monoclonal Antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4

The cDNA sequences encoding the heavy and light chain variable regions of the 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 monoclonal antibodies were obtained from the 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 hybridomas, respectively, using standard PCR techniques and were sequenced using standard DNA sequencing techniques.

The nucleotide and amino acid sequences of the heavy chain variable region of 17D8 are shown in Figure 1A and in SEQ ID NO: 57 and 1, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 17D8 are shown in Figure 1B and in SEQ ID NO: 64 and 8, respectively.

Comparison of the 17D8 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 17D8 heavy chain utilizes a VH segment from human germline VH 3-33, an undetermined D segment, and a JH segment from human germline JH 4b. The alignment of the 17D8 VH sequence to the germline VH 3-33 sequence is shown in Figure 8. Further analysis of the 17D8 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 1A and 8, and in SEQ ID NOs: 15, 22 and 29, respectively.

Comparison of the 17D8 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 17D8 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 4. The alignment of the 17D8 VL sequence to the germline VK L6 sequence is shown in Figure 9. Further analysis of the 17D8 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 1B and 9, and in SEQ ID NOs: 36, 43 and 50, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 2D3 are shown in Figure 2A and in SEQ ID NO: 58 and 2, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 2D3 are shown in Figure 2B and in SEQ ID NO: 65 and 9, respectively.

Comparison of the 2D3 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 2D3 heavy chain utilizes a VH segment from human germline VH 3-33, a D segment from human germline 7-27, and a JH segment from human germline JH 4b. The alignment of the 2D3 VH sequence to the germline VH 3-33 sequence is shown in Figure 8. Further analysis of the 2D3 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 2A and 8, and in SEQ ID NOs: 16, 23 and 30, respectively.

Comparison of the 2D3 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 2D3 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 4. The alignment of the 2D3 VL sequence to the germline VK L6 sequence is shown in Figure 9. Further analysis of the 2D3 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 2B and 9, and in SEQ ID NOs: 37, 44 and 51, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 4H1 are shown in Figure 3A and in SEQ ID NO: 59 and 3, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 4H1 are shown in Figure 3B and in SEQ ID NO: 66 and 10, respectively.

Comparison of the 4H1 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 4H1 heavy chain utilizes a VH segment from human germline VH 3-33, an undetermined D segment, and a JH segment from human germline JH 4b. The alignment of the 4H1 VH sequence to the germline VH 3-33 sequence is shown in Figure 8. Further analysis of the 4H1 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 3A and 8, and in SEQ ID NOs: 17, 24 and 31, respectively.

Comparison of the 4H1 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 4H1 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 1. The alignment of the 4H1 VL sequence to the germline VK L6 sequence is shown in Figure 10. Further analysis of the 4H1 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 3B and 10, and in SEQ ID NOs: 38, 45 and 52, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 5C4 are shown in Figure 4A and in SEQ ID NO: 60 and 4, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 5C4 are shown in Figure 4B and in SEQ ID NO: 67 and 11, respectively.

Comparison of the 5C4 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 5C4 heavy chain utilizes a VH segment from human germline VH 3-33, an undetermined D segment, and a JH segment from human germline JH 4b. The alignment of the 5C4 VH sequence to the germline VH 3-33 sequence is shown in Figure 8. Further analysis of the 5C4 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 4A and 8, and in SEQ ID NOs: 18, 25 and 32, respectively.

Comparison of the 5C4 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 5C4 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 1. The alignment of the 5C4 VL sequence to the germline VK L6 sequence is shown in Figure 10. Further analysis of the 5C4 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 4B and 10, and in SEQ ID NOs: 39, 46 and 53, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 4A11 are shown in Figure 5A and in SEQ ID NO: 61 and 5, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 4A11 are shown in Figure 5B and in SEQ ID NO: 68 and 12, respectively.

Comparison of the 4A11 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 4A11 heavy chain utilizes a VH segment from human germline VH 4-39, a D segment from human germline 3-9, and a JH segment from human germline JH 4b. The alignment of the 4A11 VH sequence to the germline VH 4-39 sequence is shown in Figure 11. Further analysis of the 4A11 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 5A and 11, and in SEQ ID NOs: 19, 26 and 33, respectively.

Comparison of the 4A11 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 4A11 light chain utilizes a VL segment from human germline VK L15 and a JK segment from human germline JK 1. The alignment of the 4A11 VL sequence to the germline VK L6 sequence is shown in Figure 12. Further analysis of the 4A11 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 5B and 12, and in SEQ ID NOs: 40, 47 and 54, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 7D3 are shown in Figure 7A and in SEQ ID NO: 62 and 6, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 7D3 are shown in Figure 7B and in SEQ ID NO: 69 and 13, respectively.

Comparison of the 7D3 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 7D3 heavy chain utilizes a VH segment from human germline VH 3-33, a human germline 7-27 D segment, and a JH segment from human germline JH 4b. The alignment of the 7D3 VH sequence to the germline VH 3-33 sequence is shown in Figure 8. Further analysis of the 7D3 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 6A and 8, and in SEQ ID NOs: 20, 27 and 34, respectively.

Comparison of the 7D3 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 7D3 light chain utilizes a VL segment from human germline VK L6 and a JK segment from human germline JK 4. The alignment of the 7D3 VL sequence to the germline VK L6 sequence is shown in Figure 9. Further analysis of the 7D3 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 6B and 9, and in SEQ ID NOs: 41, 48 and 55, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 5F4 are shown in Figure 7A and in SEQ ID NO: 63 and 7, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 5F4 are shown in Figure 7B and in SEQ ID NO: 70 and 14, respectively.

Comparison of the 5F4 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 5F4 heavy chain utilizes a VH segment from human germline VH 4-39, a D segment from human germline 3-9, and a JH segment from human germline JH 4b. The alignment of the 5F4 VH sequence to the germline VH 4-39 sequence is shown in Figure 11. Further analysis of the 5F4 VH sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figures 7A and 11, and in SEQ ID NOs: 21, 28 and 35, respectively.

Comparison of the 5F4 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 5F4 light chain utilizes a VL segment from human germline VK L15 and a JK segment from human germline JK 1. The alignment of the 5F4 VL sequence to the germline VK L6 sequence is shown in Figure 12. Further analysis of the 5F4 VL sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figures 7B and 12, and in SEQ ID NOs: 42, 49 and 56, respectively.

Example 3: Characterization of Binding Specificity and Binding Kinetics of Anti-PD-1 Human Monoclonal Antibodies

In this example, binding affinity and binding kinetics of anti-PD-1 antibodies were examined by Biacore analysis. Binding specificity, and cross-competition were examined by flow cytometry.

Binding affinity and kinetics

Anti-PD-1 antibodies were characterized for affinities and binding kinetics by Biacore analysis (Biacore AB, Uppsala, Sweden). Purified recombinant human PD-1 fusion protein was covalently linked to a CM5 chip (carboxy methyl dextran coated chip) via primary amines, using standard amine coupling chemistry and kit provided by Biacore. Binding was measured by flowing the antibodies in HBS EP buffer (provided by Biacore AB) at a concentration of 267 nM at a flow rate of 50 μl/min. The antigen-antibody association kinetics was followed for 3 minutes and the dissociation kinetics was followed for 7 minutes. The association and dissociation curves were fit to a 1:1 Langmuir binding model using BIAevaluation software (Biacore AB). To minimize the effects of avidity in the estimation of the binding constants, only the initial segment of data corresponding to association and dissociation phases were used for fitting. The K_D, k_{on} and k_{off} values that were determined are shown in Table 2.

Table 2. Biacore binding data for PD-1 human monoclonal antibodies.

Sample #	Sample ID	Affinity K_D x 10^{-9} (M)	On rate k_{on} x 10^5 (1/Ms)	Off rate k_{off} x 10^{-4} 1/s
1	17D8	0.16	2.56	0.45
2	2D3	1.20	3.77	4.52
3	4H1	5.46	3.15	1.72
4	5C4	0.73	4.32	3.15
5	4A11	0.13	0.76	0.099
6	7D3	2.49	18.2	4.54
7	5F4	2.91	8.74	2.54

Binding specificity by flow cytometry

Chinese hamster ovary (CHO) cell lines that express recombinant human PD-1 at the cell surface were developed and used to determine the specificity of PD-1 human monoclonal antibodies by flow cytometry. CHO cells were transfected with expression plasmids containing full length cDNA encoding transmembrane forms of PD-1. Binding of the 5C4 and 4H1 anti-PD-1 human monoclonal antibodies was assessed by incubating the transfected cells with the anti-PD-1 human monoclonal antibodies at a concentration of 20 μg/ml. The cells were washed and binding was detected with a FITC-labeled anti-human IgG Ab. Flow cytometric analyses were performed using a FACScan flow cytometry (Becton Dickinson, San Jose, CA). The results are depicted in Figures 13A (5C4) and 13B (4H1). The anti-PD-1 human monoclonal antibodies bound to the CHO cells transfected with PD-1 but not to CHO cells that were not transfected with human PD-1. These data demonstrate the specificity of anti-PD-1 human monoclonal antibodies for PD-1.

Binding specificity by ELISA against other CD28 family members

A comparison of the binding of anti-PD-1 antibodies to CD28 family members was performed by standard ELISA using four different CD28 family members to examine the specificity of binding for PD-1.

Fusion proteins of CD28 family members, ICOS, CTLA-4 and CD28 (R&D Biosystems) were tested for binding against the anti-PD-1 human monoclonal antibodies 17D8, 2D3, 4H1, 5C4, and 4A11. Standard ELISA procedures were performed. The anti-PD-1 human monoclonal antibodies were added at a concentration of 20 μg/ml. Goat-anti-human IgG (kappa chain-specific) polyclonal antibody conjugated with horseradish peroxidase (HRP) was used as secondary antibody. The results are shown in Figure 14. Each of the anti-PD-1 human monoclonal antibodies 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 bound with high specificity to PD-1, but not to the other CD28 family members.

Example 4: Characterization of anti-PD-1 antibody binding to PD-1 expressed on the surface of human and monkey cells

Anti-PD-1 antibodies were tested for binding to cells expressing PD-1 on their cell surface by flow cytometry.

Activated human T-cells, monkey peripheral blood mononuclear cells (PBMC), and CHO cells transfected with PD-1 were each tested for antibody binding. Human T cells and cynomolgous PBMC were activated by anti-CD3 antibody to induce PD-1 expression on T cells prior to binding with a human anti-PD-1 monoclonal antibody. Binding of the 5C4 and 4H1 anti-PD-1 human monoclonal antibodies was assessed by incubating the transfected cells with either IgG1 or IgG4 forms of the anti-PD-1 human monoclonal antibodies at different concentrations. The cells were washed and binding was detected with a FITC-labeled anti-human IgG Ab. Flow cytometric analyses were performed using a FACScan flow cytometry (Becton Dickinson, San Jose, CA). The results are shown in Figures 15A (activated human T cells), 15B (cynomolgous monkey PBMC) and 15C (PD-1-transfected CHO cells). The anti-PD-1 monoclonal antibodies 5C4 and 4H1 bound to activated human T cells, activated monkey PBMCs, and CHO cells transfected with human PD-1, as measured by the mean fluorescent intensity (MFI) of staining. These data demonstrate that the anti-PD-1 HuMAbs bind to both human and cynomolgous monkey cell surface PD-1.

Example 5: Effect of human anti-PD-1 antibodies on cell proliferation and cytokine production in a Mixed Lymphocyte Reaction

A mixed lymphocyte reaction was employed to demonstrate the effect of blocking the PD-1 pathway to lymphocyte effector cells. T cells in the assay were tested for proliferation, IFN-gamma secretion and IL-2 secretion in the presence or absence of an anti-PD-1 HuMAb antibody.

Human T-cells were purified from PBMC using a human CD4+ T cell enrichment column (R&D systems). Each culture contained 10^5 purified T-cells and 10^4 allogeneic dendritic cells in a total volume of 200 µl. Anti-PD-1 monoclonal antibody 5C4, 4H1, 17D8, 2D3 or a Fab fragment portion of 5C4 was added to each culture at different antibody concentrations. Either no antibody or an isotype control antibody was used as a negative control. The cells were cultured for 5 days at 37°C. After day 5, 100 µl of medium was taken from each culture for cytokine measurement. The levels of IFN-gamma and other cytokines were measured using OptEIA ELISA kits (BD Biosciences). The cells were labeled with ^{3}H-thymidine, cultured for another 18 hours, and analyzed for cell proliferation. The results are shown in Figures 16A (T cell proliferation), 16B (IFN-γ secretion) and 16C (IL-2 secretion). The anti-PD-1 human monoclonal antibodies promoted T-cell proliferation, IFN-gamma secretion and IL-2 secretion in a concentration dependent manner. The 5C4-Fab fragment also promoted T-cell proliferation, IFN-gamma secretion and IL-2 secretion in a concentration dependent manner. In contrast, cultures containing the isotype control antibody did not show an increase in T cell proliferation, IFN-gamma or IL-2 secretion.

Example 6: Blocking of ligand binding to PD-1 by human anti-PD-1 antibodies

Anti-PD-1 HuMAbs were tested for the ability to block binding of the ligands PD-L1 and PD-L2 to PD-1 expressed on transfected CHO cells by using a flow cytometry assay.

PD-1 expressing CHO cells were suspended in FACS buffer (PBS with 4% fetal calf serum). Various concentrations of the anti-PD-1 HuMAbs 5C4 and 4H1 were added to the cell suspension and incubated at 4°C for 30 minutes. Unbound antibody was washed off and either FITC-labeled PD-L1 fusion protein or FITC-labeled PD-L2 fusion protein was added into the tubes and incubated at 4°C for 30 minutes. Flow cytometric analyses were performed using a FACScan flowcytometer (Becton Dickinson, San Jose, CA). The results are depicted in Figures 17A (blocking of PD-L1) and 17B (blocking of PD-L2). The anti-PD-1 monoclonal antibodies 5C4 and 4H1 blocked binding of PD-L1 and PD-L2 to CHO cells transfected with human PD-1, as measured by the mean fluorescent intensity (MFI) of staining. These data demonstrate that the anti-PD-1 HuMAbs block binding of ligand (both PD-L1 and PD-L2) to cell surface PD-1.

Example 7: Effect of human anti-PD-1 antibodies on the release of cytokines in human blood

The anti-PD-1 HuMAbs were mixed with fresh human whole blood in order to determine whether the anti-PD-1 HuMAbs alone stimulated the release of certain cytokines from human blood cells.

500 μl of heparinized-fresh human whole blood, was added into each well. Either 10 μg or 100 μg of an anti-PD-1 HuMAb (4H1 or 5C4,the latter either as an IgG1 or IgG4 isotype) was added to each well. Some wells were incubated with anti-CD3 antibody as a positive control, or a human IgG1 or human IgG4 antibody as isotype-matched negative controls. The cells were incubated at 37 °C for either 6 or 24 hours. The cells were spun down and the plasma was collected for measurement of the cytokines IFN-gamma, TNF-alpha, IL-2, IL-4, IL-6, IL-10 and IL-12 using a cytokine cytometric bead array assay (BD Biosciences). The concentration of each cytokine (pg/ml) is shown in Tables 3a, with a 6 hour incubation, and 3b, with a 24 hour incubation, below. The results show that treatment with the human anti-PD-1 antibodies 5C4 and 4H1 alone did not stimulate human blood cells to release any of the cytokines IFN-gamma, TNF-alpha, IL-2, IL-4, IL-6, IL-10 and IL-12.

Table 3a. Cytokine production following 6 hour incubation

Ab	IFN-gamma (pg/ml)	TNF-alpha (pg/ml)	IL-10 (pg/ml)	IL-6 (pg/ml)	IL-4 (pg/ml)	IL-2 (pg/ml)
No Ab	12.3	2	3	5	3.6	1.9
10 mg/ml anti-CD3	5000	530	82.6	510.4	37.2	467.9
100 mg/ml anti-CD3	5000	571	91.3	530	43.9	551.5
10 mg/ml hIgG1	7	1.8	2.8	4.4	2.6	1.5
100 mg/ml hIgG1	0	2.2	2.7	6	2.6	1.4
10 mg/ml hIgG4	5.4	1.4	2.5	4.5	2.1	1.3
100 mg/ml hIgG4	6.4	2.3	3	32.6	2.9	1.4
10 mg/ml 4H1	6.2	1.8	2.4	4.1	2.8	1.6
100 mg/ml 4H1	11.8	2	2.6	3.5	2.6	1.7
10 mg/ml 5C4 IgG1	4.2	1.6	2.3	3.9	2.5	1.3
100 mg/ml 5C4 IgG1	0	1.4	2.2	3.6	2.1	1.2
10 mg/ml 5C4 IgG4	8.3	2.5	1.9	4.8	1.6	1.5
100 mg/ml 5C4 IgG4	3.6	1.7	2.4	3.9	2.3	1.5

Table 3b. Cytokine production following 24 hour incubation

Ab	IFN-gamma (pg/ml)	TNF-alpha (pg/ml)	IL-10 (pg/ml)	IL-6 (pg/ml)	IL-4 (pg/ml)	IL-2 (pg/ml)
No Ab	11.2	2	6.1	5.9	2.6	1.7
10 mg/ml anti-CD3	5000	565.9	432	5000	64.5	1265.3
100 mg/ml anti-CD3	5000	535	461	5000	73.8	1334.9
10 mg/ml hIgG1	0	0	0	0	0	0
100 mg/ml hIgG1	11.5	1.7	7.9	60.8	2.9	1.5
10 mg/ml hIgG4	24.6	3.1	8.3	63.4	3.1	2.3
100 mg/ml hIgG4	11.2	1.8	8	27.7	3.1	2.4
10 mg/ml 4H1	27.3	2.9	8	13.9	5.3	2.6
100 mg/ml 4H1	17.5	2.5	4.4	7	4	2.1
10 mg/ml 5C4 IgG1	9.1	2	7.6	68.5	3.5	1.8
100 mg/ml 5C4 IgG1	12.9	1.9	6.1	25.3	2.9	1.7
10 mg/ml 5C4 IgG4	14	1.9	4.4	3.3	2.6	1.9
100 mg/ml 5C4 IgG4	0	0	0	0	0	0

Example 8: Effect of anti-PD-1 antibodies on the apoptosis of T-cells

The effect of anti-PD-1 antibodies on the induction of apoptosis of T-cells was measured using an annexin V staining test.

T cells were cultured in a mixed lymphocyte reaction, as described above in Example 5. The anti-PD-1 antibody 5C4 was added to the tube at a concentration of 25 μg/ml. A non-specific antibody was used as a control. Annexin V and propidium iodide were added according to standard protocol (BD Biosciences). The mixture was incubated for 15 minutes in the dark at room temperature and then analyzed using a FACScan flowcytometer (Becton Dickinson, San Jose, CA). The results are shown in Figure 18. The anti-PD-1 antibody 5C4 did not have an effect on T-cell apoptosis.

Example 9: Effect of anti-PD-1 antibodies on cytokine secretion by viral-stimulated PBMC cells from a virus positive donor

In this example, peripheral blood mononuclear cells (PBMC) from a donor positive for CMV were isolated and exposed to a CMV lysate in the presence or absence of anti-PD-1 antibodies to examine the effect of the antibodies on cytokine secretion simulated by antigen.

$2x10^5$ human PMBCs from a CMV positive donor were cultured in a total volume of 200 µl and added into each well along with a lysate of CMV-infected cells. The anti-PD-1 HuMAb 5C4 was added to each well in various concentrations for 4 days. After day 4, 100 µl of medium was taken from each culture for cytokine measurement. The level of IFN-gamma was measured using OptEIA ELISA kits (BD Biosciences). The cells were labeled with ^{3}H-thymidine, cultured for another 18 hours, and analyzed for cell proliferation. The cell proliferation was analyzed using the Cell Titer-Glo reagent (Promega). The results are shown in Figure 19. The anti-PD-1 HuMab 5C4 increased IFN gamma secretion in a concentration dependent manner. These results shows that anti-PD-1 HuMAbs can stimulate IFN-gamma release in a memory T cell response from PBMC cells previously stimulated against an antigen.

Example 10: Effect of anti-PD-1 antibody on secondary antibody response to antigen

Mice were immunized and rechallenged with a TI -antigen (DNP-Ficoll) and also treated with a rat anti-mouse-PD-1 antibody, or a control antibody to examine the effect of the anti-PD-1 antibody on antibody titers.

Female C57BL6 mice were divided into two groups, with 6 mice/group. One group was treated with a control rat IgG and the other with a rat anti-mouse PD-1 antibody. The mice were immunized with 5µg of DNP-Ficoll (a T1-antigen) in 50µl CFA by i.p. at day 0. Either the control rat IgG antibody or the rat-mPD-1 antibody (200µg/mouse) was given by i.p. at days -1, 0 and 2. Four weeks later, mice were rechallenged with 5µg of DNP-Ficoll in 50µl IFA by i.p. at day 0. Rat anti-mPD-1 antibody or control antibody (200µg/mouse) was given by i.p. at days 0 and 1. Antibody titers were measured by standard ELISA assay at day 7 following the boost. The results are shown in Table 4 below. In the mice treated with the anti-mPD-1 antibody, both IgM and IgG3 isotypes showed the greatest increase in titer following challenge with the T1-antigen, as compared to mice treated with a control antibody. These results demonstrate that anti-PD-1 treatment can increase antibody titers in response to T1-antigen.

Table 4. Murine secondary response following treatment with anti-PD-1 antibody

Antibody Isotype	Control group	Rat anti-mouse PD-1 antibody	P value
IgM	606	1200	0.026
IgG	9	15.55	0.18
IgG1	1.2	1.1	0.83
IgG2b	5.05	9.26	0.18
IgG3	21.9	81.2	0.03

* Results shown are average concentration of antibody isotype (μg/ml)

Example 11: Treatment of *in vivo* tumor model using anti-PD-1 antibodies

Mice implanted with a cancerous tumor were treated *in vivo* with anti-PD-1 antibodies to examine the *in vivo* effect of the antibodies on tumor growth. As a positive control, an anti-CTLA-4 antibody was used, since such antibodies have been shown to inhibit tumor growth *in vivo*.

In this experiment, the anti-PD-1 antibody used was a chimeric rat anti-mouse-PD-1 antibody generated using well known laboratory techniques. To generate the rat anti-mouse PD-1 antibody, rats were immunized with mouse cells transfected to express a recombinant mouse PD-1 fusion protein (R&D Systems Catalog No. 1021-PD) and monoclonal antibodies were screened for binding to mouse PD-1 antigen by ELISA assay. The rat anti-PD-1 antibody V regions were then recombinantly linked to a murine IgG1 constant region using standard molecular biology techniques and rescreened for binding to mouse PD-1 by ELISA and FACS. The chimeric rat anti-mouse-PD-1 antibody used herein is referred to as 4H2.

For the tumor studies, female AJ mice between 6-8 weeks of age (Harlan Laboratories) were randomized by weight into 6 groups. The mice were implanted subcutaneously in the right flank with 2 x 10^6 SA1/N fibrosarcoma cells dissolved in 200 μl of DMEM media on day 0. The mice were treated with PBS vehicle, or antibodies at 10 mg/kg. The animals were dosed by intraperitoneal injection with approximately 200 μl of PBS containing antibody or vehicle on days 1, 4, 8 and 11. Each group contained 10 animals and the groups consisted of: (i) a vehicle group, (ii) control mouse IgG, (iii) control hamster IgG, (iv) hamster anti-mouse CTLA-4 antibody and (v) the chimeric anti-PD-1 antibody 4H2. The mice were monitored twice weekly for tumor growth for approximately 6 weeks. Using an electronic caliper, the tumors were measured three dimensionally (height x width X length) and tumor volume was calculated. Mice were euthanized when the tumors reached tumor end point (1500 mm^3) or show greater than 15% weight loss. The results are shown in Figure 20. The anti-PD-1 antibody extended the mean time to reaching the tumor end point volume (1500 mm^3) from ~25 days in the control groups to ~40 days. Thus, treatment with an anti-PD-1 antibody has a direct *in vivo* inhibitory effect on tumor growth.

Example 12: Generation of Chimeric (Rat–Mouse) anti-PD-1 Antibody 4H2

Rat monoclonal antibody against mouse PD-1 antibodies (rat anti-mPD-1) were generated from rats immunized with mPD-1-hFc fusion protein using standard hybridoma production methods (see Kohler and Milstein (1975) *Nature* 256:495; and Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor New York). Eight hybridomas were subcloned, and antibodies were isolated and screened for their ability to block mouse PD-L2 (mPD-L2) binding to mPD-1. Several anti-mPD-1 antibodies capable of blocking mPD-L2 binding to mPD-1 were identified (*see, e.g.*, activity of 4H2, Figure 41) and the binding affinity of several of these antibodies to mPD-1-Fc fusion protein was determined by ELISA (Figure 42).

Antibody 4H2.B3 was further characterized, which is referred to interchangeably herein as "4H2." CHO cells expressing mouse PD-1 were constructed and incubated with 4H2 anti-mPD-1 antibody at a concentration ranging from 200 μg/ml to 0.012 μg/ml to determine the binding affinity of 4H2 to PD-1. Binding of anti-mPD-1 antibody to the PD-1 expressing CHO cells was detected by incubating with donkey-anti-rat IgG, FITC conjugated and measured by FACS. The anti-mPD-1 antibody had an EC_{50} (50% effective concentration) of about 0.38 μg (Figure 43) and a K_D of 4.7×10^{-9} M. To examine the inhibition of PD-L1 binding to PD-1, the same assay was performed except that the cells were also incubated with 0.16 μg mPD–L1-hFc fusion protein, then binding of PD-L1 to the PD-1 expressing CHO cells was detected by incubating with goat-anti-human IgG (Fc specific), FITC conjugated and measuring binding signal by FACS (MFI, mean fluorescence intensity). The anti-mPD-1 antibody had an EC_{50} of about 0.72 μg (Figure 44).

For use in the mouse tumor models, the 4H2 rat anti-mPD-1 needed to be modified so the mouse immune system would not neutralize the immunotherapeutic antibody (*i.e.*, so the antibody would have better pharmacokinetics) and to avoid antibody-dependent cellular cytotoxicity (ADCC) by reducing Fc receptor interactions (*i.e.*, so blockade by anti-PD-1 could be evaluated with being compromised by ADCC effects). The original rat anti-mPD-1 antibody, 4H2, was determined to be a rat IgG2a isotype. Hence, the Fc-portion of the 4H2 antibody was replaced with an Fc-portion from a mouse IgG1 isotype. Using the assay described above,the binding affinity of the rat–mouse chimeric 4H2 to mPD-1 was found to be comparable to the rat 4H2.B3 anti-mPD-1 antibody (Figure 45). Similarly, inhibition of PD-L1 binding to PD-1 was comparable for both antibodies (Figure 46). Thus, the rat-mouse chimeric 4H2 anti-mPD-1 antibody was used to examine the therapeutic efficacy of anti-PD-1 in combination with anti-CTLA-4.

Example 13: *In Vivo* Efficacy of Combination Therapy (anti-CTLA-4 and anti-PD-1 Antibodies) on Tumor Establishment and Growth

MC38 colorectal cancer cells (PD-L1$^-$) (available from Dr. N. Restifo, National Cancer Institute, Bethesda, MD; or Jeffrey Schlom, National Institutes of Health, Bethesda, MD) were

implanted in C57BL/6 mice (2 x 10^6 cells/mouse). On day 0 (*i.e.*, the day the MC38 cells were implanted in the mice), each of four groups of 10 mice each was injected intraperitoneally (IP) with one of the following: (1) mouse IgG (control), (2) anti-CTLA-4 monoclonal antibody 9D9 (mouse anti-mouse CTLA-4, obtained from J. Allison, Memorial Sloan–Kettering Cancer Center, New York, NY), (3) anti-PD-1 monoclonal antibody 4H2 (chimeric antibody in which a rat anti-mouse PD-1 was modified with a mouse Fc region, as described in Example 6), or (4) anti-CTLA-4 antibody 9D9 and anti-PD-1 antibody 4H2. Antibody injections were then further administered on days 3, 6 and 10. The single antibody treatments were dosed at 10 mg/kg, and the combination of anti-CTLA-4 antibody and anti-PD-1 antibody was dosed at 5 mg/kg of each antibody (*i.e.*, 10 mg/kg of total antibody). Using an electronic caliper, the tumors were measured three dimensionally (height x width x length) and tumor volume was calculated. Mice were euthanized when the tumors reached a designated tumor end-point. The results are shown in Table 5 and Figure 21.

Table 5. Percentage of Tumor-Free Mice Following Anti–PD-1 and/or Anti–CTLA–4 Treatment

Treatment	Total mice studied	Tumor-free mice (%)
mIgG1	10	0
anti-CTLA-4	10	1 (10)
anti-PD-1	10	3 (30)
anti-CTLA-4 + anti-PD-1	10	6 (60)

Eight mice in the IgG group reached the tumor end-point by about day 30 and two mice (86066 and 87260) in the IgG group had ulcerated tumors (Figure 21A). In the anti-CTLA-4 antibody alone group, seven mice reached the tumor end-point by about day 60, one mouse had an ulcerated tumor (84952), one mouse had a tumor with a volume of less than 1500 mm^3 (85246), and one mouse was tumor-free (86057) (Figure 21B). In the anti-PD-1 antibody alone group, six mice reached the tumor end-point by about day 60, one mouse had an ulcerated tumor (86055), and three mice were tumor-free (84955, 85239 and 86750) (Figure 21C). In the anti-CTLA-4 antibody and anti-PD-1 antibody combination group, four mice reached the tumor end-point by about Day 40, and six mice were tumor-free (84596, 85240, 86056, 86071, 86082 and 86761) (Figure 21D).

Figure 22 shows that the mean tumor volume measured at day 21 was about 2955 mm^3 for the IgG control group; about 655 mm^3 for the CTLA-4 antibody alone group, about 510 mm^3 for the PD-1 antibody alone group, and about 280 mm^3 for the anti-CTLA-4 antibody and anti-PD-1 antibody combination group. Figure 23 shows that the median tumor volume measured at day 21 was about 2715 mm^3 for the IgG group; about 625 mm^3 for the CTLA-4 antibody alone group; about 525 mm^3 for the PD-1 antibody alone group; and about 10 mm^3

for the CTLA-4 antibody and PD-1 antibody combination group (and down to 0 mm^3 by day 32).

This study indicates that, in a murine tumor model, CTLA-4 antibody treatment alone and PD-1 antibody treatment alone have a modest effect on tumor growth, and that the combination treatment of CTLA-4 antibody and PD-1 antibody has a significantly greater effect on tumor growth. It is interesting to note that the combination treatment with CTLA-4 antibody and PD-1 antibody had a more significant effect on tumor growth at a dose of 5 mg/kg of each antibody as compared to the effect of either antibody alone when each is administered at a higher dose of 10 mg/kg.

Example 14: *In Vivo* Efficacy of Combination Therapy (anti-CTLA-4 and anti-PD-1 Antibodies) on Established Tumor Growth

MC38 colorectal cancer cells (PD-L1$^-$) were implanted in C57BL/6 mice (2 x 10^6 cells/mouse) for a time sufficient (about 6 to 7 days) to permit the formation of tumors. On day 6 post-implantation (day -1), tumor measurements were taken and mice were randomized based on mean tumor volume (about 250 mm^3) into 11 groups for subsequent antibody therapy. At day 0 (*i.e.*, one week after the MC38 cells were implanted), mice were injected IP with (1) mouse IgG (control), (2) anti-CTLA-4 monoclonal antibody 9D9, (3) anti-PD-1 monoclonal antibody 4H2, or (4) anti-CTLA-4 monoclonal antibody 9D9 and anti-PD-1 antibody monoclonal antibody 4H2, at a concentration of 10 mg/kg per mouse. Antibody injections were also administered on days 3, 6 and 10. The monoclonal antibody compositions used had low levels of endotoxin and did not significantly aggregate. Using an electronic caliper, the tumors were measured three dimensionally (height x width x length) and tumor volume was calculated. Tumor measurements were taken on day 0 (tumors at the beginning of treatment had a volume of about 125 mm^3), and on days 3, 6, 10, 13, 17 and 20 post-antibody injection. Mice were euthanized when the tumors reached a designated tumor end-point (a particular tumor volume such as 1500 mm^3 and/or when the mice showed greater than about 15% weight loss).

All eleven mice in the IgG group reached the tumor end-point by about day 17 (Figure 24A). In the anti-CTLA-4 antibody alone group, seven of eleven mice reached the tumor end-point by about day 12 (Figure 24B). In the anti-PD-1 antibody alone group, four mice reached the tumor end-point by about day 13 and two mice were tumor-free (Figure 24C). In the anti-CTLA-4 antibody and anti-PD-1 antibody combination group, one mouse reached the tumor end-point by about day 17, one mouse reached the tumor end-point by about day 45 and nine mice were tumor-free on day 45 (Figure 24D).

Figure 25 shows that the mean tumor volume measured at day 10 was about 1485 mm^3 for the IgG control group; about 1010 mm^3 for the CTLA-4 antibody alone group; about 695 mm^3 for the PD-1 antibody alone group; and about 80 mm^3 for the anti-CTLA-4 antibody and anti-PD-1 antibody combination group. Figure 26 shows that the median tumor volume

measured at day 10 was about 1365 mm^3 for the IgG group; about 1060 mm^3 for the anti-CTLA-4 antibody alone group; about 480 mm^3 for the anti-PD-1 antibody alone group; and about 15 mm^3 for the anti-CTLA-4 antibody and anti-PD-1 antibody combination group (which was down to 0 mm^3 by day 17).

This study indicates that, in a murine tumor model, treatment with the combination of CTLA-4 antibody and PD-1 antibody has a significantly greater effect on tumor growth than either antibody alone, even when a tumor is already well established.

Example 15: Dose Titration of Combination Therapy (anti-CTLA-4 and anti-PD-1 Antibodies) on Established Tumor Growth

MC38 colorectal cancer cells (PD-L1$^-$) were implanted in C57BL/6 mice (2×10^6 cells/mouse) for a time sufficient (about 6 to 7 days) to permit the formation of tumors as described in Example 3. Groups of 10 mice were injected IP at days 0, 3, 6 and 10 as follows: Group (A) mouse IgG (control, 20 mg/kg), Group (B) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg) and mouse IgG (10 mg/kg), Group (C) anti-CTLA-4 monoclonal antibody 9D9 (10 mg/kg) and mouse IgG (10 mg/kg), Group (D) anti-CTLA-4 monoclonal antibody 9D9 (10 mg/kg) and anti-PD-1 antibody monoclonal antibody 4H2 (10 mg/kg), Group (E) anti-CTLA-4 monoclonal antibody 9D9 (3 mg/kg) and anti-PD-1 antibody monoclonal antibody 4H2 (3 mg/kg), or Group (F) anti-CTLA-4 monoclonal antibody 9D9 (1 mg/kg) and anti-PD-1 antibody monoclonal antibody 4H2 (1 mg/kg). Using an electronic caliper, the tumors were measured three dimensionally (height x width x length) and tumor volume was calculated. Tumor measurements were taken at the beginning of treatment (*i.e.*, on day 0 tumors had an average volume of about 90 mm^3), and on days 3, 6, 10, 13, 17 and 20 post-antibody treatment. Mice were euthanized when the tumors reached a designated tumor end-point (a particular tumor volume such as 1500 mm^3 and/or when the mice showed greater than about 15% weight loss).

Figure 27A shows that all 10 control mice had reached a tumor end-point. Figure 27B shows that the group treated with 10 mg/kg anti-PD-1 antibody (Group B) had 6 mice that reached the tumor end–point and 4 mice with tumors having a volume of about 750 mm^3 or less. Figure 27C shows that the group treated with 10 mg/kg anti-CTLA-4 antibody (Group C) had 3 mice that reached the tumor end-point and 7 mice with tumors having a volume of about 1000 mm^3 or less. Figure 27D shows that the group treated with a combination of 10 mg/kg anti-PD-1 antibody with 10 mg/kg anti-CTLA-4 antibody (Group D) had 2 mice with tumors having a volume of about 1000 mm^3 or less, and 8 mice that were tumor free. Figure 27E shows that the group treated with a combination of 3 mg/kg anti-PD-1 antibody with 3 mg/kg anti-CTLA-4 antibody (Group E) had one mouse that had reached the tumor end-point, 7 mice with tumors having a volume of about 500 mm^3 or less, and 2 mice that were tumor free. Figure 27F shows that the group treated with a combination of 1 mg/kg anti-PD-1 antibody with 1 mg/kg anti-CTLA-4 antibody (Group F) had 4 mice that had reached the

tumor end-point, 5 mice with tumors having a volume of about 1100 mm^3 or less, and one mouse that was tumor free.

Figures 27G and 27H show the tumor volumes in mice treated sequentially with anti–PD-1 antibody first and anti-CTLA-4 antibody second, and vice versa. The mice of Figure 27G first received 10 mg/kg anti-CTLA-4 on each of days 0 and 3, and then received 10 mg/kg anti-PD-1 antibody on each of days 6 and 10. The mice of Figure 27H first received 10 mg/kg anti-PD-1 antibody on each of days 0 and 3, and then received 10 mg/kg anti-CTLA-4 antibody on each of days 6 and 10. For group G at day 27, 8 mice reached the tumor end-point, one mouse had a very small tumor (which, after a significant delay, eventually grew out) and one mouse was tumor free. For group H at day 27, 8 mice reached the tumor end-point and 2 were tumor free.

Figure 28 shows that the mean tumor volume measured at day 10 was about 1250 mm^3 for the IgG control group; about 470 mm^3 for the PD-1 antibody with the IgG control; about 290 mm^3 for the CTLA-4 antibody with the IgG control (measured at day 6); about 40 mm^3 for the anti-CTLA-4 antibody (10 mg/kg) and anti-PD-1 antibody (10 mg/kg) combination group; about 165 mm^3 for the anti-CTLA-4 antibody (3 mg/kg) and anti-PD-1 antibody (3 mg/kg) combination group; and about 400 mm^3 for the anti-CTLA-4 antibody (1 mg/kg) and anti-PD-1 antibody (1 mg/kg) combination group. Figure 29 shows that the median tumor volume measured at day 13 was about 1680 mm^3 for the IgG control group; about 400 mm^3 for the PD-1 antibody with the IgG control; about 660 mm^3 for the CTLA-4 antibody with the IgG control; 0 mm^3 for the anti-CTLA-4 antibody (10 mg/kg) and anti-PD-1 antibody (10 mg/kg) combination group; about 90 mm^3 for the anti-CTLA-4 antibody (3 mg/kg) and anti-PD-1 antibody (3 mg/kg) combination group; and about 650 mm^3 for the anti-CTLA-4 antibody (1 mg/kg) and anti-PD-1 antibody (1 mg/kg) combination group. For the combination treatment of the anti-PD-1 antibody with the anti-CTLA-4 antibody, the number of mice per group that were tumor free at day 27 of the study was 8/10 (10 mg/kg), 2/10 (3 mg/kg) and 1/10 (1 mg/kg) (data not shown).

This study indicates that, in a murine tumor model, treatment with the combination of CTLA-4 antibody and PD-1 antibody functions in a dose dependent manner and has a significantly greater effect on tumor growth than both antibodies alone, even at a lower dose and even when a tumor is already well established. Moreover, the antibodies may be administered sequentially (anti-CTLA-4 antibody first and anti-PD-1 antibody second, or vice versa) and the combination is still superior to the antibody monotherapies.

Example 16: *In Vivo* Efficacy of Combination Therapy (anti-CTLA-4 and anti-PD-1 Antibodies) on Fibrosarcoma Establishment and Growth

SA1/N fibrosarcoma cells (PD-L1$^-$) (Leach *et al.* (1996) *Science* 271:1734-1736) were implanted subcutaneously in A/J mice (2 x 10^6 cells/mouse) on day 0. On days 1, 4, 7 and 11 post-implantation, mice were injected IP as follows: Group (A) PBS alone (referred to as the

"vehicle"); Group (B) mouse IgG (control, 10 mg/kg per mouse), Group (C) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg per mouse), Group (D) anti-CTLA-4 monoclonal antibody 9D9 (10 mg/kg or 0.2 mg/kg per mouse), and Group (E) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg per mouse) in combination with anti-CTLA-4 monoclonal antibody 9D9 (0.2 mg/kg per mouse). The study lasted 41 days and tumor measurements were taken on various days throughout the course of the study (*see* Figure 29). Tumor volume was calculated by measuring tumors in three dimensions (height x width x length) using an electronic caliper. Mice were euthanized when the tumors reached a designated tumor end-point-a volume of 1500 mm^3 and/or an ulcerated tumor.

Figures 30A and 30B show that 19 out of the 20 control (9/10 in Group A and 10/10 in Group B) mice had either reached a tumor end-point or had developed ulcerated tumors. Figure 30C shows that the group treated with 10 mg/kg anti-PD-1 antibody (Group C) had 6 mice that reached a tumor end-point (2 with a volume greater than 1500 mm^3 and 4 with an ulcerated tumor) and 4 mice that were tumor free. Figure 30D shows that the group treated with 10 mg/kg anti-CTLA-4 antibody (Group D) had 5 mice that reached a tumor end-point (2 with a volume greater than 1500 mm^3 and 3 with an ulcerated tumor), one mouse with a small tumor (volume of about 70 mm^3) and 4 mice that were tumor free. Figure 30E shows that the group treated with 0.2 mg/kg anti-CTLA-4 antibody (Group E) had 10 mice that reached a tumor end-point (6 with a volume greater than 1500 mm^3 and 4 with an ulcerated tumor). Figure 30F shows that the group treated with a combination of 10 mg/kg anti-PD-1 antibody with 0.2 mg/kg anti-CTLA-4 antibody (Group F) had 2 mice that reached a tumor end–point (one with a volume greater than 1500 mm^3 and one with an ulcerated tumor) and 8 mice that were tumor free.

Figures 31 and 32 show the mean and median tumor volume, respectively, that developed in treated and untreated mice over the course of this study. The tumor growth inhibition in mice treated with these antibodies, as compared to mice treated with the control antibody mouse IgG, is summarized in Table 6.

Table 6. Tumor Growth Inhibition and Tumor Free Mice Following Anti-PD-1 and/or Anti-CTLA-4 Treatment

Group†	Median Tumor Volume – mm^3 (Day 15)	TGI* (%) (Day 15)	Median Tumor Volume – mm^3 (Day 19)	TGI (%) (Day 19)	No. of Tumor Free Mice (Day 41)
A	985	–	1140	–	0/10
B	635	–	1060	–	0/10
C	465	27	310	71	4/10
D	235	63	90	91	4/10
E	600	6	805	24	0/10
F	330	48	90	92	8/10

* TGI = tumor growth inhibition; the median could be calculated only when fewer than 50% of the mice reached the tumor end point.

† Groups are as defined in Figure 30. A = vehicle (PBS); B = mouse IgG; C = anti-PD-1, 10 mg/kg; D = anti-CTLA-4, 10 mg/kg; E = anti-CTLA-4, 0.2 mg/kg; and F = anti-PD-1, 10 mg/kg with anti-CTLA-4, 0.2 mg/kg.

These data further indicate that the combination therapy comprising anti-PD-1 and anti-CTLA-4 antibodies is substantially more effective than treatment with either antibody alone. Indeed, the combination is still more effective than single antibody treatments even when the combination therapy contains a subtherapeutic dose of anti-CTLA-4 antibody. These data also indicate that surprisingly the presence or absence of PD-L1 on the tumor may have no effect on the efficacy of treatment with this antibody combination, although the presence of PD-L1 may influence the effect of the antibody monotherapies in that expression of PD-L1 on the tumor may also lead to inhibition of anti-tumor T cell responses (*see* Figure 40).

Example 17: *In Vivo* Efficacy and Dose Titration of Combination Therapy (anti-CTLA-4 and anti-PD-1 Antibodies) on PD-L1$^-$ Fibrosarcoma Growth

SA1/N fibrosarcoma cells (PD-L1$^-$) were implanted subcutaneously in A/J mice (2 x 10^6 cells/mouse) on day 0 for a time sufficient (about 7 days) to permit the establishment of a tumor. On days 7, 10, 13 and 16 post-implantation, ten groups of 8 mice having an average tumor volume of 110 mm^3 were injected IP as follows: Group (A) PBS alone (referred to as the "vehicle"); Group (B) mouse IgG (control, 10 mg/kg per mouse); Group (C) anti-CTLA-4 monoclonal antibody 9D9 (0.25 mg/kg); Group (D) anti-CTLA-4 monoclonal antibody 9D9 (0.5 mg/kg per mouse); Group (E) anti-CTLA-4 monoclonal antibody 9D9 (5 mg/kg); Group (F) anti-PD-1 monoclonal antibody 4H2 (3 mg/kg per mouse); Group (G) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg per mouse); Group (H) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg per mouse) in combination with anti-CTLA-4 monoclonal antibody 9D9 (0.25 mg/kg per mouse); Group (I) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg per mouse) in combination with anti-CTLA-4 monoclonal antibody 9D9 (0.5 mg/kg per mouse); and Group

(J) anti-PD-1 monoclonal antibody 4H2 (3 mg/kg per mouse) in combination with anti-CTLA-4 monoclonal antibody 9D9 (0.5 mg/kg per mouse).

On days 10, 13, 16 and 19 post-implantation, two groups of 6 mice having an average tumor volume of 255 mm^3 were injected IP as follows: Group (K) mouse IgG (control, 10 mg/kg per mouse); and Group (L) anti-PD-1 monoclonal antibody 4H2 (10 mg/kg per mouse) in combination with anti-CTLA-4 monoclonal antibody 9D9 (1 mg/kg per mouse). The study lasted 51 days and tumor measurements were taken on various days throughout the course of the study (*see* Figures 33-38). Tumor volume was calculated by measuring tumors in three dimensions (height x width x length) using an electronic caliper. Mice were euthanized when the tumors reached a designated tumor end-point-a volume of 1500 mm^3 and/or an ulcerated tumor.

Figure 33 shows the response to immunostimulatory antibody treatment in mice with tumors having an initial volume of about 110 mm^3 (*i.e.*, at the time of the first antibody treatment. Figures 33A and 33B show that all 16 control mice (Groups A and B) reached a tumor end-point (15 with a tumor volume greater than 1500 mm^3 and 1 with an ulcerated tumor). Figures 33C-33E show that tumor bearing mice respond to treatment with anti-CTLA-4 antibody in a dose-dependent manner (*e.g.*, Group C receiving 0.25 mg/kg had 7/8 mice reach the tumor end-point and one mouse with a tumor volume less than 200 mm^3, whereas Group E receiving 5 mg/kg had 6/8 mice reach the tumor end-point and two mice were tumor free). Figures 33F and 33G show that mice responded about the same regardless of the anti-PD-1 antibody dose (Group F received 3 mg/kg and Group G received 10 mg/kg). In contrast, the mice receiving a combination treatment of 10 or 3 mg/kg anti-PD-1 antibody with 0.25 or 0.5 mg/kg anti-CTLA-4 antibody (Groups H, I and J) showed a significant reduction in tumor growth. For example, Figure 33J shows that the group treated with a combination of 3 mg/kg anti-PD-1 antibody with 0.5 mg/kg anti-CTLA-4 antibody (Group J) had 2 mice that had ulcerated tumors, 2 mice with a tumor volume less than 500 mm^3, and 4 mice that were tumor free. The unexpected synergistic effect of an anti-PD-1 antibody combined with an anti-CTLA-4 antibody, along with the surprising effectiveness of subtherapeutic levels of anti-CTLA-4 antibody in the combination, are shown in Figures 34 (mean tumor volume) and 35 (median tumor volume).

Figure 36 shows the response to immunostimulatory antibody treatment in mice with larger tumors, those having an initial volume of about 250 mm^3 (*i.e.*, at the time of the first antibody treatment). Figure 36A shows that all 6 control mice (Group K) reached a tumor end-point (4 with a tumor volume greater than 1500 mm^3 and 2 with an ulcerated tumor). Figure 36B shows that the group treated with a combination of 10 mg/kg anti-PD-1 antibody with 1 mg/kg anti-CTLA-4 antibody (Group L) had one mouse with an ulcerated tumor, 4 mice with a tumor volume greater than 1500 mm^3, and one mouse that was tumor free. The mean and median tumor volumes are shown in Figures 37 and 38.

The tumor growth inhibition in mice treated with these antibodies, as compared to mice treated with the control antibody mouse IgG, is summarized in Table 7 and Figure 39.

Table 7. Tumor Growth Inhibition Following Anti-PD-1 and/or Anti-CTLA-4 Treatment

Group	Mean Tumor Volume – mm^3 (Day 23)	TGI* (Mean)	Median Tumor Volume – mm^3 (Day 23)	TGI (Median)	Tumor Free Mice (Day 51)	No. Mice at Tumor End Point
A	700	–	1,380	–	–	–
B	1,710	–	1,360	–	–	–
C	1,050	39%	925	32	–	–
D	770	55%	505	63	–	–
E	155	91%	100	93	2/8	6/8
F	1,050	39%	675	50	–	7/8
G	1,070	37%	1,145	16	–	6/8
H	85	95%	25	98	4/8	3/8
I	75	96%	60	95	4/8	1/8
J	80	95%	5	99	4/8	0/8
K	1,900	–	2,125	–	–	–
L	1,115	41	1,090	49	1/6	–

* TGI = tumor growth inhibition; the median could only be calculated when fewer than 50% of the mice reached the tumor end point.

† Groups are as defined in Figures 33 and 36. For smaller initial tumor: A = vehicle (PBS); B = mouse IgG, 10 mg/kg; C = anti-CTLA-4, 0.25 mg/kg; D = anti-CTLA-4, 0.5 mg/kg; E = anti-CTLA-4, 5 mg/kg; F = anti-PD-1, 3 mg/kg; G = anti-PD-1, 10 mg/kg; H = anti-PD-1, 10 mg/kg with anti-CTLA-4, 0.25 mg/kg; I = anti-PD-1, 10 mg/kg with anti-CTLA-4, 0.5 mg/kg; and J = anti-PD-1, 3 mg/kg with anti-CTLA-4, 0.5 mg/kg. For larger initial tumor: K = mouse IgG, 10 mg/kg; and L = anti-PD-1, 10 mg/kg with anti-CTLA-4, 0.25 mg/kg.

Together these data indicate that the combination therapy comprising anti-PD-1 and anti-CTLA-4 antibodies is substantially more effective than treatment with either antibody alone. In addition, surprisingly the dose of each antibody can be reduced without affecting the synergistic efficacy of this combination of immunostimulatory therapeutic antibodies. The combination therapy still seems to be effective even when the tumor mass is more mature (*i.e.*, larger).

Example 18: Tumor Immunity in Mice Following anti-PD-1 Antibody Treatment and Re-Challenge with PD-L1$^-$ Fibrosarcoma Cells

Mice that survived tumor-free from a challenge with tumor cells and treatment with anti-PD-1 antibody (*i.e.*, treatment similar to the efficacy studies described in Examples 5 and 6) were then re-challenged with tumor cells to investigate immunity to tumor formation after such a treatment. Briefly, in the initial challenge, SA1/N fibrosarcoma cells (PD-L1$^-$) were implanted subcutaneously in A/J mice (1×10^6 cells/mouse) on day 0. On days 1, 4, 7, 10, 14, 17 and 20 post-implantation, groups of mice were injected IP with either mouse IgG (control, 10 mg/kg per mouse) or with one of various doses of anti-PD-1 monoclonal antibody 4H2 (30,

10, 3, 1 and 0.3 mg/kg per mouse). Tumor formation and volume was monitored with a precision electronic caliper twice a week until the study was complete. A group of 8 mice were tumor-free after the anti-PD1 antibody treatment (4 that were treated with 30 mg/kg, 2 with 3 mg/kg, one with 1 mg/kg, and one with 0.3 mg/kg).

The eight treated, tumor-free A/J mice were re-challenged by subcutaneously implanting 1 x 10^6 SA1/N fibrosarcoma cells/mouse. As a control, nine naïve mice were subcutaneously implanted with 1 x 10^6 SA1/N fibrosarcoma cells/mouse. Tumor formation and volume was monitored with a precision electronic caliper twice a week until day 62 post-implantation. All nine naïve (control) mice reached the tumor end-point by day 22 post-implantation of the fibrosarcoma cells. In contrast, the eight tumor-free mice re-challenged with fibrosarcoma cells did not develop tumors up to 62 days post-implantation. Figure 47 shows the mean tumor volume for the naïve and re-challenged mice. These results demonstrate that treatment with an immunostimulatory antibody, such as anti-PD-1, provides the treated subject with immunity to further tumor formation, even in the presence of cells capable of forming a tumor.

Example 19: Tumor Immunity in Mice Following Single Antibody Therapy (anti-PD-1) or Combination Antibody Therapy (anti-CTLA-4 and anti-PD-1 Re-Challenged with PD-L1$^-$ Colorectal Cancer Cells

Mice that survived tumor-free from a challenge with tumor cells and treatment with either anti-PD-1 antibody alone or anti-PD-1 antibody combined with anti-CTLA-4 antibody (*i.e.*, treatment similar to the efficacy studies described in Examples 2-4) were then re-challenged with tumor cells to investigate immunity to tumor formation after such treatments. Briefly, in the initial challenge, MC38 colorectal cancer cells (PD-L1$^-$) were implanted in C57BL/6 mice (2 x 10^6 cells/mouse) on day 0. On days 0, 3, 6 and 10 post-implantation, groups of mice were injected IP with one of the following treatments: (1) mouse IgG (control, 10 mg/kg per mouse), anti-PD-1 monoclonal antibody 4H2, or (3) anti-PD-1 monoclonal antibody 4H2 in combination with anti-CTLA-4 monoclonal antibody 9D9. Tumor growth was monitored with a precision electronic caliper as described in Example 15. A group of 11 mice were tumor-free after the anti-PD1 antibody treatment (2 total) or the combination anti-PD-1/anti-CTLA-4 antibody treatment (9 total).

The 11 treated, tumor-free C57BL/6 mice were re-challenged by implantation of 2 x 10^7 MC38 colorectal cancer cells/mouse (*i.e.*, a dose of cells 10 x greater than the initial challenge). As a control, seven naïve mice were implanted with 2 x 10^7 MC38 colorectal cancer cells/mouse. Tumor formation and volume was monitored with a precision electronic caliper for the duration of the re-challenge experiment (at least 20 days). Figure 48 shows that all seven naïve (control) mice developed a tumor and reached the tumor end-point by day 18 post-implantation of the colorectal cancer cells. In contrast, all 11 tumor-free mice re-challenged with colorectal cancer cells did not develop tumors up to 18 days post-implantation.

Figure 49 shows the mean tumor volume for the naïve and re-challenged mice. These data indicate that, similar to the antibody monotherapy, the combination antibody therapy resulting in PD-1 and CTLA-4 blockade produces a persistent immunity to tumor relapse.

Example 20: *In Vivo* Efficacy of Combination Therapy (anti-CTLA-4 and anti-PD-1 Antibodies) on Established Tumor Growth

CT26 colorectal cancer cells were implanted in BALB/Cmice (2×10^6 cells/mouse) for a time sufficient (about 10 days) to permit the formation of tumors. On day 10 post-implantation, tumor measurements were taken and mice were randomized based on mean tumor volume (about 250 mm^3) into 5 groups for subsequent antibody therapy. At day 0 (*i.e.*, 10 days after the CT26 cells were implanted), mice were injected IP with (1) mouse IgG (control), (2) anti-CTLA-4 monoclonal antibody 9D9, (3) anti-PD-1 monoclonal antibody 4H2, or (4) anti-CTLA-4 monoclonal antibody 9D9 and anti-PD-1 antibody monoclonal antibody 4H2, at a concentration of 10 mg/kg per mouse. Antibody injections were also administered on days 3, 6 and 10. The monoclonal antibody compositions used had low levels of endotoxin and did not significantly aggregate. Using an electronic caliper, the tumors were measured three dimensionally (height x width x length) and tumor volume was calculated. Tumor measurements were taken on day 0 (tumors at the beginning of treatment had a volume of about 125 mm^3), and on days 3, 6, 10, 13, 17 and 20 post-antibody injection. Mice were euthanized when the tumors reached a designated tumor end-point (a particular tumor volume such as 1500 mm^3 and/or when the mice showed greater than about 15% weight loss). The results are shown in Figure 50. This study indicates that, in a murine tumor model, treatment with the combination of CTLA-4 antibody and PD-1 antibody has a significantly greater effect on tumor growth than either antibody alone, even when a tumor is already well established.

Example 21: Effect of human anti-PD-1 antibody on function of T regulatory cells

T regulatory cells are lymphocytes that suppress the immune response. In this example, T regulatory cells were tested for its inhibitory function on proliferation and IFN-gamma secretion of CD4+CD25- T cells in the presence or absence of an anti-PD-1 human monoclonal antibody.

T regulatory cells were purified from PBMC using a CD4+CD25+ regulatory T cell isolation kit (Miltenyi Biotec). T regulatory cells were added into a mixed lymphocyte reaction (see above) containing purified CD4+CD25- T cells and allogeneic dendritic cells in a 2:1 ratio of CD4+CD25- to T regulatory cells. Anti-PD-1 monoclonal antibody 5C4 was added at a concentration of 10 μg/ml. Either no antibody or an isotype control antibody was used as a negative control. Culture supernatants were harvested on Day 5 for cytokine measurement using a Beadlyte cytokine detection system (Upstate). The cells were labeled with ^3H-thymidine, cultured for another 18 hours, and analyzed for cell proliferation. The results are shown in Figures 51A (T cell proliferation) and 51B (IFN-gamma secretion). The

addition of anti-PD-1 human monoclonal antibody 5C4 partially released inhibition imposed by Treg cells on proliferation and IFN-gamma secretion of CD4+CD25- T cells, indicating that anti-PD-1 antibodies have an effect on T regulatory cells.

Example 22: Effect of human anti-PD-1 antibody on T cell activation

In this example, effect of blockade of PD-1 pathway by anti-PD-1 antibody 5C4 on T cell activation was examined. Purified human CD4+ T cells (Dynal CD4 T cell purification kit) were activated with 1μg/ml soluble anti-CD3 antibody (BD) in the presence of autologous monocytes or monocyte-derived dendritic cells (DCs). Monocytes were purified using Miltenyi CD14 monocyte purification kit, and DCs was generated in vitro after culture of monocytes with GM-CSF and IL-4 (PeproTech) for 7 days. After three days of activation in the presence or absence of titrated anti-PD-1 antibody or irrelevant isotype control mAb, culture supernatants were harvested for ELISA analysis of IFNγ secretion while tritiated thymidine was added during the final 18 hours of the assay in order to measure T cell proliferation. The results shown in Figures 52A and 52B demonstrate that PD-1 blockade by anti-PD-1 antibody resulted in enhanced T cell proliferation and IFN-γ secretion. Synergic effect by anti-PD-1 antibody and anti-CTLA-4 antibody on T cell activation (specifically on IFN-γ secretion) in the presence of monocytes was also observed.

Example 23: Assessment of ADCC activity of anti-PD-1 antibody

In this example, an antibody-dependent cellular cytotoxicity (ADCC) assay was performed to evaluate whether anti-PD-1 antibody could induce ADCC to target cells. Two versions of 5C4, one with an Fc region of human IgG1 (5C4-IgG1) and the other with an Fc region of human IgG4 (5C4-IgG4), were tested in the assay. The Delfia Cell Cytotoxicity Kit from Perkin Elmer was used for the assay. Briefly, purified human CD4 T cells (Dynal CD4 T cell purification kit) were activated by plate-bound anti-CD3 antibody (BD) to induce PD-1 expression. Target activated CD4 T cells were then labeled with BATDA reagent. Labeled CD4 T cells were added to a V-bottom 96-well plate, followed by the addition of human PBMC (an effector to target (E/T) cell ratio of 50:1) and designed antibody. After incubation for 1 hour at 37°C, the plate was spun down. Supernatant was transferred into a flat bottom 96-well plate and the plate was read using a RubyStar plate reader. Results showed that 5C4-IgG4 did not mediate ADCC on activated CD4 T cells, while 5C4-IgG1 did mediate ADCC on activated CD4 T cells (Figure 53), indicating that ADCC activity is related to its Fc region of the anti-PD-1 antibody.

Example 24: Assessment of Complement-dependent Cytotoxicity of anti-PD-1 antibody

In this example, complement dependant cytotoxicity (CDC) of anti-PD-1 antibody was examined. Two versions of 5C4, one with Fc region of human IgG1 (5C4-IgG1) and the other with Fc region of human IgG4 (5C4-IgG4), were tested in the assay. Briefly, purified human

CD4 T cells (Dynal CD4 T cell purification kit) were activated by plate-bound anti-CD3 antibody (BD) to induce PD-1 expression. Serial dilutions of anti-PD-1 antibody (5C4) and control antibodies from 50 μg/mL to 640 pg/mL were tested for CDC in the presence of human complement (Quidel-A113). Alamar blue (Biosource International) was used to measure cytotoxicity. The plate was read on a fluorescent plate reader (EX530 EM590). Viable cell counts are proportional to fluorescence units. Results showed that neither 5C4-IgG1 or 5C4-IgG4 mediated CDC on activated CD4 T cells, while the positive control antibody (anti-HLA-ABC antibody) did (Figure 54).

Example 25: Assessment of PD-1 expression on human T cells

In this example, human PBMCs from different donors were examined for PD-1 expression on various cell subsets by FACS. Biotinylated anti-PD-1 antibody, which has displayed a much higher sensitivity than commercially available anti-PD-1 antibody on detection of PD-1 molecules on cell surface, was used in the assay. Bound antibody was detected using an PE-conjugated streptavidin. Flow cytometric analyses were performed using a FACScan flow cytometry (Becton Dickinson) and Flowjo software (Tree Star). PD-1 expression was detected on some peripheral human T cells, but not on B cells or monocytes. Further examination of T cell subsets indicates that PD-1 is expressed on CD4 and CD8 memory and effector T cells, but absent on naïve CD4 or CD8 T cells.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims. The invention is, therefore, to be limited only by the terms of the appended claims along with the full scope of equivalents to which the claims are entitled.

Claims

1. An isolated human monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody binds to PD-1 and wherein the antibody exhibits at least one of the following properties:
   a) binds to human PD-1 with a K_D of 1×10^{-7} M or less;
   b) does not substantially bind to human CD28, CTLA-4 or ICOS;
   c) increases T-cell proliferation in an Mixed Lymphocyte Reaction (MLR) assay;
   d) increases interferon-gamma production in an MLR assay; or
   e) increases interleukin-2 (IL-2) secretion in an MLR assay.

2. The antibody of claim 1 which is full-length antibody of an IgG1 or IgG4 isotype.

3. The antibody of claim 1 which is an antibody fragment or a single chain antibody.

4. The antibody of claim 1, wherein the antibody binds to human PD-1 with a K_D of 5×10^{-8} M or less.

5. The antibody of claim 1, wherein the antibody binds to human PD-1 with a K_D of 1×10^{-8} M or less.

6. The antibody of claim 1, wherein the antibody binds to human PD-1 with a K_D of 5×10^{-9} M or less.

7. The antibody of claim 1, wherein the antibody binds to human PD-1 with a K_D of between 1×10^{-8} M and 1×10^{-10} M.

8. An isolated human monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody cross-competes for binding to PD-1 with a reference antibody comprising:
   a) a human heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7; and
   b) a human light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14.

9. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 1 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 8.

10. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 2 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 9

11. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 3 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 10.

12. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 4 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 11.

13. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 5 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 12.

14. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 6 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 13.

15. The antibody of claim 8, wherein the human heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 7 and the human light chain variable region comprises the amino acid sequence of SEQ ID NO: 14.

16. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene, wherein the antibody specifically binds to PD-1.

17. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 4-39 gene, wherein the antibody specifically binds to PD-1.

18. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L6 gene, wherein the antibody specifically binds to PD-1.

19. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L15 gene, wherein the antibody specifically binds to PD-1.

20. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:
a) a heavy chain variable region of a human V_H 3-33 gene; and
b) a light chain variable region of a human Vk L6 gene;
wherein the antibody specifically binds to PD-1.

21. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:
a) a heavy chain variable region of a human V_H 4-39 gene; and
b) a light chain variable region of a human Vk L15 gene;
wherein the antibody specifically binds to PD-1.

22. An isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region that comprises CDR1, CDR2, and CDR3 sequences; and a light chain variable region that comprises CDR1, CDR2, and CDR3 sequences, wherein:
a) the heavy chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35, and conservative modifications thereof;
b) the light chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56, and conservative modifications thereof; and
c) the antibody specifically binds to PD-1.

23. The antibody of claim 22, wherein the heavy chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28, and conservative modifications thereof; and the light chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and conservative modifications thereof.

24. The antibody of claim 23, wherein the heavy chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, and conservative modifications thereof; and the light chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, and conservative modifications thereof.

25. An isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region and a light chain variable region, wherein:

a) the heavy chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7;
b) the light chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14;
c) the antibody binds to human PD-1 with a K_D of 1 x 10^{-7} M or less; and
d) the antibody does not substantially bind to human CD28, CTLA-4 or ICOS.

26. The antibody of claim 25, wherein the antibody further comprises at least one of the following properties:
a) the antibody increases T-cell proliferation in an Mixed Lymphocyte Reaction (MLR) assay;
b) the antibody increases interferon-gamma production in an MLR assay; or
c) the antibody increases interleukin-2 (IL-2) secretion in an MLR assay.

27. An isolated monoclonal antibody, or antigen-binding portion thereof, comprising:
a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21;
b) a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28;
c) a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35;
d) a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42;
e) a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49; and
f) a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56;
wherein the antibody specifically binds PD-1.

28. The antibody of claim 27, which comprises:
a) a heavy chain variable region CDR1 comprising SEQ ID NO: 15;
b) a heavy chain variable region CDR2 comprising SEQ ID NO: 22;
c) a heavy chain variable region CDR3 comprising SEQ ID NO: 29;
d) a light chain variable region CDR1 comprising SEQ ID NO: 36;
e) a light chain variable region CDR2 comprising SEQ ID NO: 43; and
f) a light chain variable region CDR3 comprising SEQ ID NO: 50.

29. The antibody of claim 27, which comprises:
    a) a heavy chain variable region CDR1 comprising SEQ ID NO: 16;
    b) a heavy chain variable region CDR2 comprising SEQ ID NO: 23;
    c) a heavy chain variable region CDR3 comprising SEQ ID NO:30;
    d) a light chain variable region CDR1 comprising SEQ ID NO: 37;
    e) a light chain variable region CDR2 comprising SEQ ID NO: 44; and
    f) a light chain variable region CDR3 comprising SEQ ID NO: 51.

30. The antibody of claim 27, which comprises:
    a) a heavy chain variable region CDR1 comprising SEQ ID NO: 17;
    b) a heavy chain variable region CDR2 comprising SEQ ID NO: 24;
    c) a heavy chain variable region CDR3 comprising SEQ ID NO: 31;
    d) a light chain variable region CDR1 comprising SEQ ID NO: 38;
    e) a light chain variable region CDR2 comprising SEQ ID NO: 45; and
    f) a light chain variable region CDR3 comprising SEQ ID NO: 52.

31. The antibody of claim 27, which comprises:
    a) a heavy chain variable region CDR1 comprising SEQ ID NO: 18;
    b) a heavy chain variable region CDR2 comprising SEQ ID NO: 25;
    c) a heavy chain variable region CDR3 comprising SEQ ID NO: 32;
    d) a light chain variable region CDR1 comprising SEQ ID NO: 39;
    e) a light chain variable region CDR2 comprising SEQ ID NO: 46; and
    f) a light chain variable region CDR3 comprising SEQ ID NO: 53.

32. The antibody of claim 27, which comprises:
    a) a heavy chain variable region CDR1 comprising SEQ ID NO: 19;
    b) a heavy chain variable region CDR2 comprising SEQ ID NO: 26;
    c) a heavy chain variable region CDR3 comprising SEQ ID NO: 33;
    d) a light chain variable region CDR1 comprising SEQ ID NO: 40;
    e) a light chain variable region CDR2 comprising SEQ ID NO: 47; and
    f) a light chain variable region CDR3 comprising SEQ ID NO: 54.

33. The antibody of claim 27, which comprises:
    a) a heavy chain variable region CDR1 comprising SEQ ID NO: 20;
    b) a heavy chain variable region CDR2 comprising SEQ ID NO: 27;
    c) a heavy chain variable region CDR3 comprising SEQ ID NO: 34;
    d) a light chain variable region CDR1 comprising SEQ ID NO: 41;
    e) a light chain variable region CDR2 comprising SEQ ID NO: 48; and
    f) a light chain variable region CDR3 comprising SEQ ID NO: 55.

34. The antibody of claim 27, which comprises:
    a) a heavy chain variable region CDR1 comprising SEQ ID NO: 21;
    b) a heavy chain variable region CDR2 comprising SEQ ID NO: 28;
    c) a heavy chain variable region CDR3 comprising SEQ ID NO: 35;
    d) a light chain variable region CDR1 comprising SEQ ID NO: 42;
    e) a light chain variable region CDR2 comprising SEQ ID NO: 49; and
    f) a light chain variable region CDR3 comprising SEQ ID NO: 56.

35. An isolated monoclonal antibody, or antigen-binding portion thereof comprising:
    a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6 and 7; and
    b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14;
    wherein the antibody specifically binds PD-1.

36. The antibody of claim 35, which comprises:
    a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1; and
    b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.

37. The antibody of claim 35, which comprises:
    a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and
    b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9.

38. The antibody of claim 35, which comprises:
    a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 3; and
    b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 10.

39. The antibody of claim 35, which comprises:
    a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4; and
    b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 11.

40. The antibody of claim 35, which comprises:
    a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5; and

b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 12.

41. The antibody of claim 35, which comprises:
a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6; and
b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 13.

42. The antibody of claim 35, which comprises:
a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and
b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14.

43. A composition comprising the antibody, or antigen-binding portion thereof, of any one of claims 1-36, and a pharmaceutically acceptable carrier.

44. An immunoconjugate comprising the antibody, or antigen-binding portion thereof, of any one of claims 1-36, linked to a therapeutic agent.

45. A composition comprising the immunoconjugate of claim 44 and a pharmaceutically acceptable carrier.

46. The immunoconjugate of claim 44, wherein the therapeutic agent is a cytotoxin.

47. A composition comprising the immunoconjugate of claim 46 and a pharmaceutically acceptable carrier.

48. The immunoconjugate of claim 44, wherein the therapeutic agent is a radioactive isotope.

49. A composition comprising the immunoconjugate of claim 48 and a pharmaceutically acceptable carrier.

50. A bispecific molecule comprising the antibody, or antigen-binding portion thereof, of any one of claims 1-36, linked to a second functional moiety having a different binding specificity than said antibody, or antigen-binding portion thereof.

51. A composition comprising the bispecific molecule of claim 50, and a pharmaceutically acceptable carrier.

52. An isolated nucleic acid molecule encoding the antibody, or antigen-binding portion thereof, of any one of claims 1-36.

53. An expression vector comprising the nucleic acid molecule of claim 52.

54. A host cell comprising the expression vector of claim 53.

55. A transgenic mouse comprising human immunoglobulin heavy and light chain transgenes, wherein the mouse expresses the antibody of any one of claims 1-36.

56. A hybridoma prepared from the mouse of claim 55, wherein the hybridoma produces said antibody.

57. A method of modulating an immune response in a subject comprising administering to the subject the antibody, or antigen-binding portion thereof, of any one of claims 1-36 such that the immune response in the subject is modulated.

58. A method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof.

59. The method of claim 58, wherein the antibody is a chimeric antibody.

60. The method of claim 58, wherein the antibody is a humanized antibody.

61. The method of claim 58, wherein the antibody is a fully human antibody.

62. The method of claim 58, wherein the tumor cells are of a cancer selected from the group consisting of melanoma, renal cancer, prostate cancer, breast cancer, colon cancer and lung cancer.

63. The method of claim 58, wherein the tumor cells are of a cancer selected from the list consisting of bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of

the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers.

64. A method of inhibiting growth of tumor cells in a subject, comprising administering to a subject the antibody, or antigen-binding portion thereof, of any one of claims 1-36 in an amount effective to inhibit growth of the tumor cells.

65. A method of treating an infectious disease in a subject comprising administering to the subject the antibody, or antigen-binding portion thereof, of any one of claims 1-36 such that the subject is treated for the infectious disease.

66. The method of claim 65, wherein the infectious disease is selected from the list consisting of: HIV, Influenza, Herpes, Giardia, Malaria, Leishmania, the pathogenic infection by the virus Hepatitis (A, B, & C), herpes virus (e.g., VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus, pathogenic infection by the bacteria chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lymes disease bacteria, pathogenic infection by the fungi Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum, and pathogenic infection by the parasites Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, and Nippostrongylus brasiliensis.

67. A method of enhancing an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) the antibody, or antigen-binding portion thereof, of any one of claims 1-36 such that an immune response to the antigen in the subject is enhanced.

68. The method of claim 67, wherein the antigen is a tumor antigen, a viral antigen, a bacterial antigen or an antigen from a pathogen.

69. A method for treating a hyperproliferative disease, which method comprises:
(a) administering a anti-PD-1 antibody to a subject, and
(b) administering a anti-CTLA-4 antibody to the subject.

70. The method of claim 69, wherein the anti-PD-1 antibody is administered at a subtherapeutic dose.

71. The method of claim 69, wherein the anti-CTLA-4 antibody is administered at a subtherapeutic dose.

72. The method of claim 69, wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are each administered at a subtherapeutic dose.

73. The method according to claim 69 wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are administered sequentially.

74. The method according to claim 73 wherein the anti-PD-1 antibody is administered before the anti-CTLA-4 antibody.

75. The method according to claim 73 wherein the anti-CTLA-4 antibody is administered before the anti-PD-1 antibody.

76. The method according to claim 73 wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are administered concurrently.

77. The method according to claim 73 wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are admixed as a single composition and administered concurrently.

78. The method according to claim 73 comprising administering to a subject (a) a composition comprising the anti-PD-1 antibody and a pharmaceutically acceptable

carrier and (b) a composition comprising the anti-CTLA-4 antibody and a pharmaceutically acceptable carrier.

79. The method of any one of claims 69 to 78 wherein the hyperproliferative disease is cancer.

80. The method of claim 79 wherein the cancer is colon cancer.

81. The method of any one of claims 68 to 79 wherein the subject is a human.

82. The method of any one of claims 68 to 79 wherein the anti-PD-1 antibody is a human sequence antibody.

83. The method of claim 82 wherein the anti-PD-1 human sequence antibody is a monoclonal antibody.

84. The method of claim 83 wherein the anti-PD-1 human sequence monoclonal antibody is 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4.

85. The method of any one of claims 68 to 79 wherein the anti-CTLA-4 antibody is a human sequence antibody.

86. The method of claim 85 wherein the anti-CTLA-4 human sequence antibody is a monoclonal antibody.

87. The method of claim 86 wherein the anti-CTLA-4 human sequence monoclonal antibody is 10D1 (MDX-010).

88. A method for altering an adverse event associated with treatment of a hyperproliferative disease with an immunostimulatory agent, which method comprises:
(a) administering an anti-PD-1 antibody to a subject, and
(b) administering a subtherapeutic dose of anti-CTLA-4 antibody to the subject.

89. The method of claim 86 wherein the anti-PD-1 antibody is administered in a subtherapeutic dose.

90. The method according to claim 88 wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are administered sequentially.

91. The method according to claim 90 wherein the anti-PD-1 antibody is administered before the anti-CTLA-4 antibody.

92. The method according to claim 90 wherein the anti-CTLA-4 antibody is administered before the anti-PD-1 antibody.

93. The method according to claim 88 wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are administered concurrently.

94. The method according to claim 88 wherein the anti-PD-1 antibody and the anti-CTLA-4 antibody are admixed as a single composition and administered concurrently.

95. The method according to claim 88 comprising administering to a subject (a) a composition comprising the anti-PD-1 antibody and a pharmaceutically acceptable carrier and (b) a composition comprising the anti-CTLA-4 antibody and a pharmaceutically acceptable carrier.

96. The method of any one of claims 88 to 95 wherein the hyperproliferative disease is cancer.

97. The method of claim 96 wherein the cancer is colon cancer.

98. The method of any one of claims 88 to 95 wherein the subject is a human.

99. The method of any one of claims 88 to 95 wherein the anti-PD-1 antibody is a human sequence antibody.

100. The method of claim 99 wherein the anti-PD-1 human sequence antibody is a monoclonal antibody.

101. The method of claim 100 wherein the anti-PD-1 human sequence monoclonal antibody is 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4.

102. The method of any one of claims 88 to 95 wherein the anti-CTLA-4 antibody is a human sequence antibody.

103. The method of claim 102 wherein the anti-CTLA-4 human sequence antibody is a monoclonal antibody.

104. The method of claim 103 wherein the anti-CTLA-4 human sequence monoclonal antibody is 10D1 (MDX-010).

105. A method for preparing an anti-PD-1 antibody comprising:
(a) providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs: 15, 16, 17, 18, 19, 20 and 21, a CDR2 sequence that is selected from the group consisting of SEQ ID NOs: 22, 23, 24, 25, 26, 27 and 28; and a CDR3 sequence that is selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34 and 35; or (ii) a light chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs: 36, 37, 38, 39, 40, 41 and 42, a CDR2 sequence that is selected from the group consisting of SEQ ID NOs: 43, 44, 45, 46, 47, 48 and 49, and a CDR3 sequence that is selected from the group consisting of SEQ ID NOs: 50, 51, 52, 53, 54, 55 and 56;

(b) altering at least one amino acid residue within at least one variable region antibody sequence, said sequence being selected from the heavy chain variable region antibody sequence and the light chain variable region antibody sequence, to create at least one altered antibody sequence; and

(c) expressing the altered antibody sequence as a protein.

Anti-PD1 17D8 VH

V segment: 3-33
D segment: unknown
J segment: JH4b

```
      Q   V   Q   L   V   E   S   G   G   D   V   V   Q   P   G   G   S   L
  1   CAG GTG CAG CTG GTG GAG TCT GGG GGA GAC GTG GTC CAG CCT GGG GGG TCC CTG

                                                          CDR1
                                                      ~~~~~~~~~~~~~~~~~~~~
      R   L   S   C   A   A   S   G   V   A   F   S   N   Y   G   M   H   W
 55   AGA CTC TCC TGT GCA GCG TCT GGA GTC GCC TTC AGT AAC TAT GGC ATG CAC TGG

                                                              CDR2
                                                          ~~~~~~~~~~~~~~~~~~~~
      V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   Y   D
109   GTC CGC CAG GCT CCC GGC AAG GGG CTG GAG TGG GTG GCA GTT ATC TGG TAT GAT

                    CDR2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      G   S   N   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R
163   GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC AGA

      D   N   S   K   N   M   L   Y   L   Q   M   N   S   L   R   A   E   D
217   GAC AAT TCC AAG AAC ATG CTC TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                        CDR3
                                    ~~~~~~~~~~~~~~~~
      T   A   M   Y   Y   C   A   R   N   D   D   Y   W   G   Q   G   T   L
271   ACG GCT ATG TAT TAC TGT GCG AGG AAC GAT GAC TAC TGG GGC CAG GGA ACC CTG

                                                  ↳ JH4b

      V   T   V   S   S
325   GTC ACC GTC TCC TCA
```

Figure 1A

RECTIFIED SHEET(RULE91)

Anti-PD1 17D8 VK

V segment: L6
J segment: JK4

```
      E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
  1   GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                              CDR1
                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
 55   GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                                  CDR2
                                                          ~~~~~~~~~~~~~~~~~~~~
      Q   Q   K   P   G   Q   A   P   R   L   I   I   Y   D   A   S   N   R
109   CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC ATC ATC TAT GAT GCA TCC AAC AGG

      CDR2
      ~~~~~~~
      A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163   GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                      CDR3
                                                                      ~~~~~~~
      L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217   CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

              CDR3
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~
      R   S   N   W   P   L   T   F   G   G   G   T   K   V   E   I   K
271   CGT AGC AAC TGG CCT CTC ACT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
                              ↳
```

Figure 1B

RECTIFIED SHEET(RULE91)

Anti-PD-1 2D3 VH

V segment: 3-33
D segment: 7-27
J segment: JH4b

```
      Q   V   Q   L   V   E   S   G   G   D   V   V   Q   P   G   R   S   L
  1  CAG GTG CAG CTG GTG GAA TCT GGG GGA GAC GTG GTC CAG CCT GGG AGG TCC CTG

                                                          CDR1
                                                      ~~~~~~~~~~~~~~~~~~~
      R   L   S   C   A   A   S   G   L   T   F   T   N   Y   G   F   H   W
 55  AGA CTC TCC TGT GCA GCG TCT GGA TTA ACC TTC ACT AAC TAT GGC TTC CAC TGG

                                                              CDR2
                                                          ~~~~~~~~~~~~~~~~~~~
      V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   Y   D
109  GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCT GTT ATA TGG TAT GAT

                      CDR2
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      G   S   K   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R
163  GGA AGT AAG AAA TAT TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA

      D   N   S   K   N   T   L   Y   L   Q   M   N   N   L   R   A   E   D
217  GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AAC CTG AGA GCC GAG GAC

                                          CDR3
                                      ~~~~~~~~~~~~~~~~
      T   A   V   Y   Y   C   A   T   G   D   D   Y   W   G   Q   G   T   L
271  ACG GCT GTG TAT TAC TGT GCG ACT GGG GAT GAC TAC TGG GGC CAG GGA ACC CTG

      V   T   V   S   S
325  GTC ACC GTC TCC TCA
```

Figure 2A

RECTIFIED SHEET(RULE91)

Anti-PD-1 2D3 VK

V segment: L6
J segment: JK4

```
     E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1    GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                CDR1
                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55   GCC ACC CTG TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                                CDR2
                                                            ~~~~~~~~~~~~~~~~~~~
     Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   T   S   N   R
109  CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT ACA TCC AAC AGG

     CDR2
     ~~~~~~~
     A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163  GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                    CDR3
                                                                    ~~~~~~~
     L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217  CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

             CDR3
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     R   S   N   W   P   L   T   F   G   G   G   T   K   V   E   I   K
271  CGT AGC AAC TGG CCG CTC ACT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
```

Figure 2B

RECTIFIED SHEET(RULE91)

Anti-PD-1 4H1 VH

V segment: 3-33
D segment: undetermined
J segment: JH4b

```
      Q   V   Y   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
  1   CAG GTG TAC TTG GTA GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                          CDR1
                                                      ~~~~~~~~~~~~~~~~~~~
      R   L   S   C   A   A   S   G   F   T   F   S   N   Y   G   M   H   W
 55   AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AGT AAC TAT GGC ATG CAC TGG

                                                              CDR2
                                                          ~~~~~~~~~~~~~~~~~~~
      V   R   Q   A   P   G   K   G   L   E   W   V   A   L   I   W   Y   D
109   GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA CTT ATA TGG TAT GAT

                    CDR2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      G   S   N   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R
163   GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA

      D   N   S   K   N   T   L   Y   L   Q   M   T   S   L   R   V   E   D
217   GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG ACC AGT CTG AGA GTC GAG GAC

                                        CDR3
                                    ~~~~~~~~~~~~~~~~
      T   A   V   Y   Y   C   A   S   N   V   D   H   W   G   Q   G   T   L
271   ACG GCT GTG TAT TAT TGT GCG AGC AAC GTT GAC CAT TGG GGC CAG GGA ACC CTG

      V   T   V   S   S
325   GTC ACC GTC TCC TCA
```

Figure 3A

RECTIFIED SHEET(RULE91)

Anti-PD-1 4H1 VK

V segment: L6
J segment: JK1

```
     E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1    GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                CDR1
                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55   GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGT AGT TAC TTA GCC TGG TAC

                                                            CDR2
                                                    ~~~~~~~~~~~~~~~~~~~~
     Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109  CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

     CDR2
     ~~~~~~~
     A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163  GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                CDR3
                                                                ~~~~~~~
     L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217  CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

             CDR3
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~
     S   S   N   W   P   R   T   F   G   Q   G   T   K   V   E   I   K
271  AGT AGC AAC TGG CCT CGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
```

Figure 3B

RECTIFIED SHEET(RULE91)

Anti-PD1 5C4 VH

V segment: 3-33
D segment: unknown
J segment: JH4b

```
     Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
  1  CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                         CDR1
                                                     ~~~~~~~~~~~~~~~~~~~
     R   L   D   C   K   A   S   G   I   T   F   S   N   S   G   M   H   W
 55  AGA CTC GAC TGT AAA GCG TCT GGA ATC ACC TTC AGT AAC TCT GGC ATG CAC TGG

                                                             CDR2
                                                         ~~~~~~~~~~~~~~~~~~~
     V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   Y   D
109  GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTT ATT TGG TAT GAT

                    CDR2
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     G   S   K   R   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R
163  GGA AGT AAA AGA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA

     D   N   S   K   N   T   L   F   L   Q   M   N   S   L   R   A   E   D
217  GAC AAT TCC AAG AAC ACG CTG TTT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                        CDR3
                                    ~~~~~~~~~~~~~~~~
     T   A   V   Y   Y   C   A   T   N   D   D   Y   W   G   Q   G   T   L
271  ACG GCT GTG TAT TAC TGT GCG ACA AAC GAC GAC TAC TGG GGC CAG GGA ACC CTG
                                            |→
                                            JH4b

     V   T   V   S   S
325  GTC ACC GTC TCC TCA
```

Figure 4A

RECTIFIED SHEET(RULE91)

Anti-PD1 5C4 VK

V segment: L6
J segment: JK1

```
      E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
  1   GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                              CDR1
                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
 55   GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGT AGT TAC TTA GCC TGG TAC

                                                          CDR2
                                                      ~~~~~~~~~~~~~~~~~~~~
      Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109   CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

      CDR2
      ~~~~~~~
      A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163   GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                  CDR3
                                                                  ~~~~~~~
      L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217   CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

              CDR3
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~
      S   S   N   W   P   R   T   F   G   Q   G   T   K   V   E   I   K
271   AGT AGC AAC TGG CCT CGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
                          └→
```

Figure 4B

RECTIFIED SHEET(RULE91)

Anti-PD-1 4A11 VH

V segment: 4-39
D segment: 3-9
J segment: JH4b

```
        Q   L   Q   L   Q   E   S   G   P   G   L   V   K   P   S   E   T   L
1       CAG CTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCG GAG ACC CTG

                                                        CDR1
                                                        ~~~~~~~~~~~~~~~~~~~~~~~
        S   L   T   C   T   V   S   G   G   S   L   S   R   S   S   F   F   W
55      TCC CTC ACC TGC ACT GTC TCT GGT GGC TCC CTC AGC AGG AGT AGT TTC TTC TGG

        CDR1                                                        CDR2
        ~~~~                                                        ~~~~~~~~~~~
        G   W   I   R   Q   P   P   G   K   G   L   E   W   I   G   S   I   Y
109     GGC TGG ATC CGT CAG CCC CCA GGG AAG GGA CTG GAG TGG ATT GGG AGT ATC TAT

                        CDR2
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        Y   S   G   S   T   Y   Y   N   P   S   L   K   S   R   V   T   I   S
163     TAT AGT GGG AGC ACC TAC TAC AAC CCG TCC CTC AAG AGT CGA GTC ACC ATA TCC


        V   D   T   S   K   N   Q   F   S   L   K   L   S   S   V   T   A   A
217     GTA GAC ACG TCC AAG AAC CAG TTC TCC CTG AAG CTG AGC TCT GTG ACC GCC GCA

                                                CDR3
                                                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        D   T   A   V   Y   Y   C   V   R   D   Y   D   I   L   T   G   D   E
271     GAC ACG GCT GTG TAT TAC TGT GTG AGA GAT TAC GAT ATT TTG ACT GGC GAC GAG

        CDR3
        ~~~~~~~
        D   Y   W   G   Q   G   T   L   V   T   V   S   S
325     GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTG TCC TCA
```

Figure 5A

RECTIFIED SHEET(RULE91)

Anti-PD-1 4A11 VK

V segment: L15
J segment: JK1

```
        D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R
1       GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT GCA TCT GTG GGA GAC AGA

                                CDR1
                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        V   S   I   T   C   R   A   S   Q   G   I   S   S   W   L   A   W   Y
55      GTC TCC ATC ACT TGT CGG GCG AGT CAG GGT ATT AGC AGC TGG TTA GCC TGG TAT

                                                                CDR2
                                                            ~~~~~~~~~~~~~~~~~~
        Q   Q   K   P   E   K   A   P   K   S   L   I   Y   A   A   S   N   L
109     CAG CAG AAA CCA GAG AAA GCC CCT AAG TCC CTG ATC TAT GCT GCA TCC AAT TTA

        CDR2
        ~~~~~~~
        R   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163     CGA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

                                                                        CDR3
                                                                    ~~~~~~~
        L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217     CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGC CAA CAG

                CDR3
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~
        Y   Y   S   Y   P   R   T   F   G   Q   G   T   K   V   E   I   K
271     TAT TAT AGT TAC CCT AGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
```

Figure 5B

RECTIFIED SHEET(RULE91)

Anti-PD-1 7D3 VH

V segment: 3-33
D segment: 7-27
J segment: JH4b

```
     Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
1    CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                        CDR1
                                                    ~~~~~~~~~~~~~~~~~~~
     R   L   S   C   T   T   S   G   I   T   F   S   S   Y   G   F   H   W
55   AGA CTC TCC TGT ACA ACG TCT GGA ATC ACC TTC AGT AGC TAT GGC TTT CAC TGG

                                                            CDR2
                                                        ~~~~~~~~~~~~~~~~~~~
     V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   Y   D
109  GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTG ATA TGG TAT GAT

                    CDR2
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     G   S   K   K   Y   Y   A   D   S   V   K   G   R   F   T   L   S   R
163  GGA AGT AAA AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC CTC TCC AGA

     D   D   S   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D
217  GAC GAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                        CDR3
                                    ~~~~~~~~~~~~~~~~
     T   A   V   Y   Y   C   V   T   G   D   D   Y   W   G   Q   G   T   L
271  ACG GCT GTG TAT TAC TGT GTT ACT GGG GAT GAC TAC TGG GGC CAG GGA ACC CTG

     V   T   V   S   S
325  GTC ACC GTC TCC TCA
```

Figure 6A

RECTIFIED SHEET(RULE91)

Anti-PD-1 7D3 VK

V segment: L6
J segment: JK4

```
        E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1       GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                CDR1
                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55      GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                                CDR2
                                                            ~~~~~~~~~~~~~~~~~~~~
        Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109     CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

        CDR2
        ~~~~~~~
        A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163     GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                        CDR3
                                                                        ~~~~~~~
        L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217     CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

                CDR3
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        R   S   N   W   P   L   T   F   G   G   G   T   K   V   E   I   K
271     CGT AGC AAC TGG CCT CTC ACT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
```

Figure 6B

RECTIFIED SHEET(RULE91)

Anti-PD-1 5F4 VH

V segment: 4-39
D segment: 3-9
J segment: JH4b

```
      Q   L   Q   L   Q   E   S   G   P   G   L   V   K   P   S   E   T   L
1     CAG CTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCG GAG ACC CTG

                                                            CDR1
                                                    ~~~~~~~~~~~~~~~~~~~~~~~
      S   L   T   C   S   V   S   G   G   S   L   S   R   S   S   Y   F   W
55    TCC CTC ACC TGC TCT GTC TCT GGT GGC TCC CTC AGC AGG AGT AGT TAC TTC TGG

      CDR1                                                          CDR2
      ~~~                                                           ~~~~~~~~~~~
      G   W   I   R   Q   P   P   G   K   G   L   E   W   I   A   S   I   F
109   GGC TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG TGG ATT GCG AGT ATC TTT

                        CDR2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      Y   S   G   E   T   Y   F   N   P   S   L   K   S   R   V   T   I   S
163   TAT AGT GGG GAA ACC TAC TTC AAT CCG TCC CTC AAG AGT CGA GTC ACC ATA TCC


      V   D   T   S   R   N   Q   F   S   L   K   L   S   S   V   T   A   A
217   GTA GAC ACG TCC AGG AAC CAG TTC TCC CTG AAG CTG AGC TCT GTG ACC GCC GCA

                                                    CDR3
                                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      D   T   A   V   Y   Y   C   A   R   D   Y   D   I   L   T   G   D   E
271   GAC ACG GCT GTG TAT TAC TGT GCG AGA GAT TAC GAT ATT TTG ACT GGC GAC GAG

      CDR3
      ~~~~~~~
      D   Y   W   G   Q   G   T   L   V   T   V   S   S
325   GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

Figure 7A

RECTIFIED SHEET(RULE91)

Anti-PD-1 5F4 VK

V segment: L15
J segment: JK1

```
         D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R
1        GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT GCA TCT GTA GGA GAC AGA

                                 CDR1
                             ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
         V   T   I   T   C   R   A   S   Q   G   I   S   S   W   L   A   W   Y
55       GTC ACC ATC ACT TGT CGG GCG AGT CAG GGT ATT AGC AGC TGG TTA GCC TGG TAT

                                                                 CDR2
                                                             ~~~~~~~~~~~~~~~~~~~
         Q   Q   K   P   E   K   A   P   K   S   L   I   Y   A   A   S   S   L
109      CAG CAG AAA CCA GAG AAA GCC CCT AAG TCC CTG ATC TAT GCT GCA TCC AGT TTG

         CDR2
         ~~~~~~~
         Q   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163      CAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

                                                                         CDR3
                                                                         ~~~~~~~
         L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217      CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGC CAA CAG

                 CDR3
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~
         Y   Y   S   Y   P   R   T   F   G   Q   G   T   K   V   E   I   K
271      TAT TAT AGT TAC CCT AGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
```

Figure 7B

RECTIFIED SHEET(RULE91)

RECTIFIED SHEET (RULE 91)

Anti-PD1 17D8, 2D3, 4H1, 5C4 and 7D3 VH region

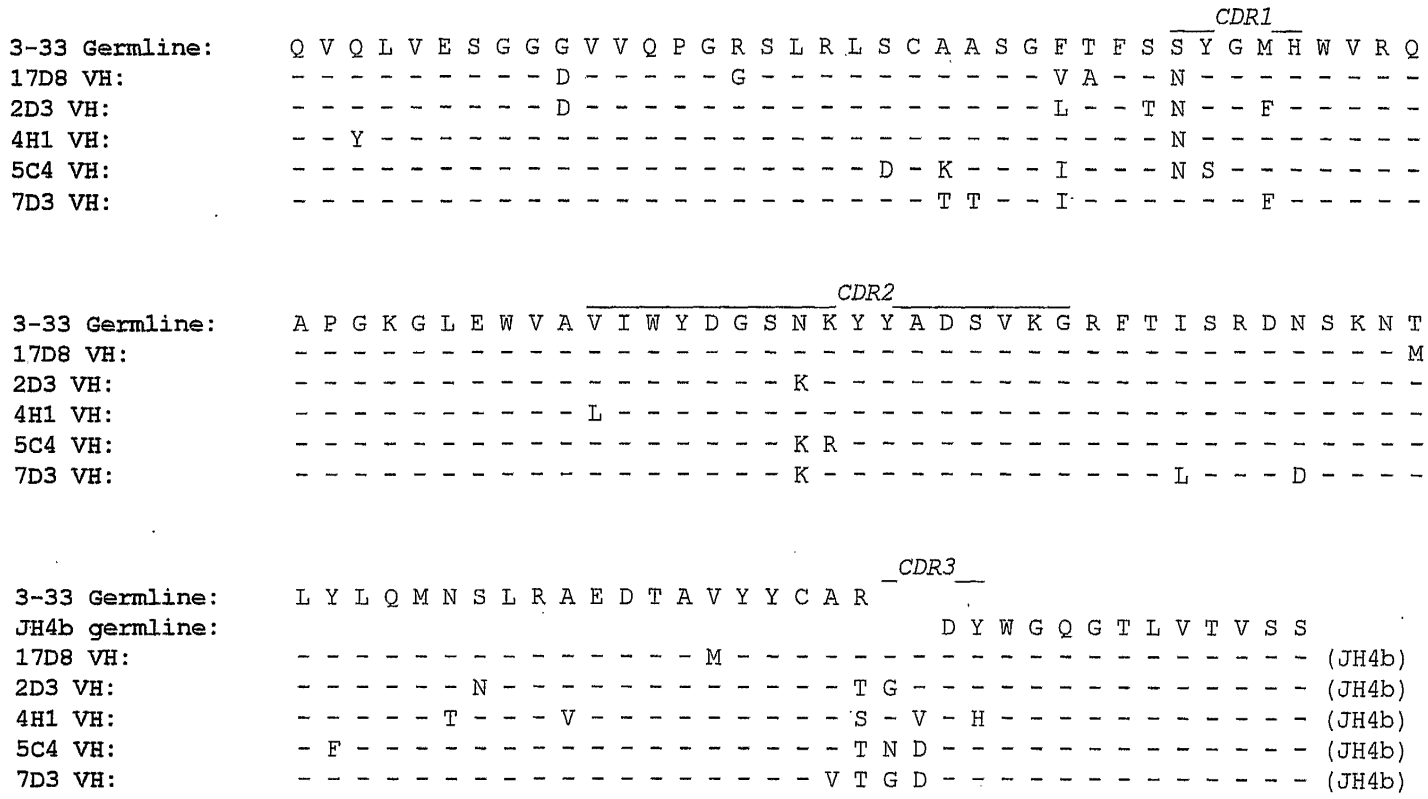


Figure 8

Anti-PD-1 17D8, 2D3 and 7D3 VK Region

```
                                                                                                                  CDR1
L6 germline: E I V L T Q S P A T L S L S P G E R A T L S C R A S Q S V S S Y L A
17D8 VK:     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
2D3 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
7D3 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                CDR2
L6 germline: W Y Q Q K P G Q A P R L L I Y D A S N R A T G I P A R F S G S G S G
17D8 VK:     - - - - - - - - - - - - I - - - - - - - - - - - - - - - - - - - - - - -
2D3 VK:      - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - - - - -
7D3 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                                CDR3
L6 germline: T D F T L T I S S L E P E D F A V Y Y C Q Q R S N W P
JK4 germline:                                                                   L T F G G G T
17D8 VK:     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
2D3 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
7D3 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

JK4 germline: K V E I K
17D8 VK:     - - - - - (JK4)
2C3 VK:      - - - - - (JK4)
7D3 VK:      - - - - - (JK4)
```

Figure 9

RECTIFIED SHEET(RULE91)

Anti-PD-1 4H1 and 5C4 VK Region

```
                                                                                                  CDR1
L6 germline: E I V L T Q S P A T L S L S P G E R A T L S C R A S Q S V S S Y L A
4H1 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
5C4 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                  CDR2
L6 germline: W Y Q Q K P G Q A P R L L I Y D A S N R A T G I P A R F S G S G S G
4H1 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
5C4 VK:      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                  CDR3
L6 germline: T D F T L T I S S L E P E D F A V Y Y C Q Q R S N W P
JK1 germline:                                                                W T F G Q G T
4H1 VK:      - - - - - - - - - - - - - - - - - - - - - - S - - - - R - - - - - -
5C4 VK:      - - - - - - - - - - - - - - - - - - - - - - S - - - - R - - - - - -

JK1 germline:K V E I K
4H1 VK:      - - - - - (JK1)
5C4 VK:      - - - - - (JK1)
```

Figure 10

RECTIFIED SHEET(RULE91)

Anti-PD-1 4A11 and 5F4 VH regions

```
                                                                          __CDR1_____
4-39 germline:   Q L Q L Q E S G P G L V K P S E T L S L T C T V S G G S I S S S S Y Y W
4A11 VH:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - L - R - - F F -
5F4 VH:          - - - - - - - - - - - - - - - - - - - - - - S - - - - - L - R - - - F -

                                                 CDR2
                 _                              ____________________________________
4-39 germline:   G W I R Q P P G K G L E W I G S I Y Y S G S T Y Y N P S L K S R V T I S
4A11 VH:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
5F4 VH:          - - - - - - - - - - - - - - A - - F - - - E - - F - - - - - - - - - - -

                                                                       ______CDR3______
4-39 germline:   V D T S K N H F S L K L S S V T A A D T A V Y Y C A R
4A11 VH:         - - - - - - Q - - - - - - - - - - - - - - - - - - V - D Y D I L T G D E
5F4 VH:          - - - - R - - - - - - - - - - - - - - - - - - - - - - D Y D I L T G D E

                 ___
JH4b germline:   D Y W G Q G T L V T V S S
4A11 VH:         - - - - - - - - - - - - - (JH4b)
5F4 VH:          - - - - - - - - - - - - - (JH4b)
```

Figure 11

RECTIFIED SHEET (RULE 91)

Anti-PD-1 4A11 and 5F4 VK region

```
                                                                                                  ____CDR1_______
L15 germline:    D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q G I S S
4A11 VK:         - - - - - - - - - - - - - - - - - - - S - - - - - - - - - - -
5F4 VK1:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                 ___CDR2______
L15 germline:    W L A W Y Q Q K P E K A P K S L I Y A A S S L Q S G V P S R F
4A11 VK:         - - - - - - - - - - - - - - - - - - - - - N - R - - - - - - -
5F4 VK1:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                                  __CDR3___
L15 germline:    S G S G S G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y N S
4A11 VK:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Y -
5F4 VK1:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Y -

                 _______
L15 germline:    Y P
JK1 germline:          T F G Q G T K V E I K
4A11 VK:         - - R - - - - - - - - - - - (JK1)
5F4 VK1:         - - R - - - - - - - - - - - (JK1)
```

Figure 12

RECTIFIED SHEET(RULE91)

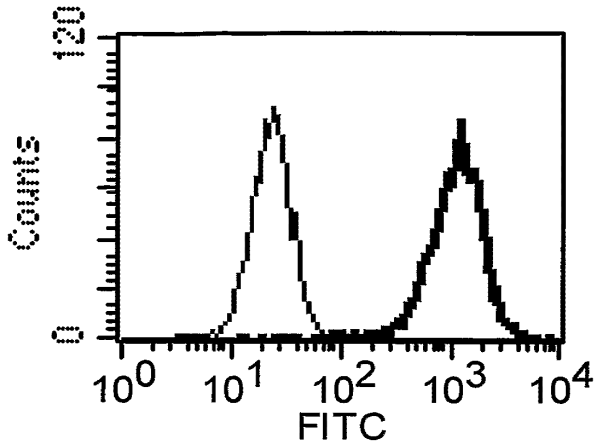


Figure 13A

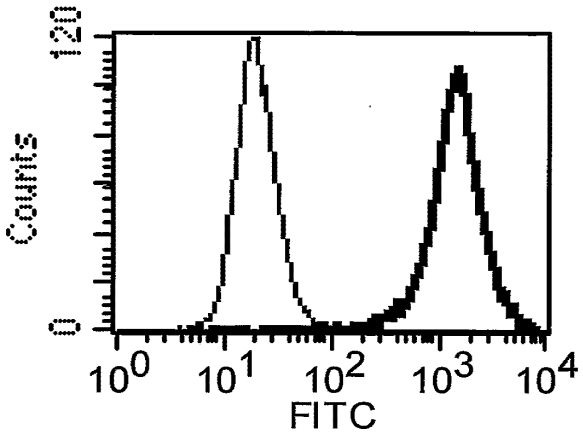


Figure 13B

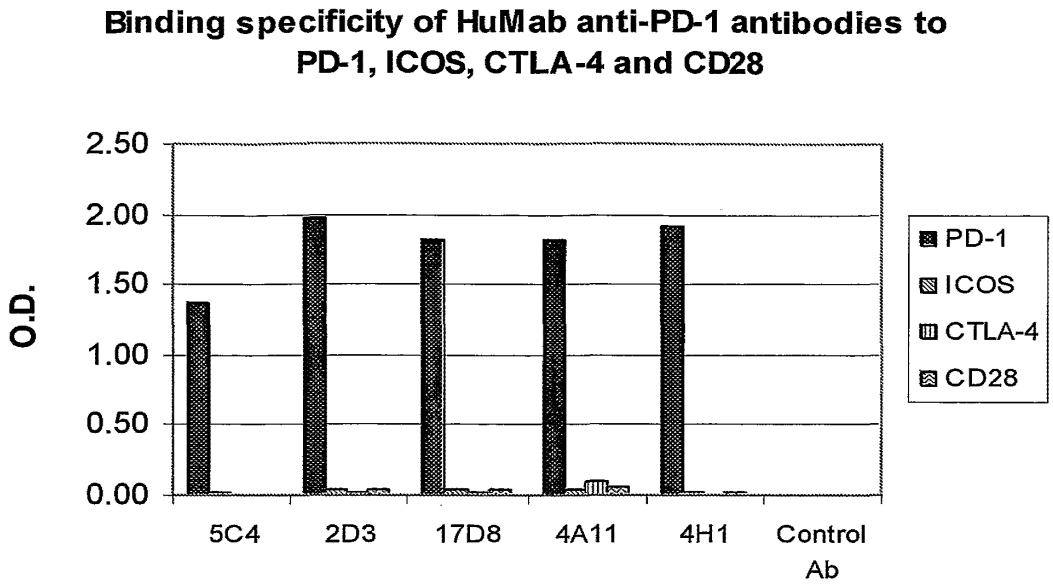

Binding specificity of HuMab anti-PD-1 antibodies to PD-1, ICOS, CTLA-4 and CD28
O.D.
2.50
2.00
1.50
1.00
0.50
0.00
5C4
2D3
17D8
4A11
4H1
Control Ab
PD-1
ICOS
CTLA-4
CD28

Figure 14

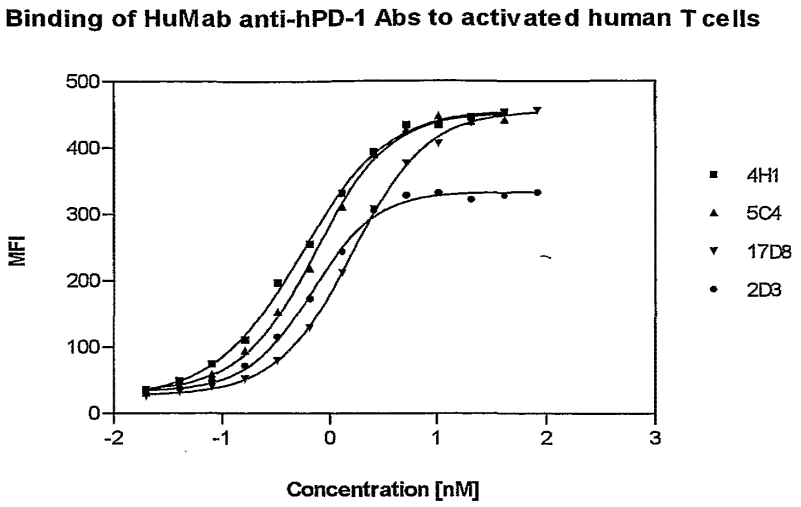


Figure 15A

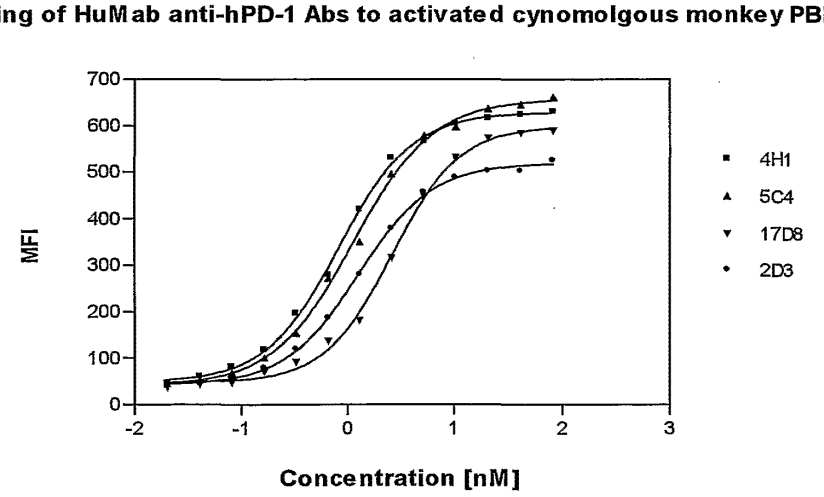


Figure 15B

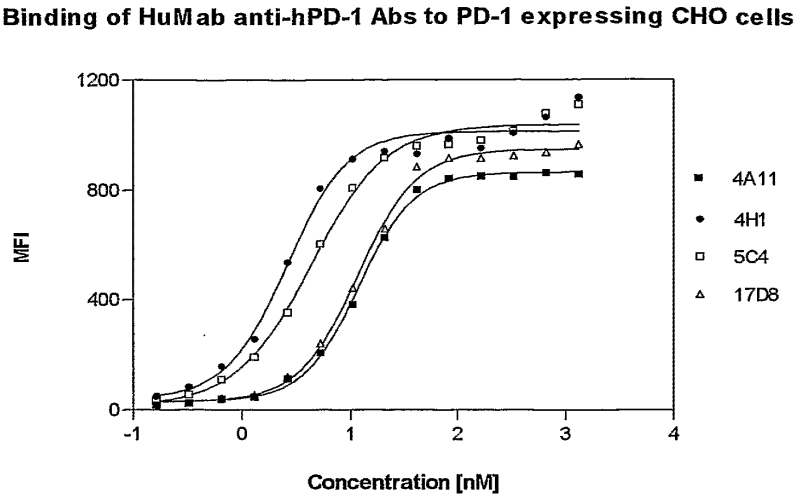

Binding of HuMab anti-hPD-1 Abs to PD-1 expressing CHO cells
MFI
1200
800
400
0
-1
0
1
2
3
4
Concentration [nM]
4A11
4H1
5C4
17D8

Figure 15C

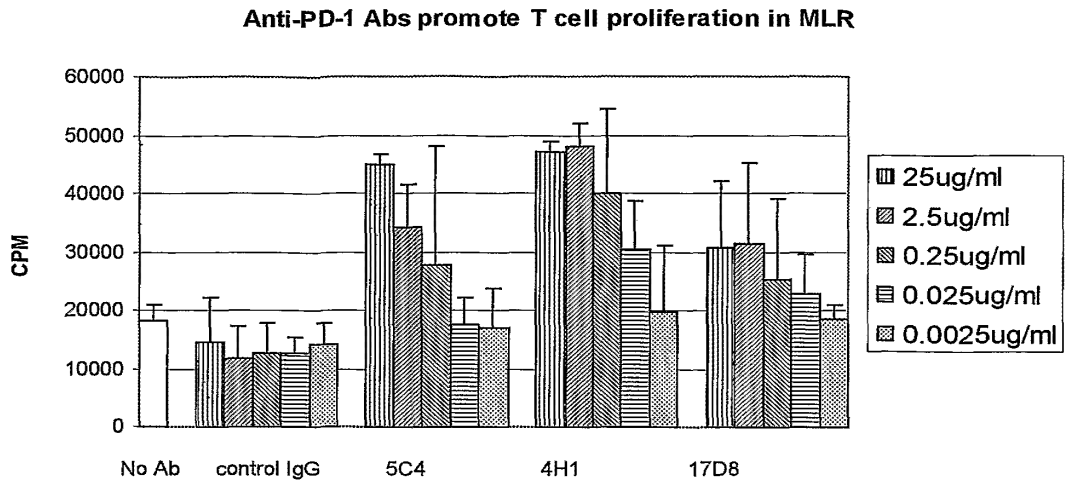


Figure 16A

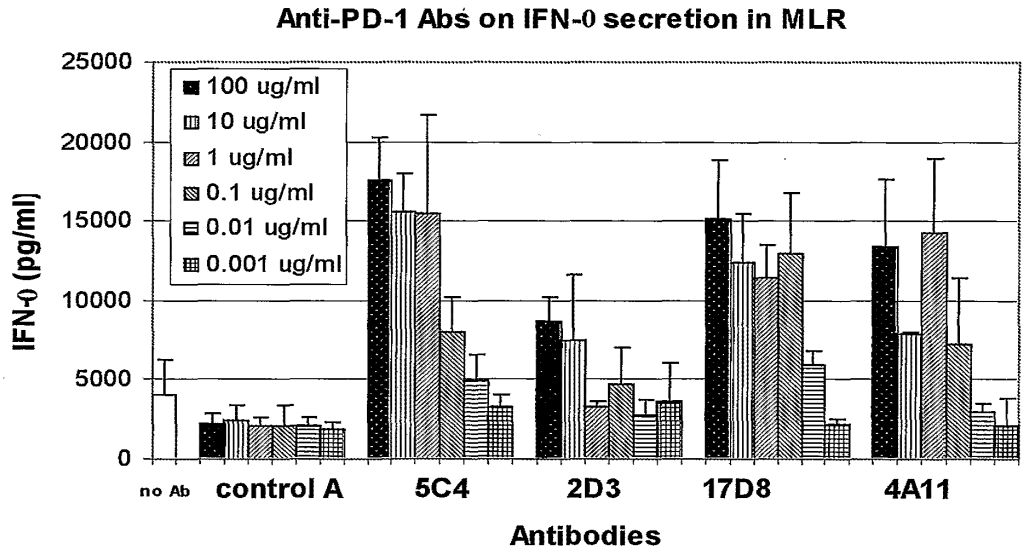


Figure 16B

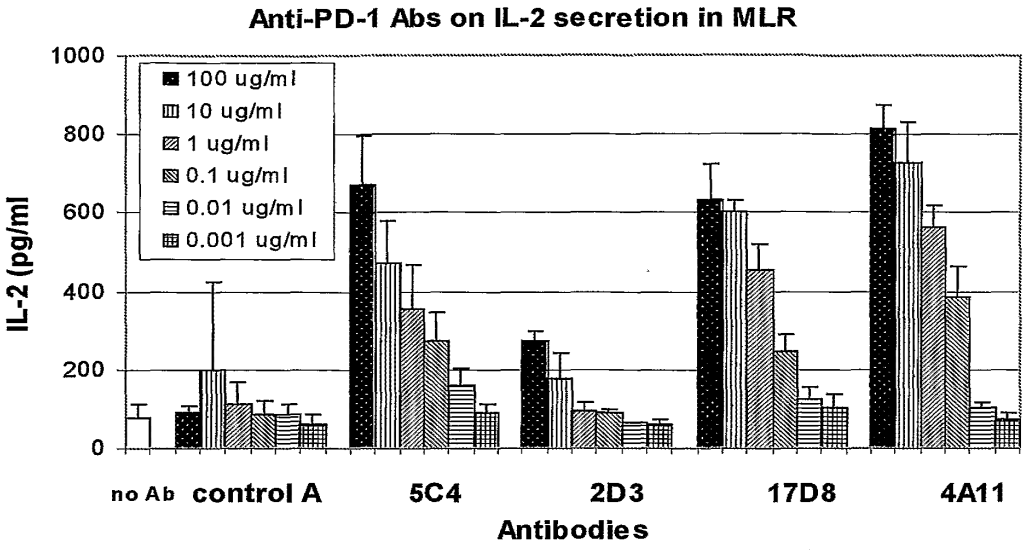

Anti-PD-1 Abs on IL-2 secretion in MLR
IL-2 (pg/ml)
1000
800
600
400
200
0
100 ug/ml
10 ug/ml
1 ug/ml
0.1 ug/ml
0.01 ug/ml
0.001 ug/ml
no Ab
control A
5C4
2D3
17D8
4A11
Antibodies

Figure 16C

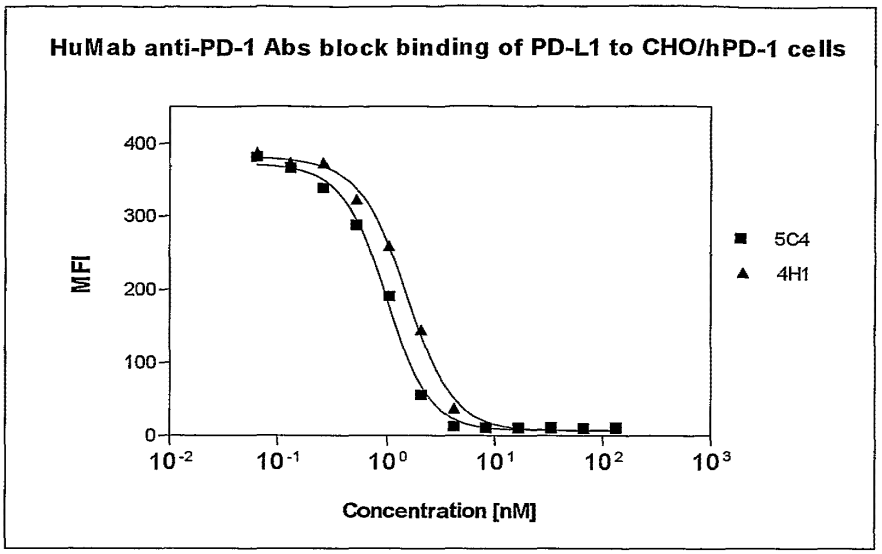


Figure 17A

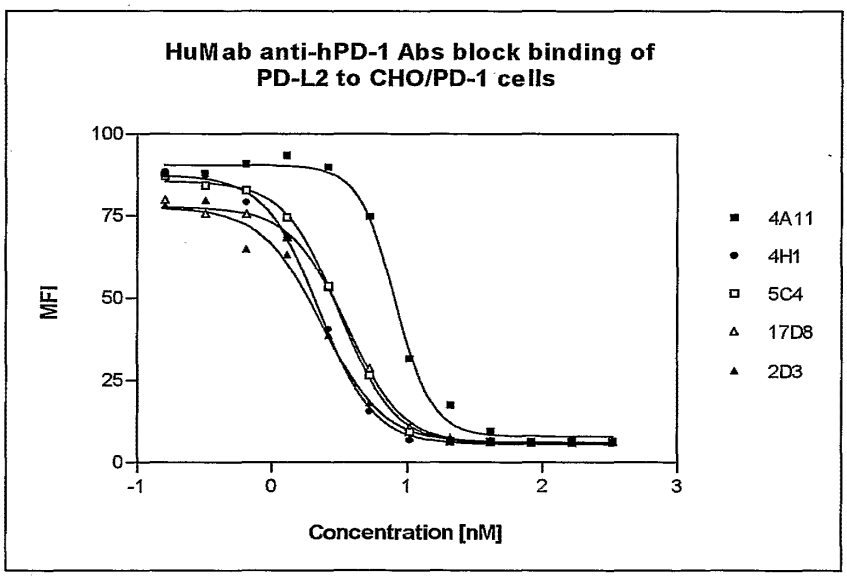


Figure 17B

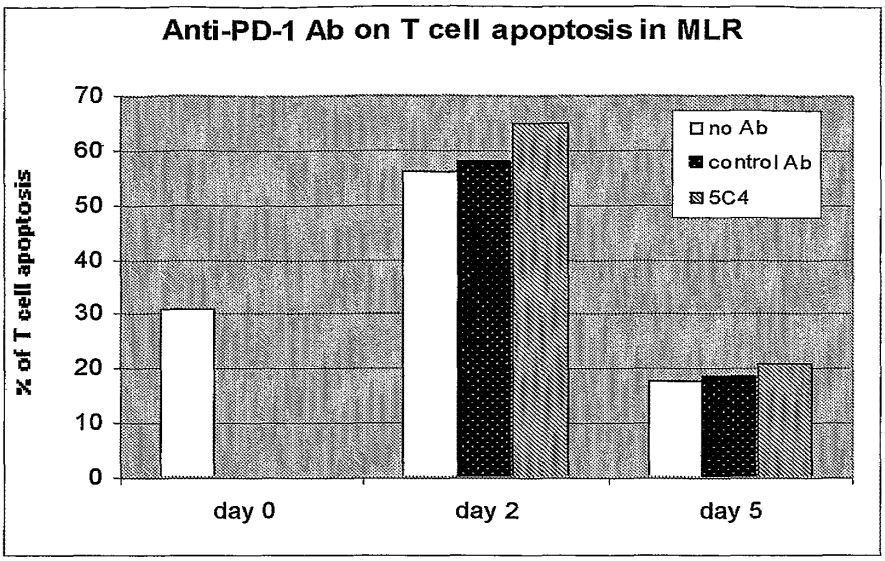


Figure 18

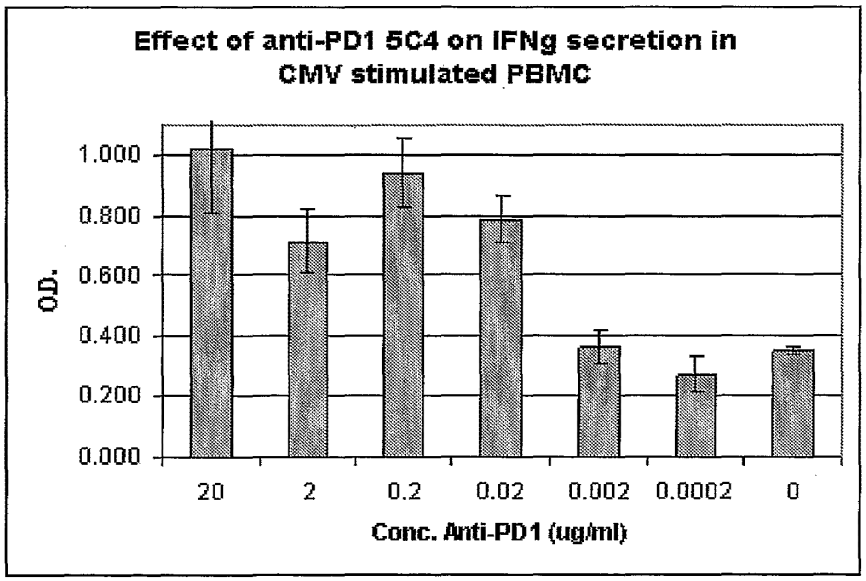


Figure 19

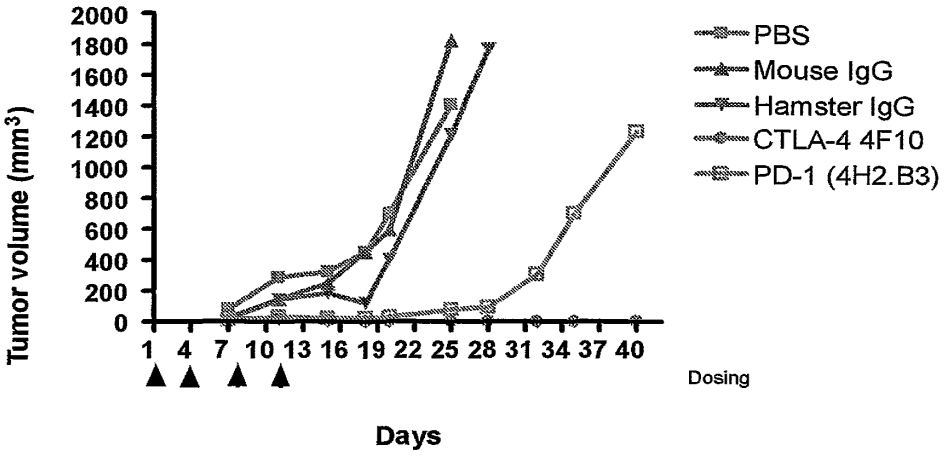

Tumor volume (mm3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40
Dosing
Days
PBS
Mouse IgG
Hamster IgG
CTLA-4 4F10
PD-1 (4H2.B3)

Figure 20

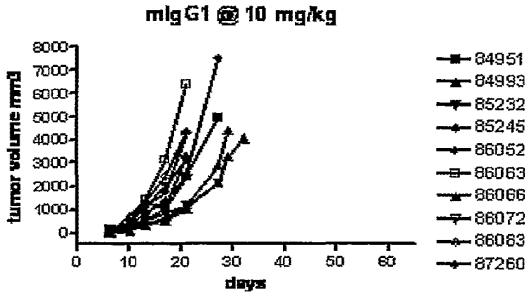


Figure 21A

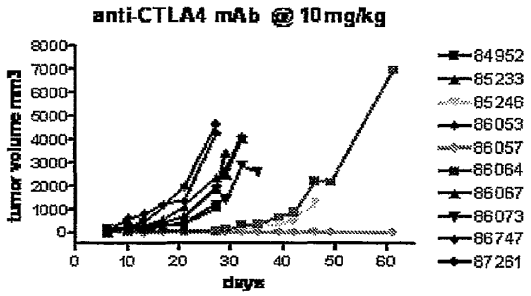


Figure 21B

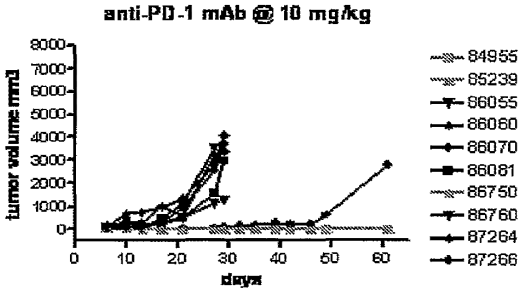


Figure 21C

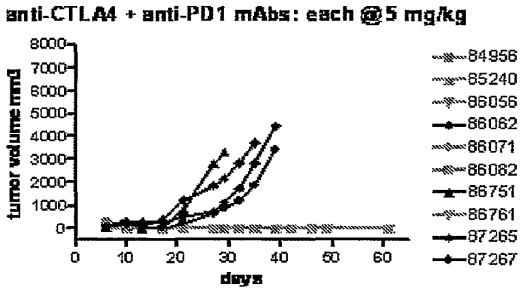


Figure 21D

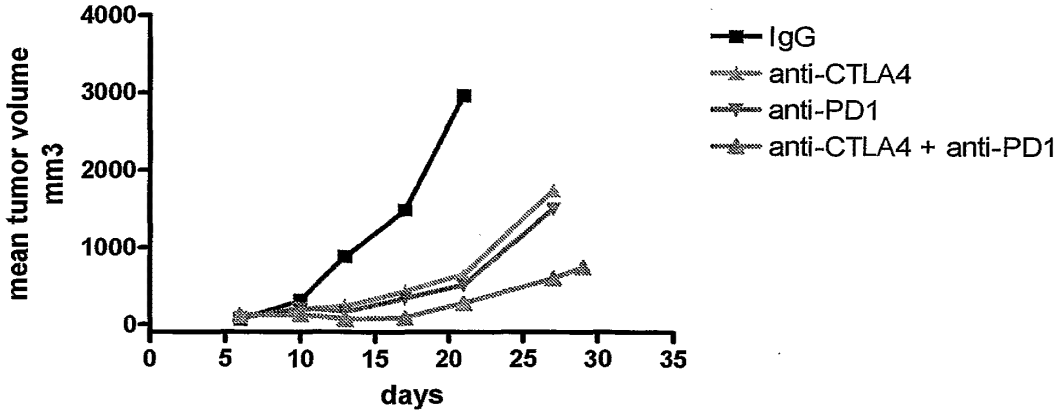


Figure 22

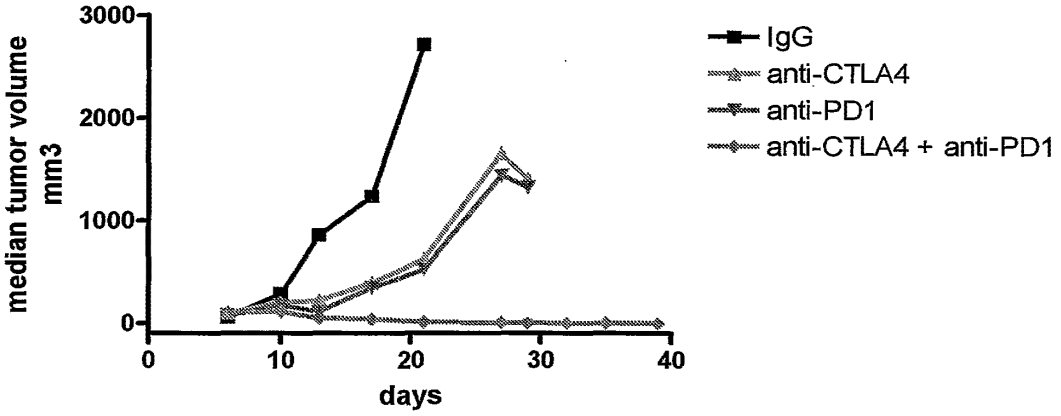


Figure 23

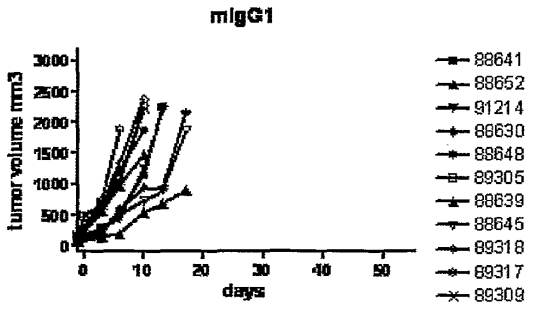


Figure 24A

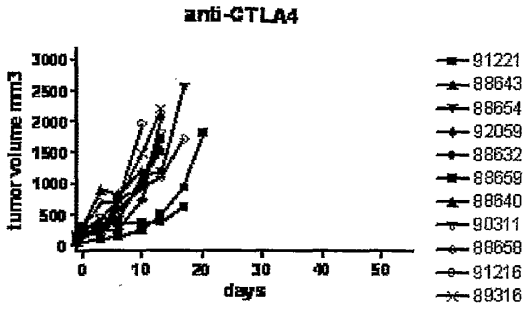


Figure 24B

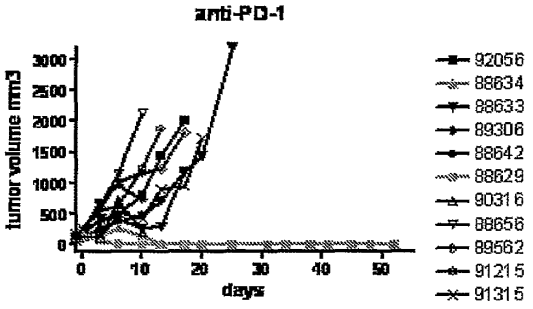


Figure 24C

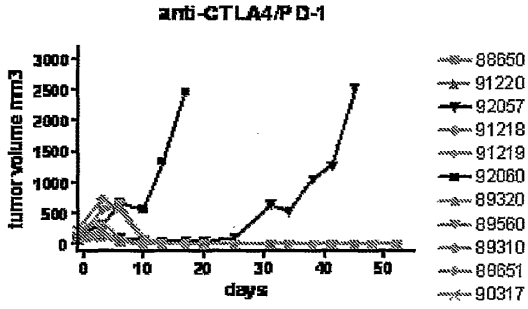


Figure 24D

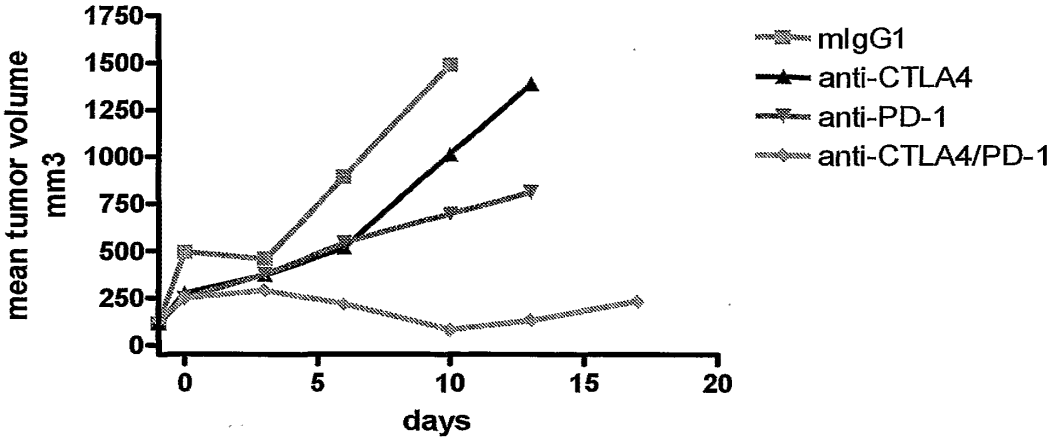


Figure 25

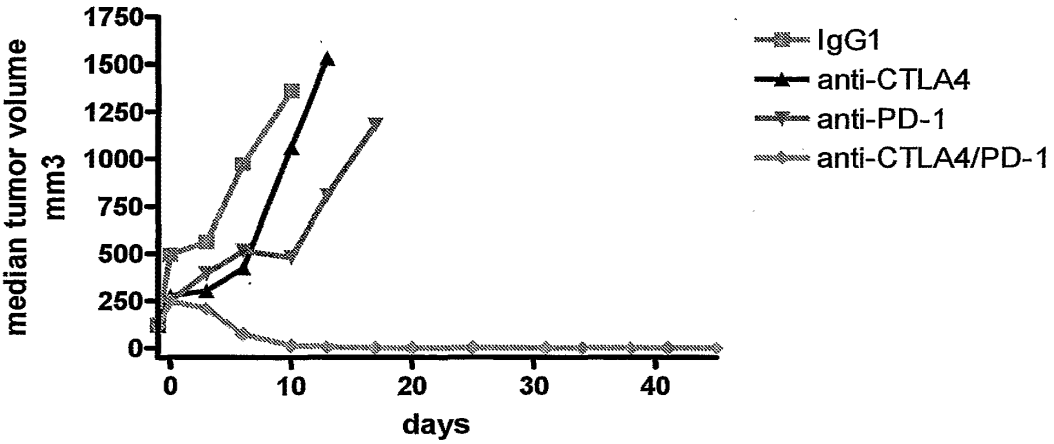


Figure 26

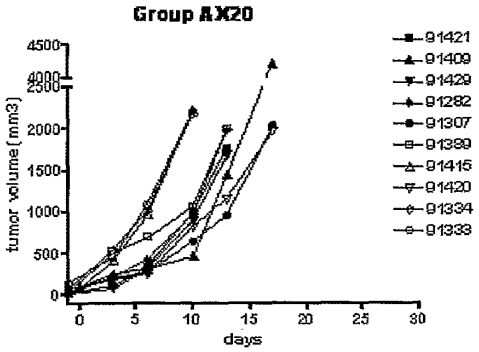


Figure 27A

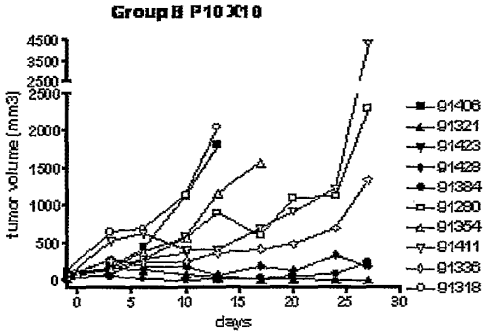


Figure 27B

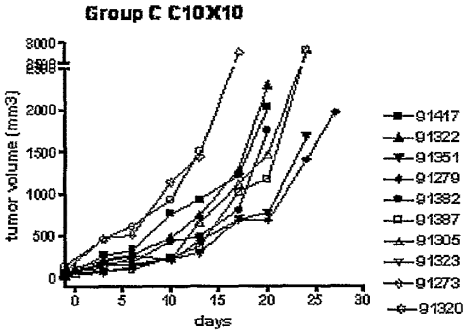


Figure 27C

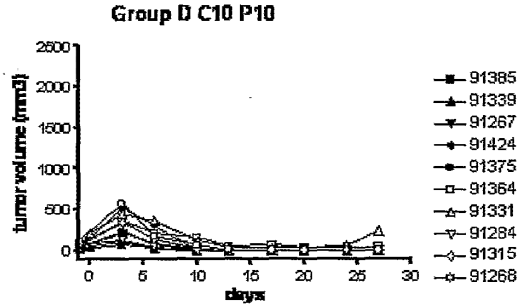
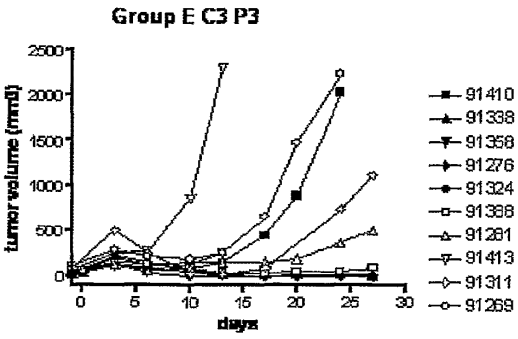


Figure 27D



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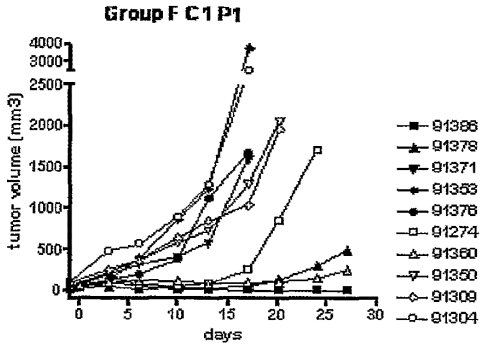

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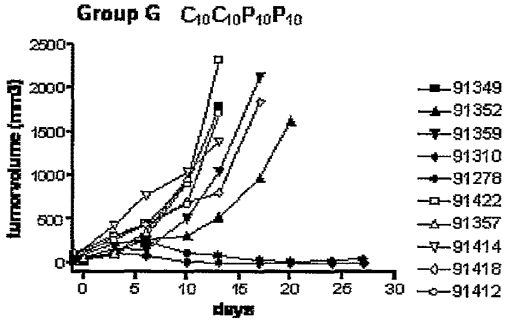

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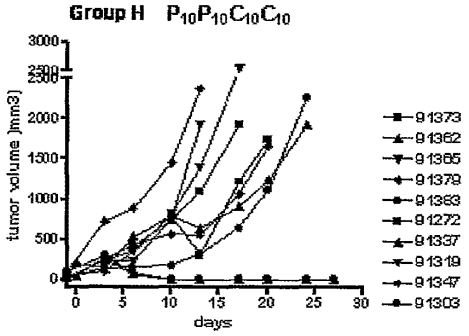

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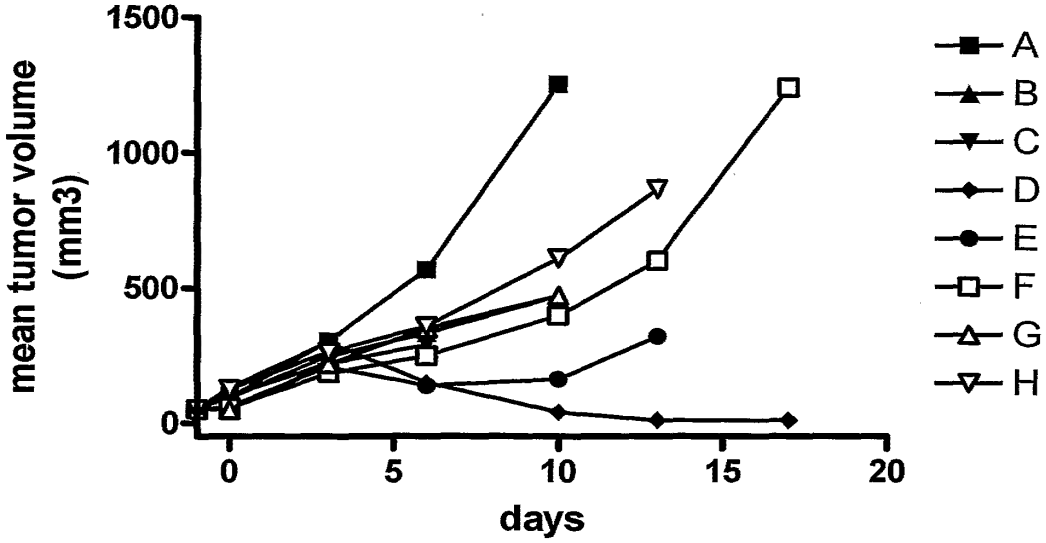

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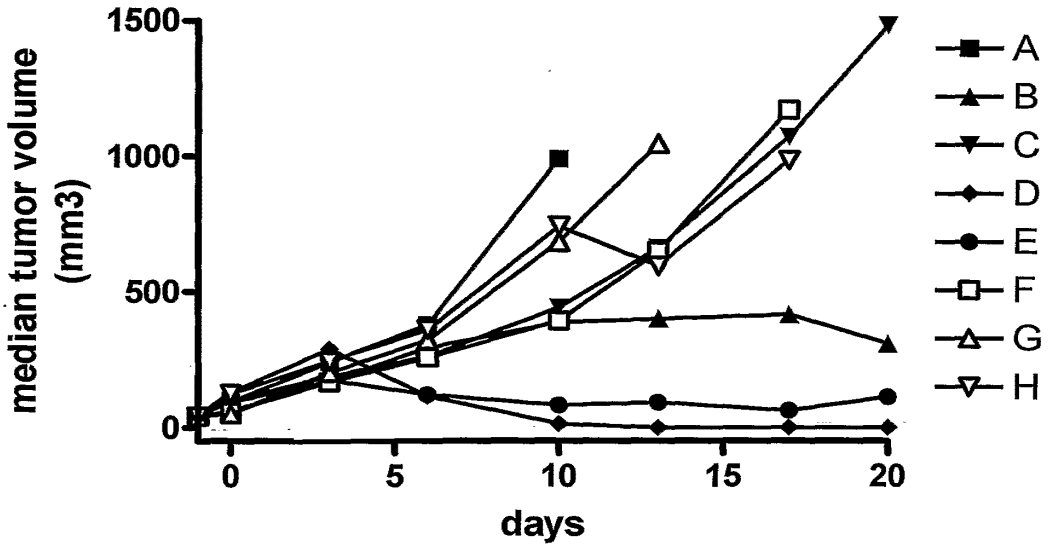

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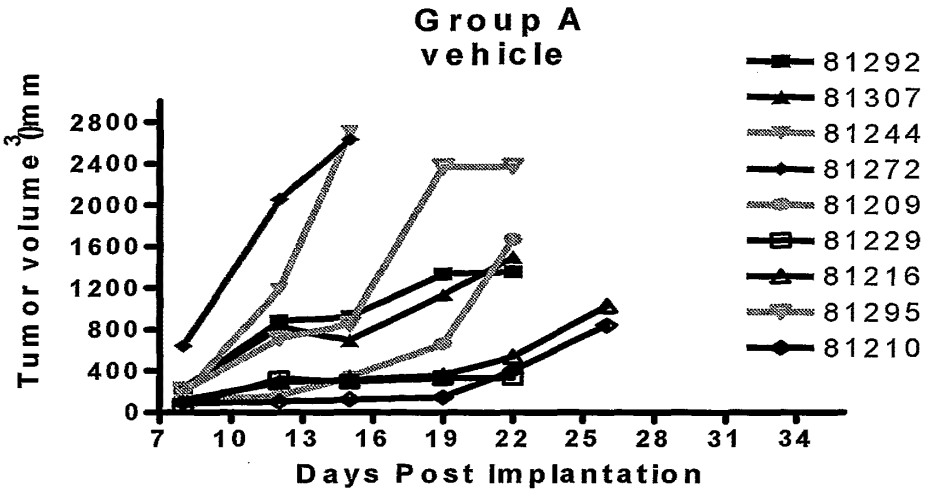

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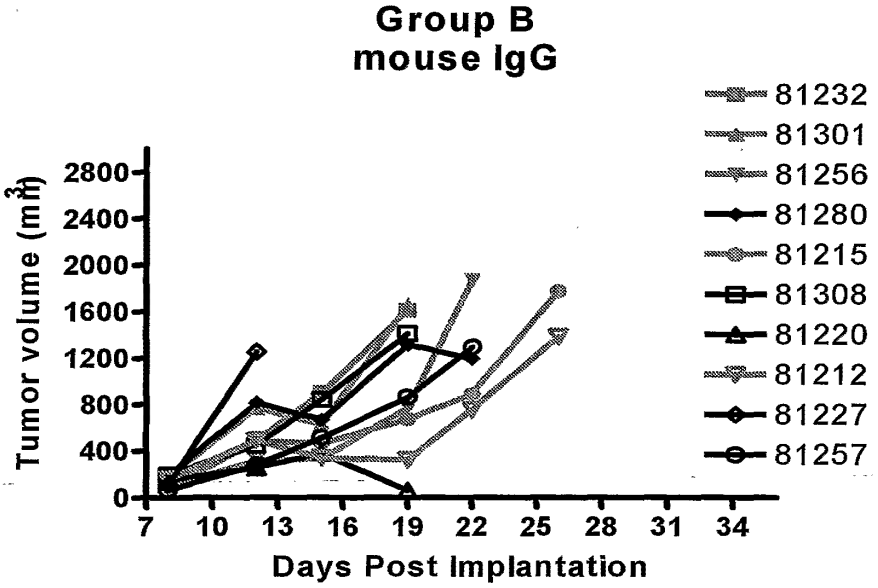

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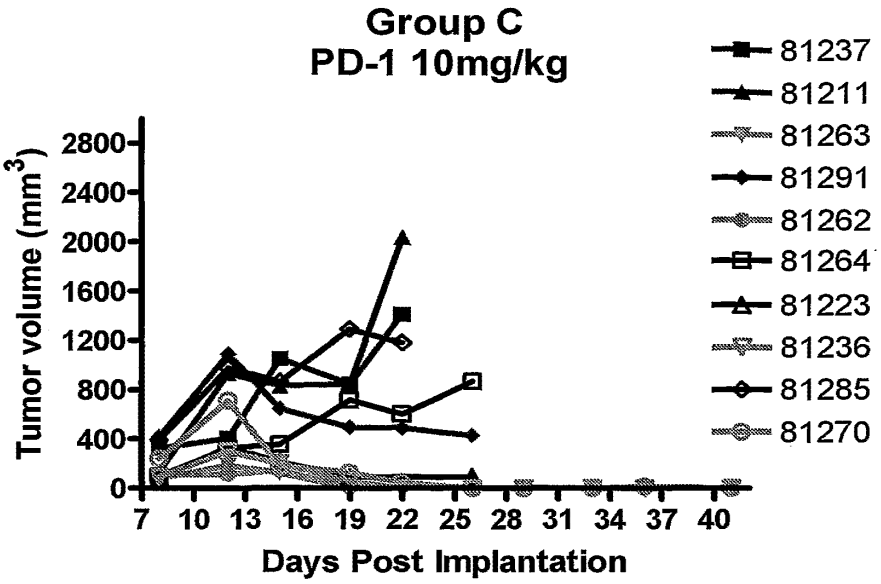

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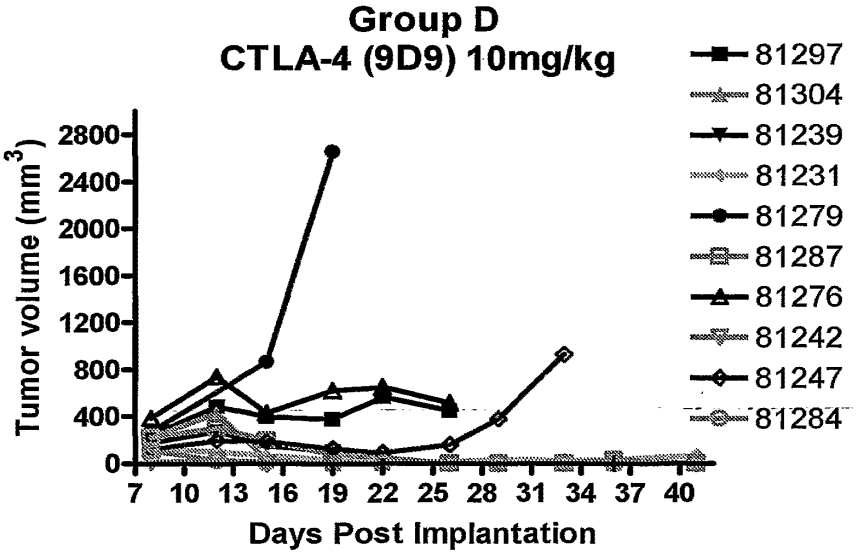

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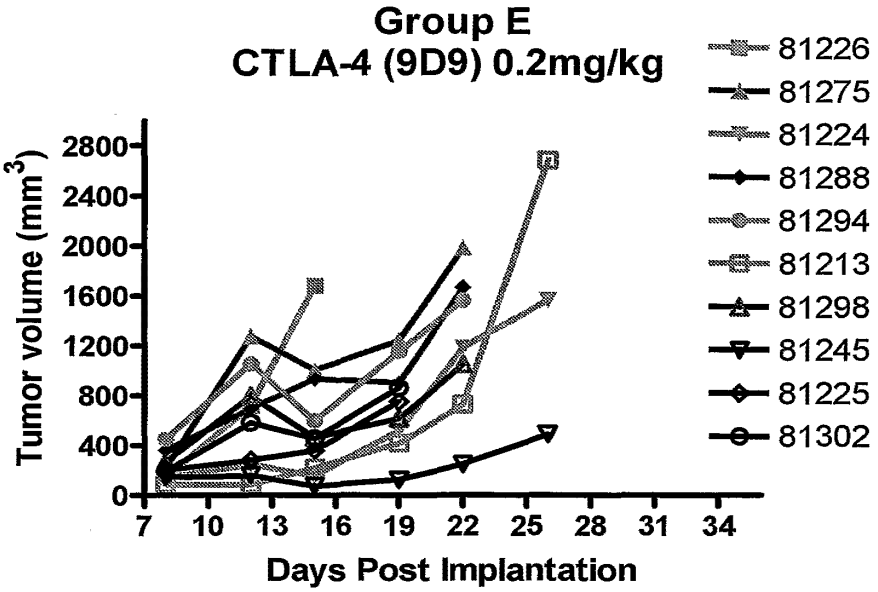

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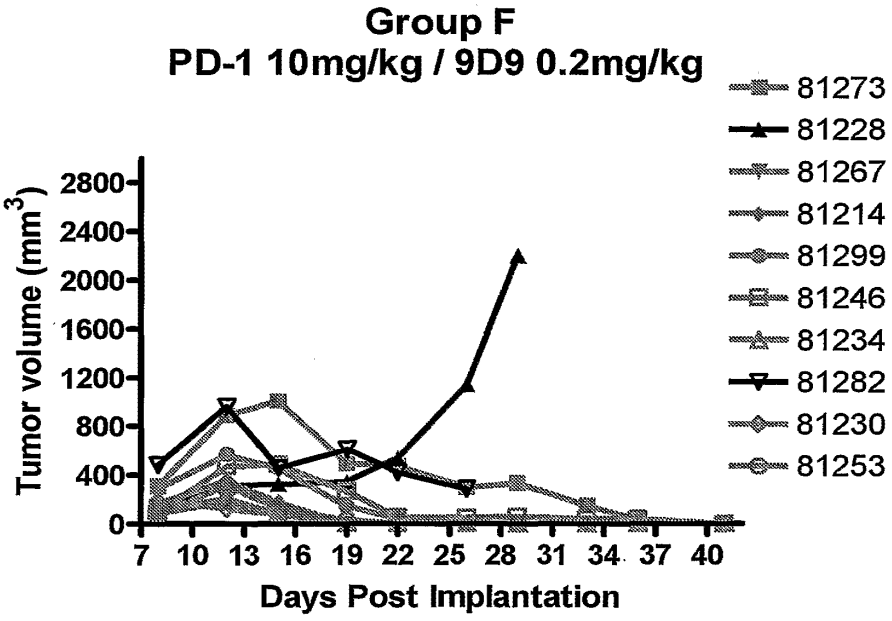

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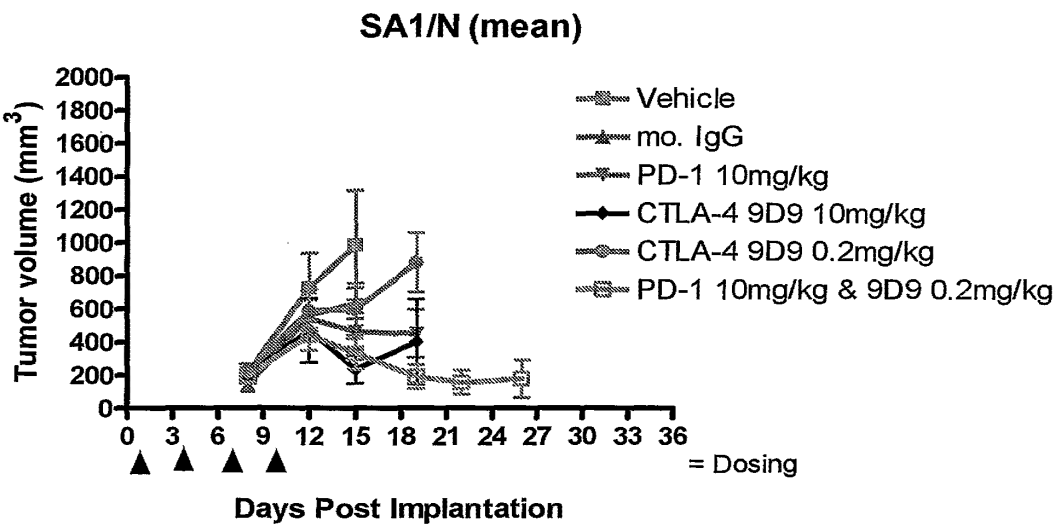

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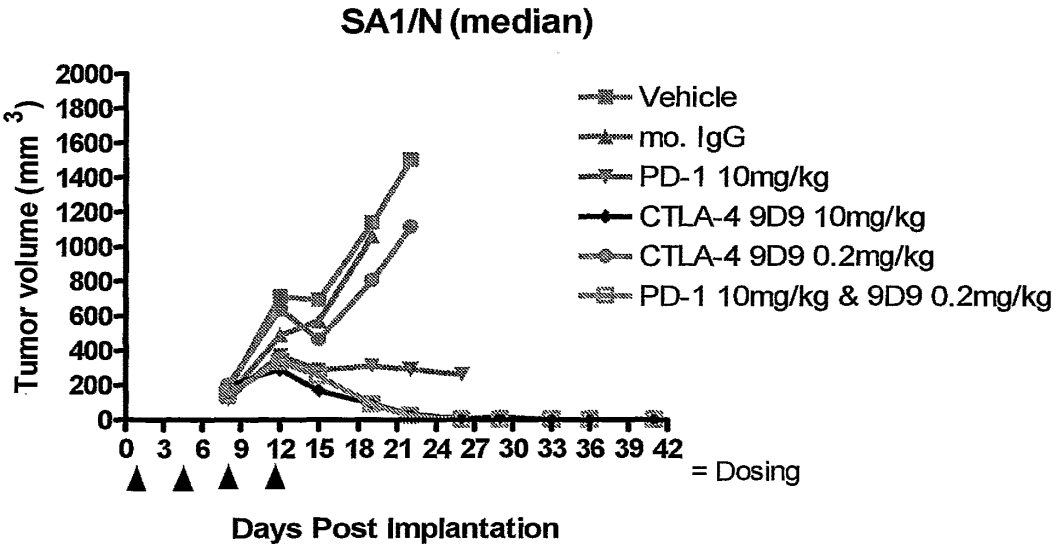

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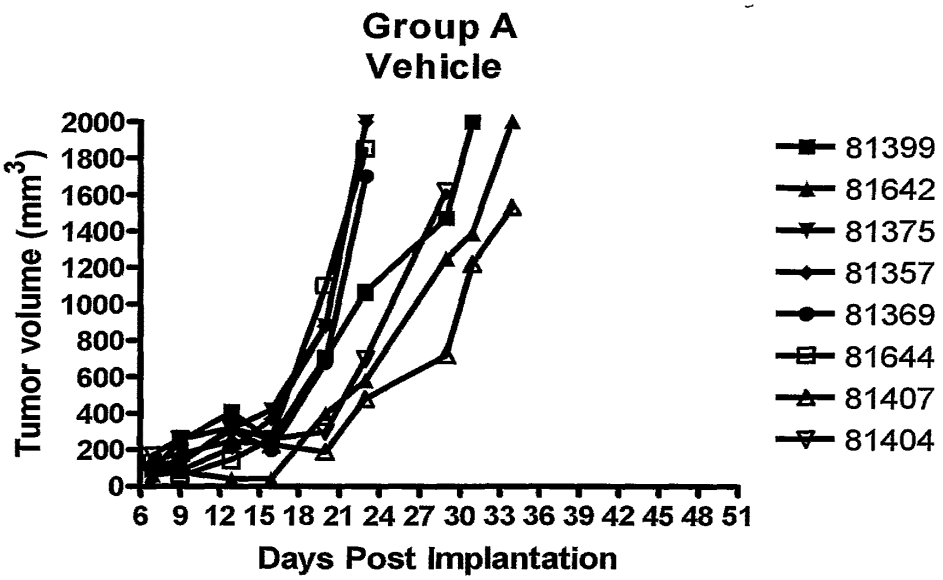

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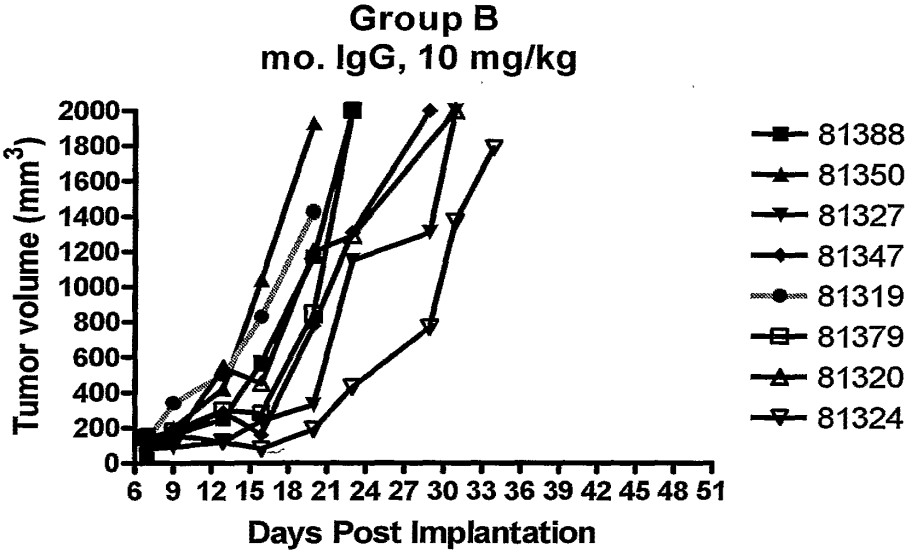

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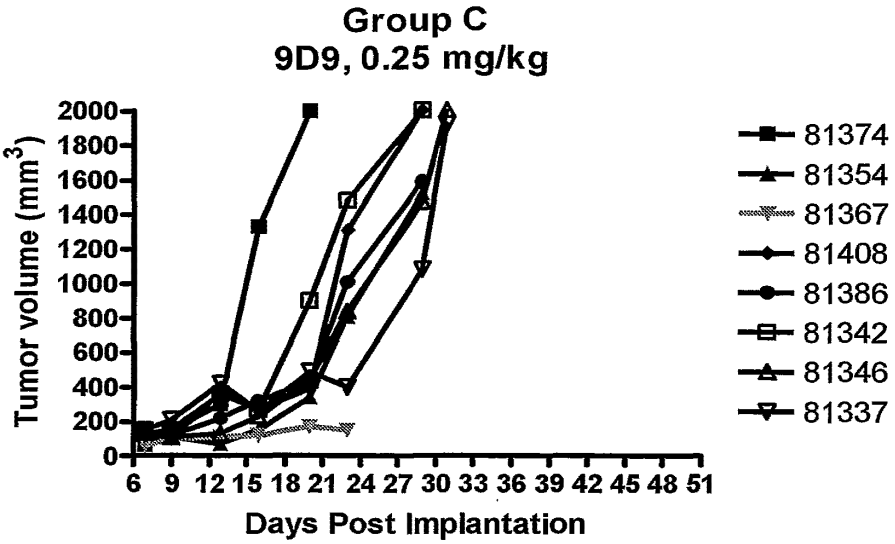

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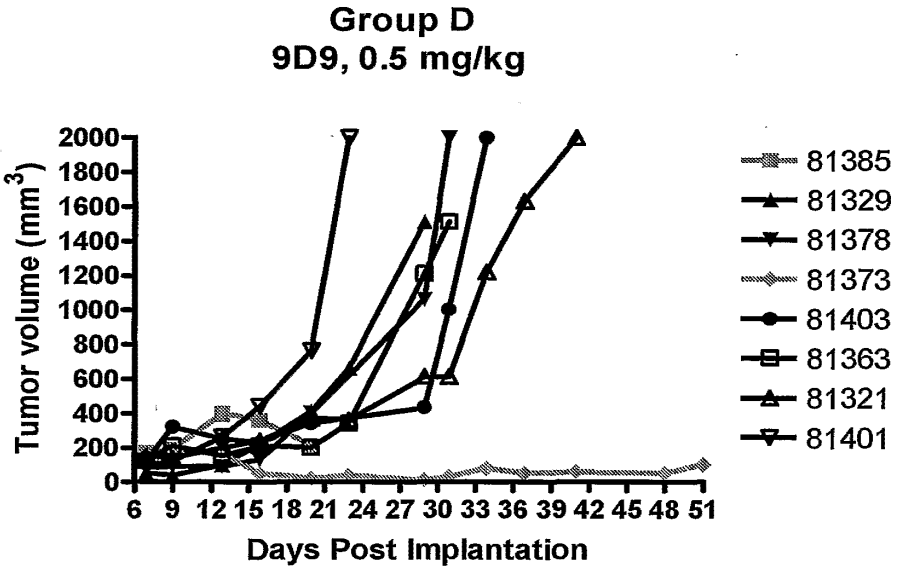

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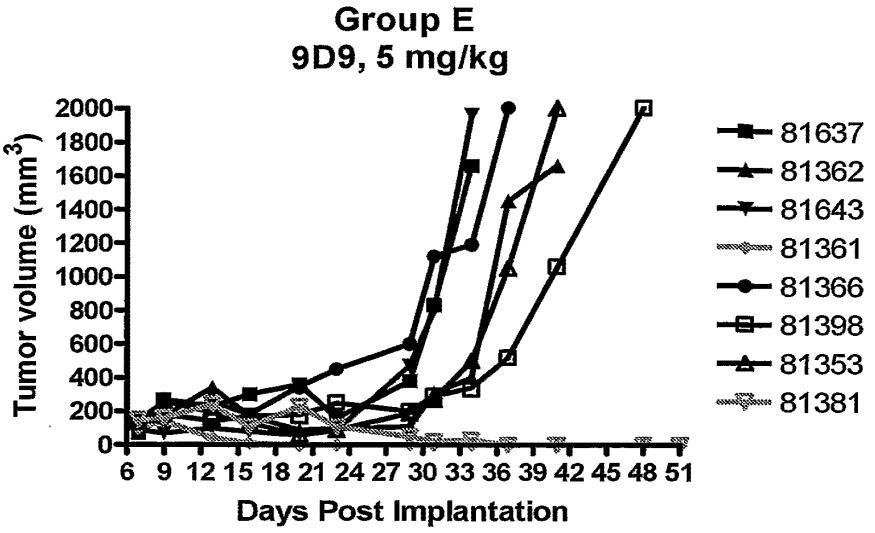

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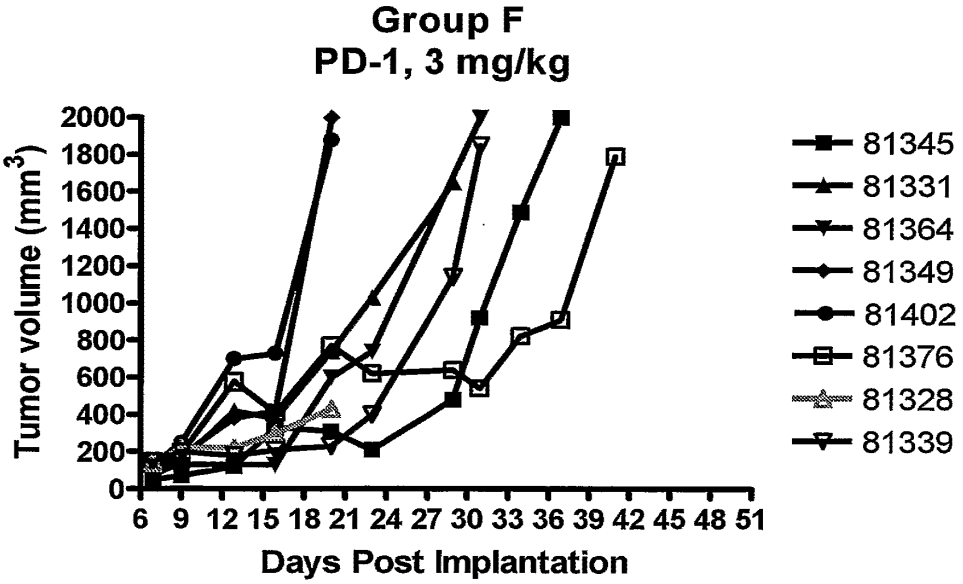

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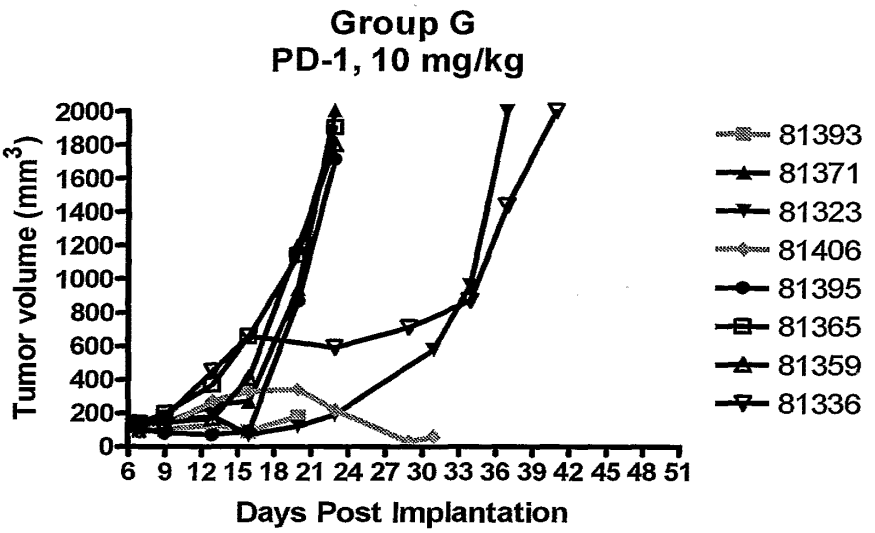

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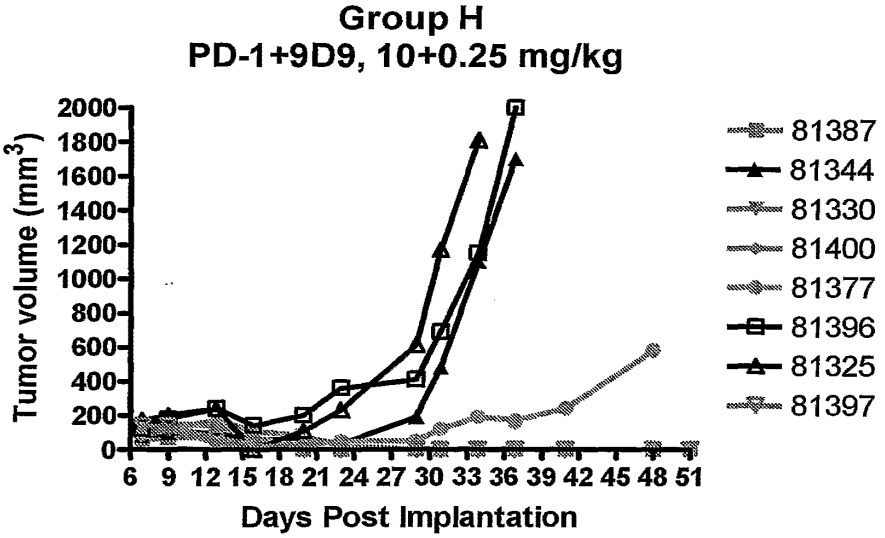

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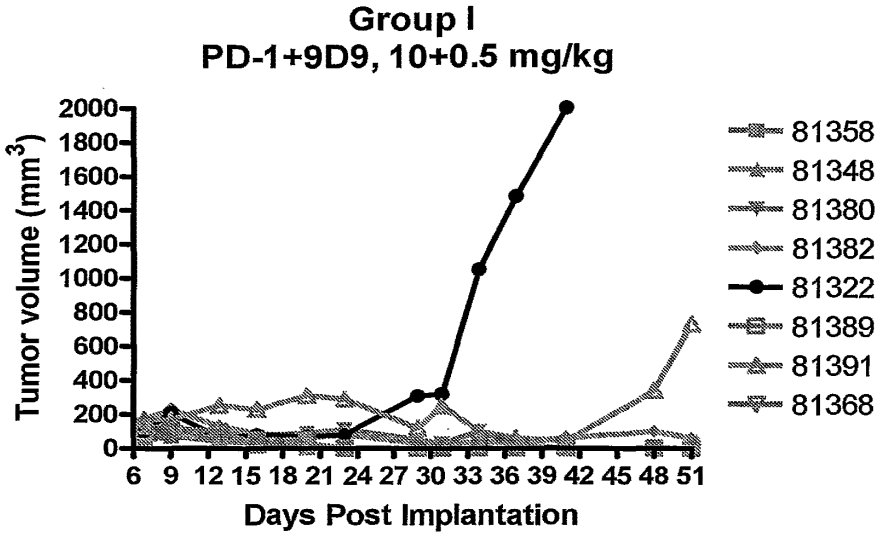

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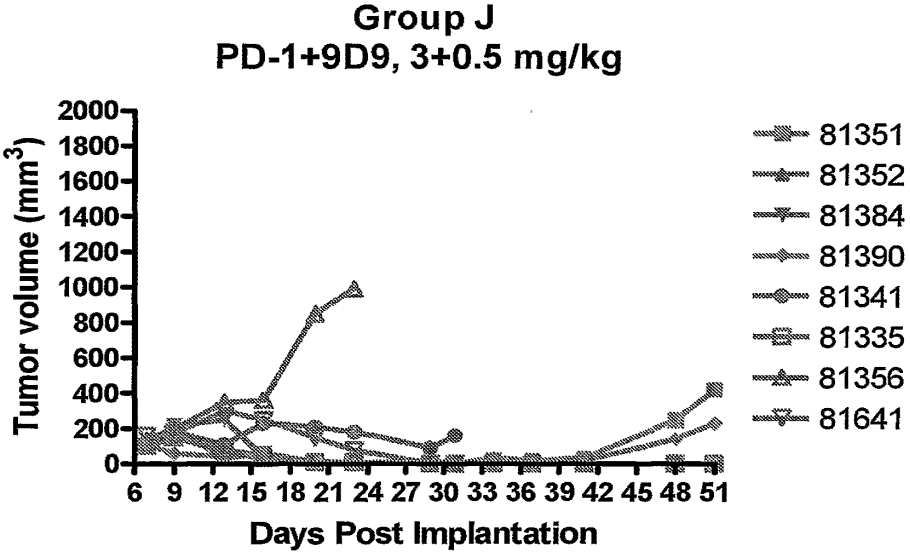

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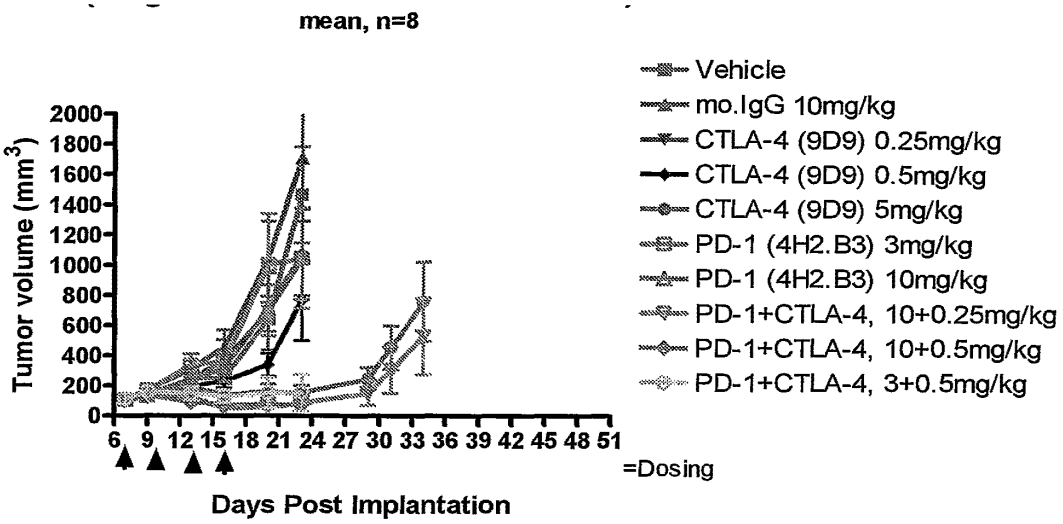

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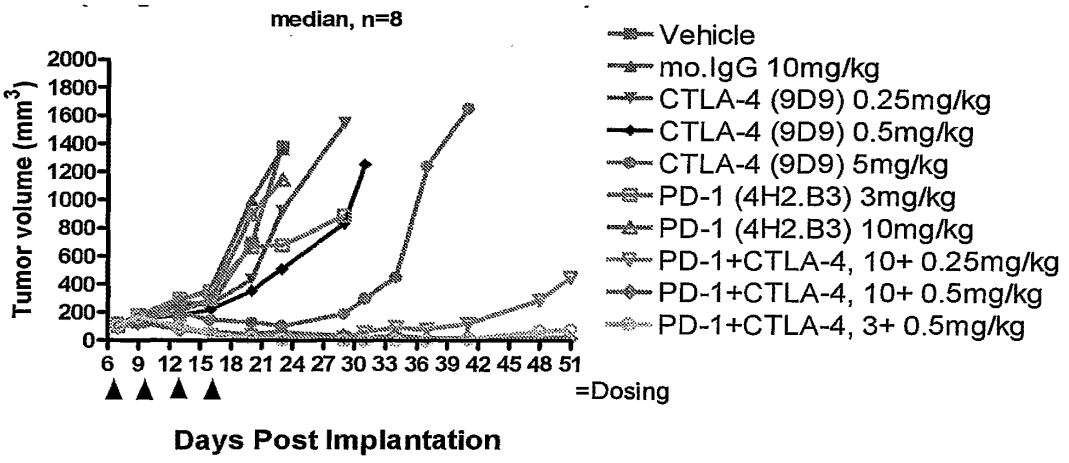

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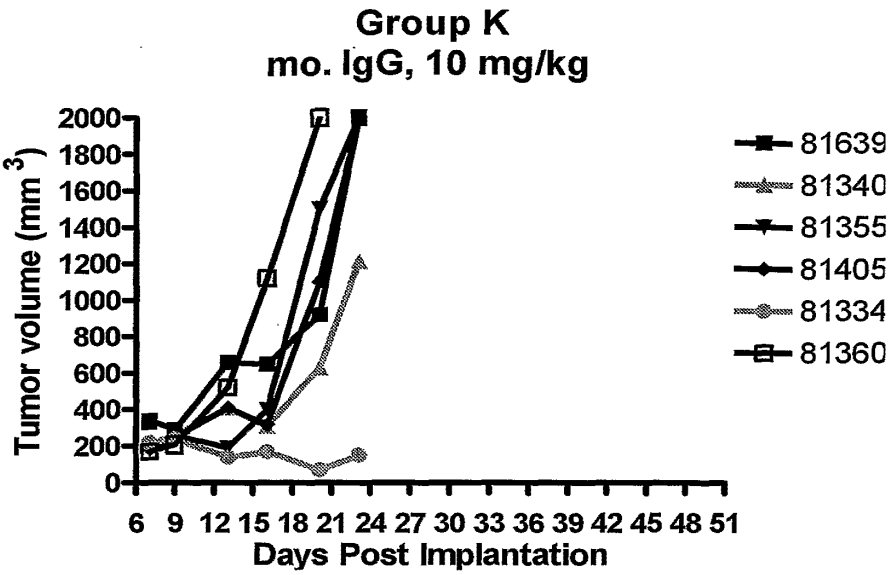

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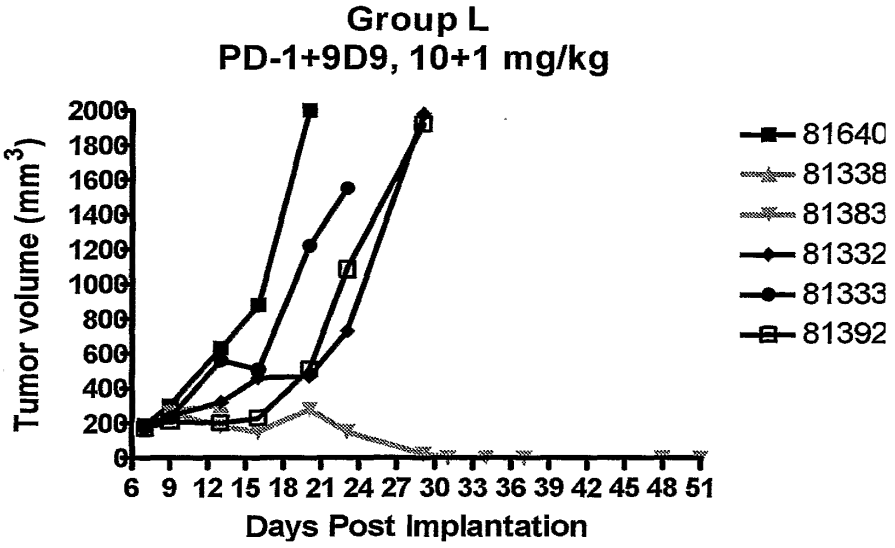

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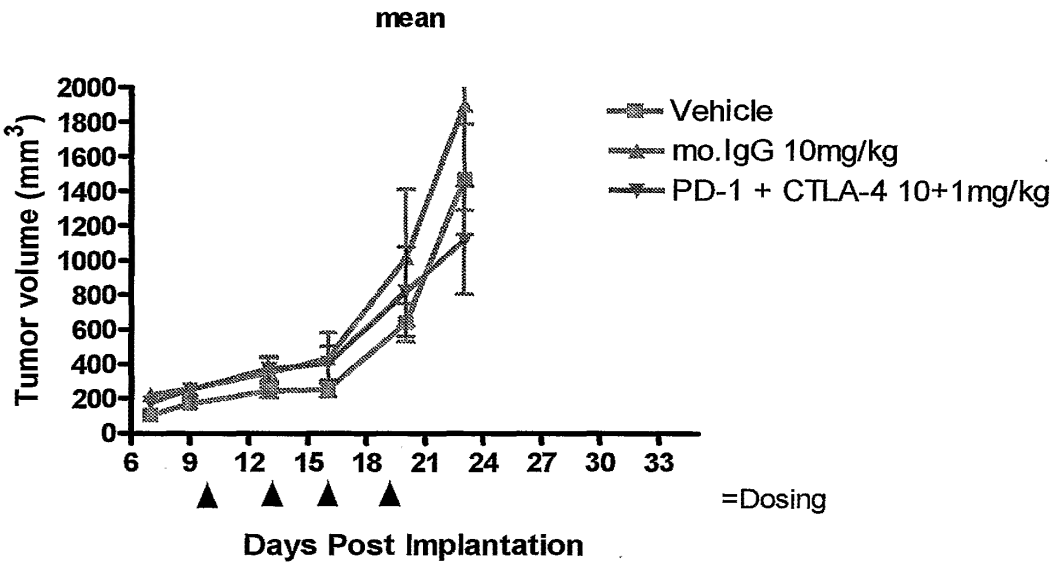

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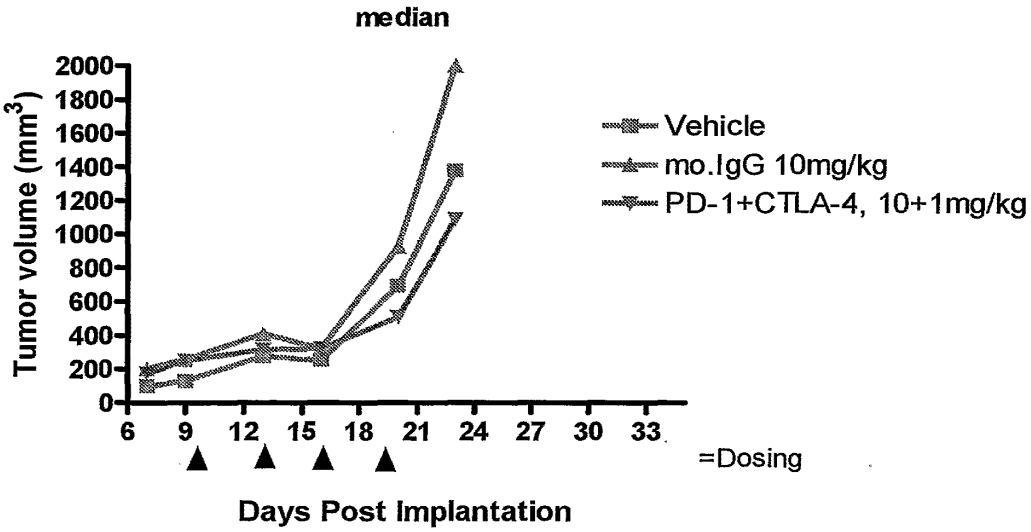

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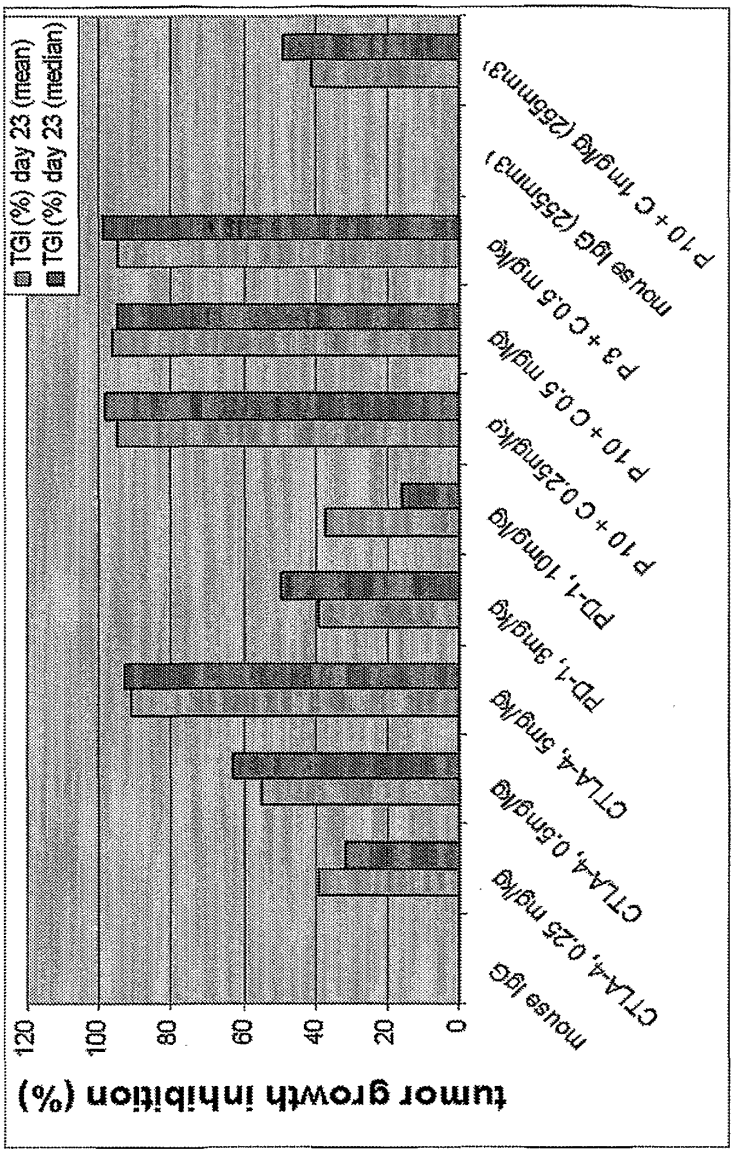
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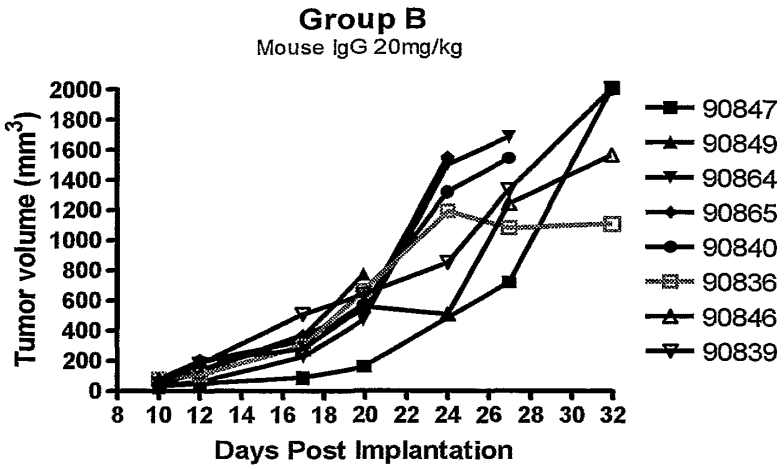
TGI (%) day 23 (median)
tumor growth inhibition (%)
120
100
80
60
40
20
0
mouse IgG
CTLA-4, 0.25 mg/kg
CTLA-4, 0.5mg/kg
CTLA-4, 5mg/kg
PD-1, 3mg/kg
PD-1, 10mg/kg
P10+C 0.25mg/kg
P10+C 0.5 mg/kg
P3+C 0.5 mg/kg
mouse IgG (265mm3)
P10+C 1mg/kg (265mm3)

Figure 39



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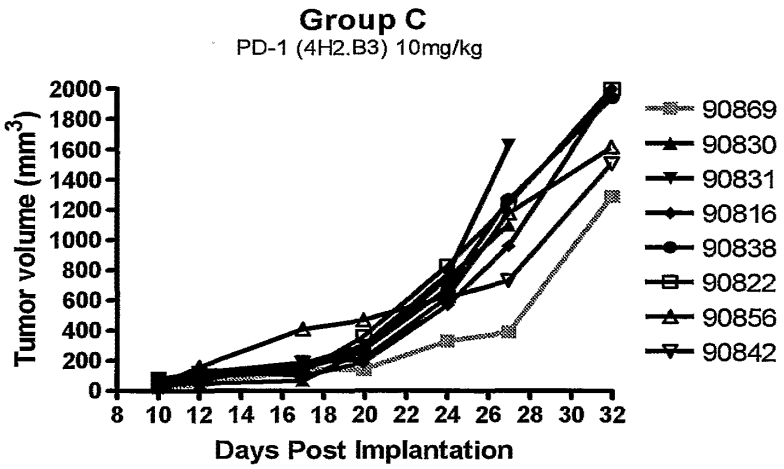

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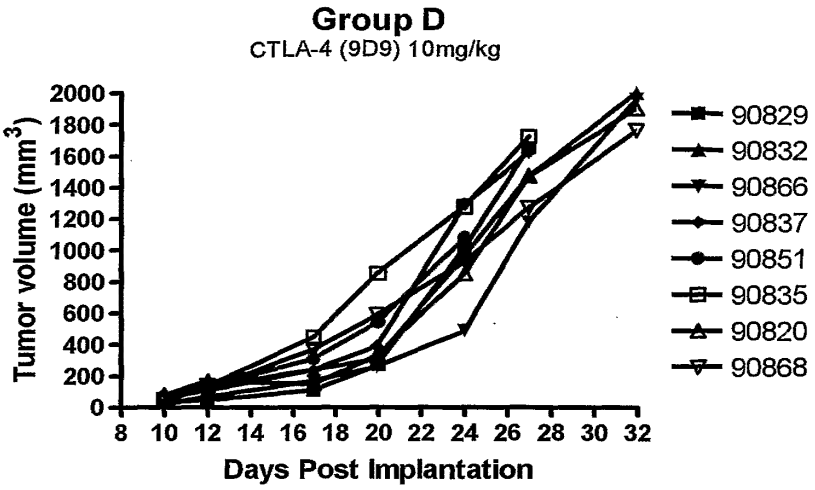
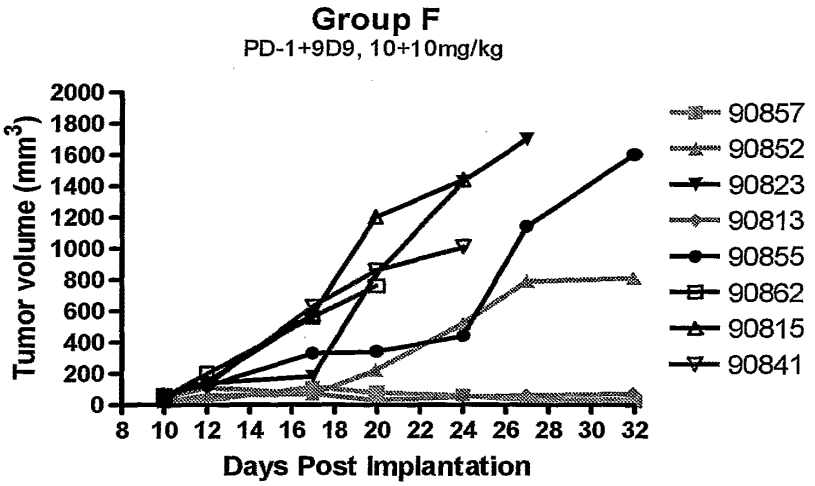


Figure 40C



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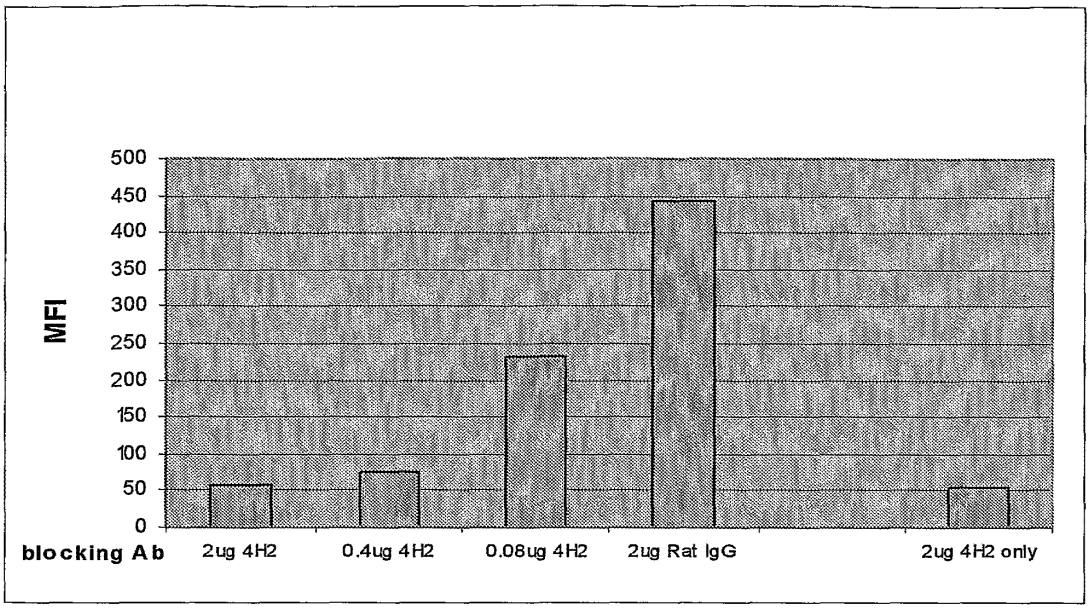

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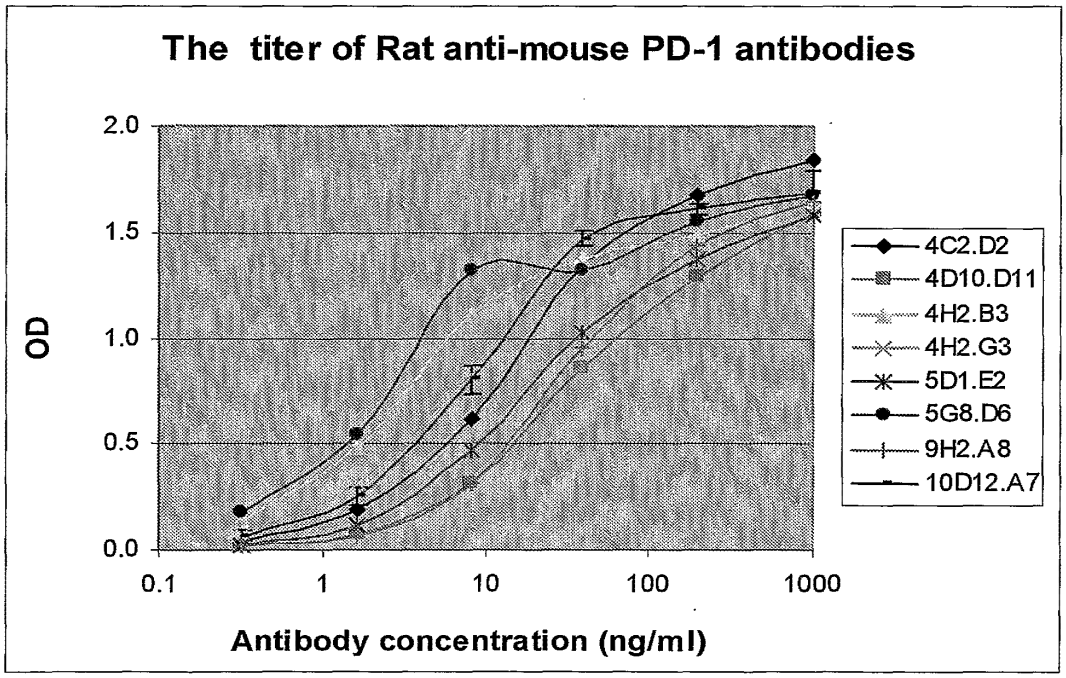

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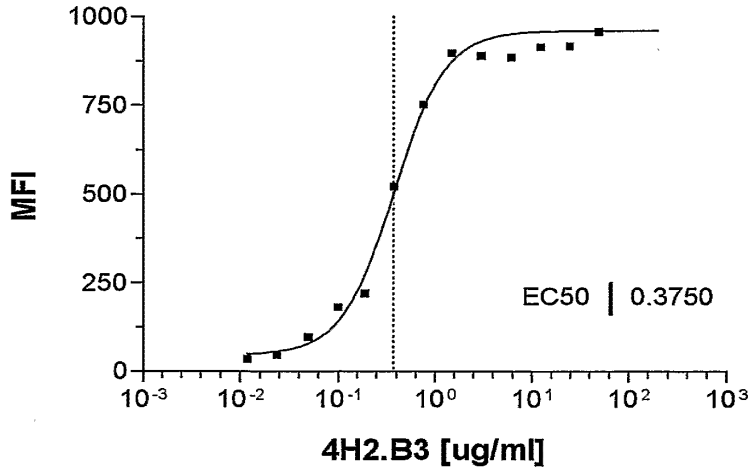

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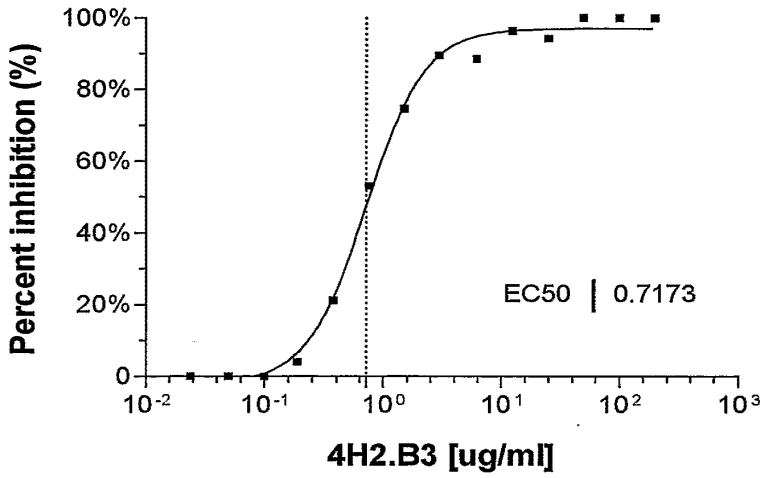

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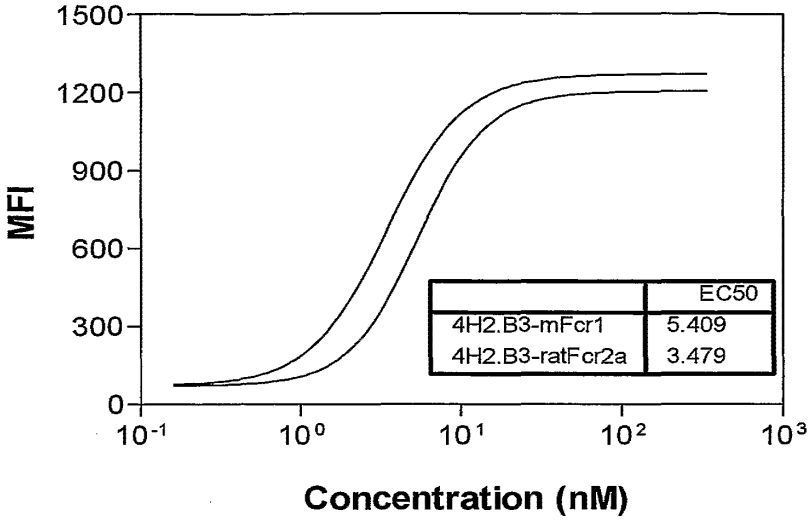

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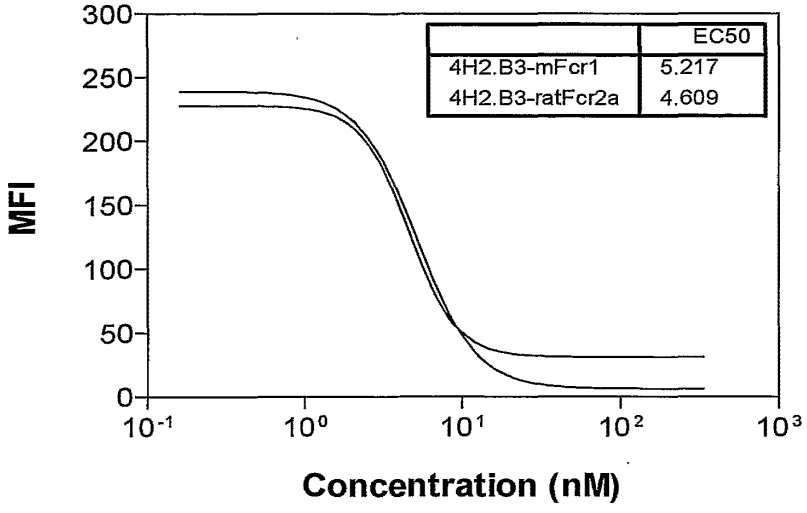

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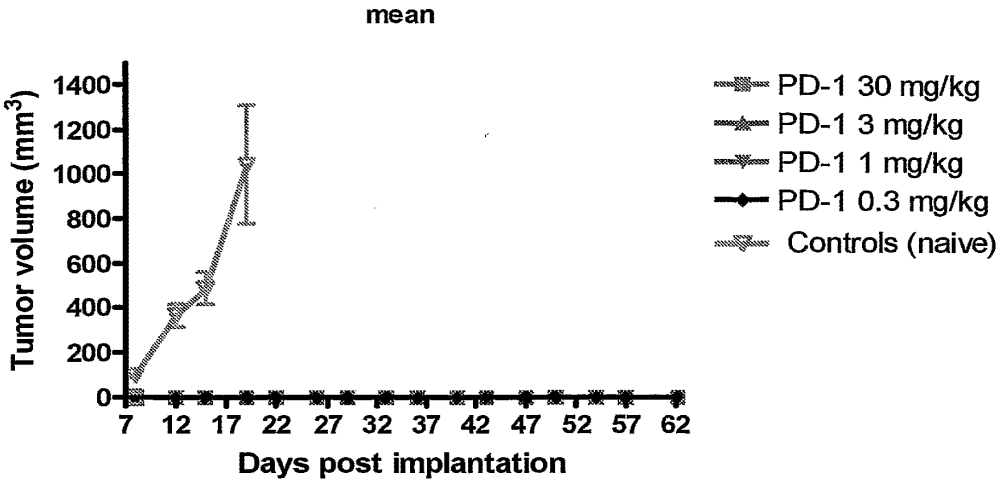

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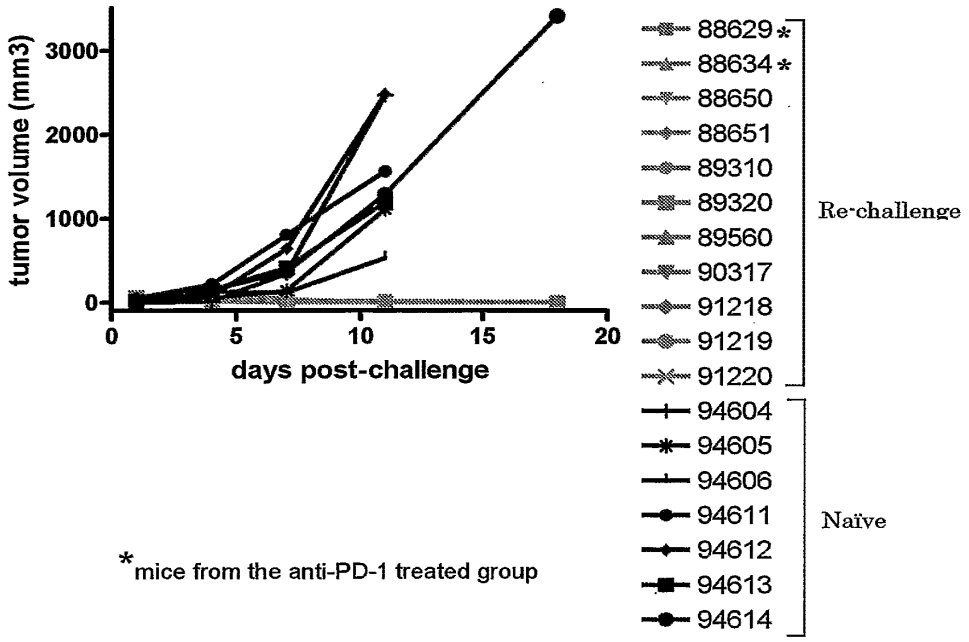

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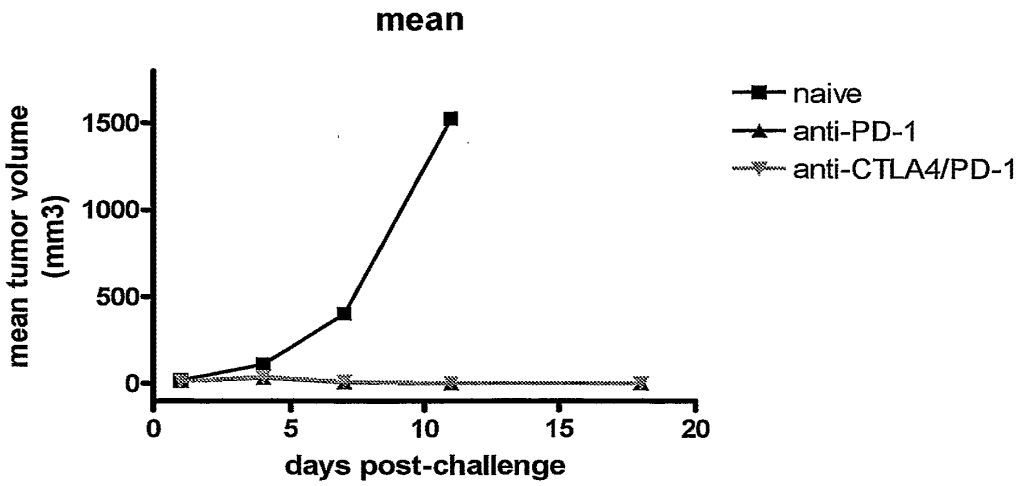

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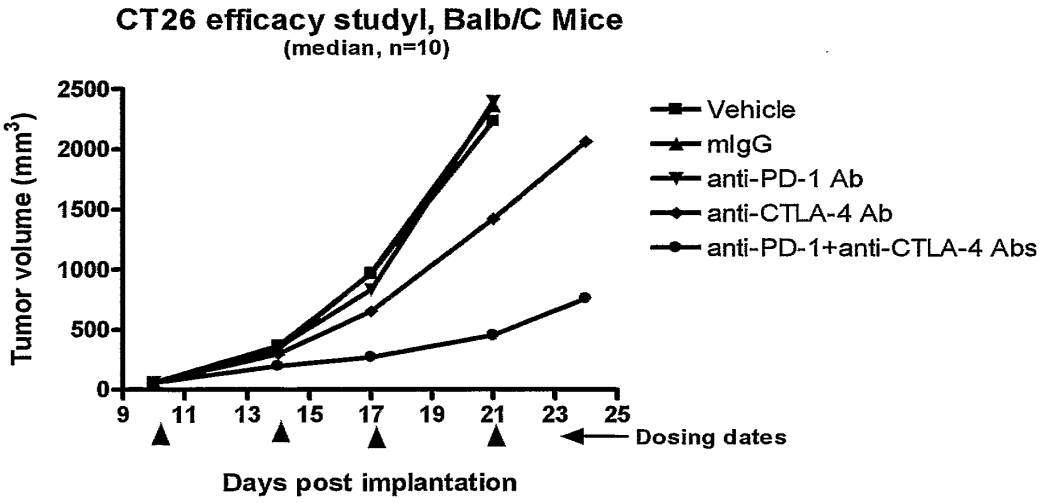

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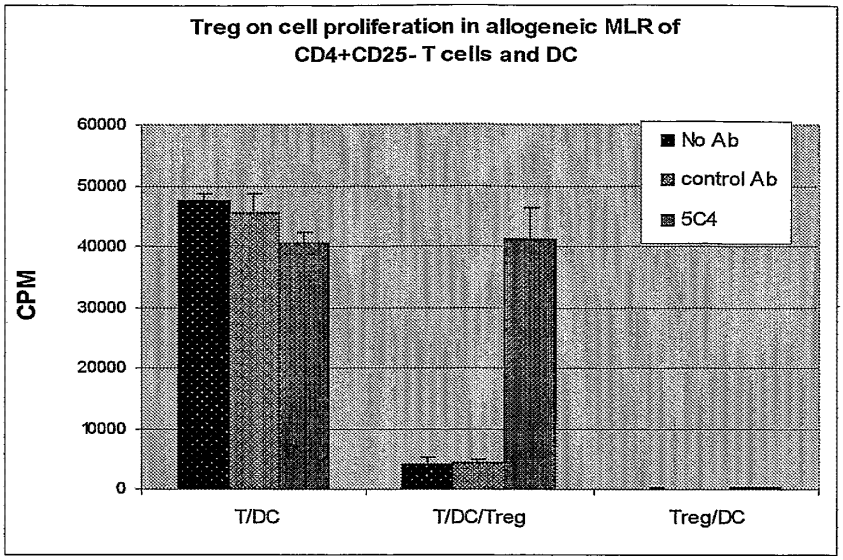

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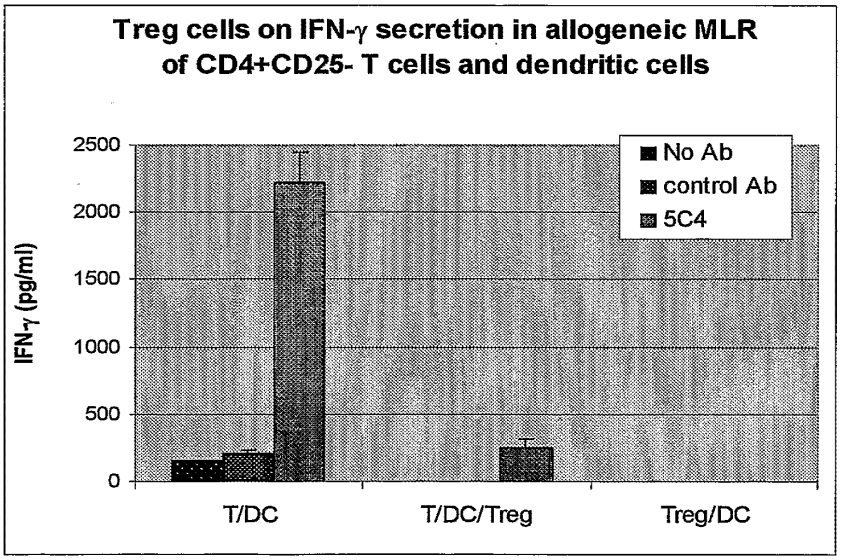

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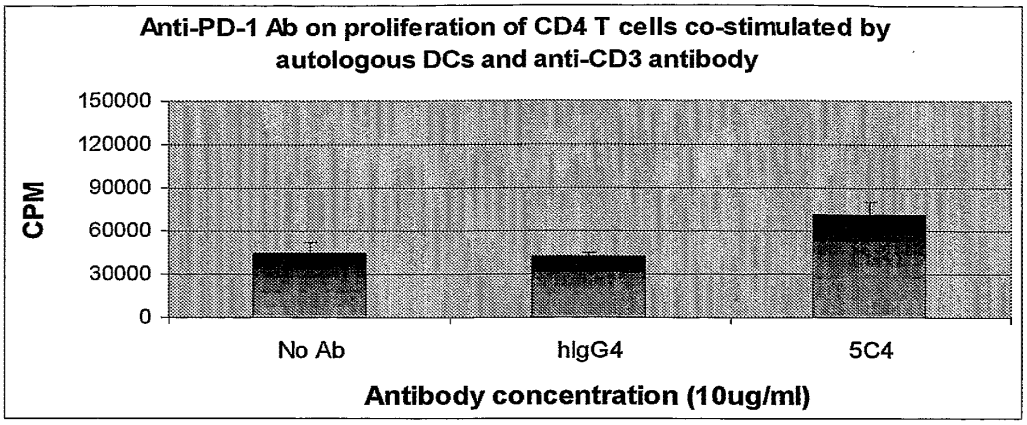

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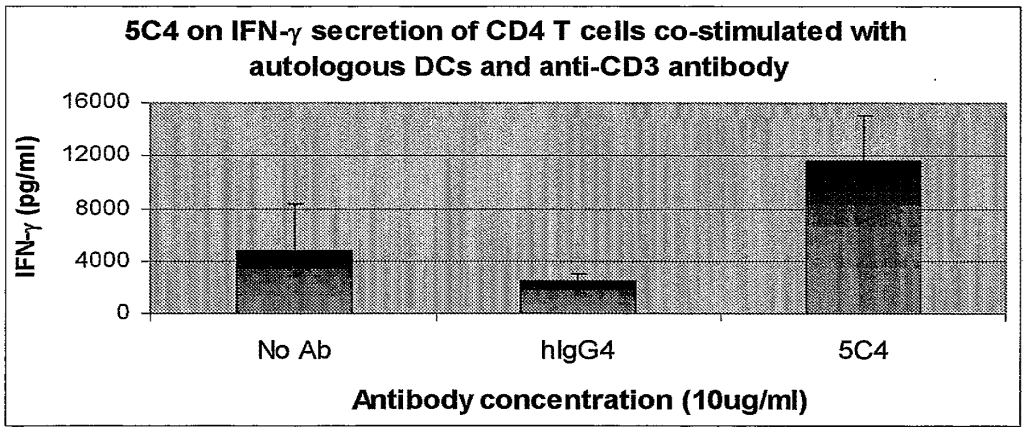

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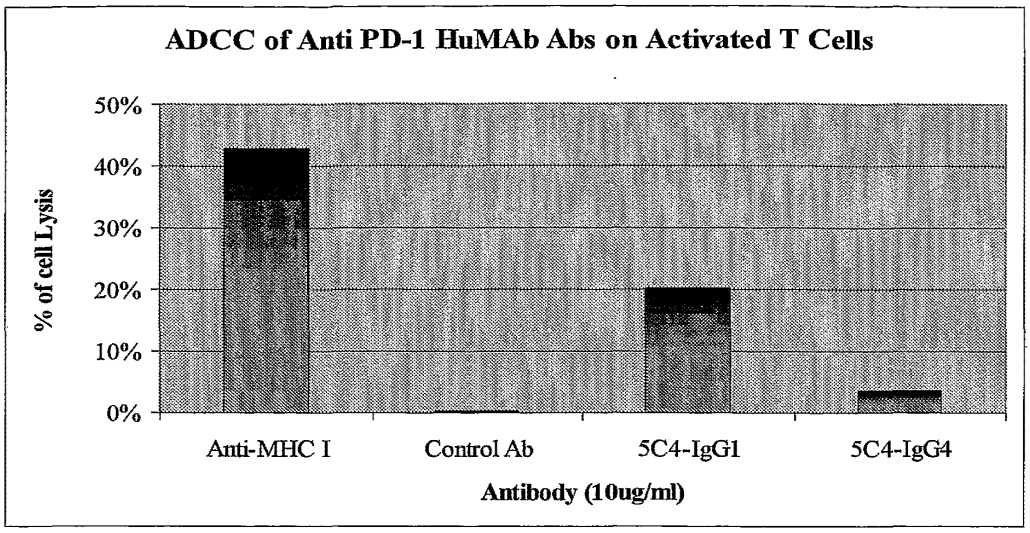

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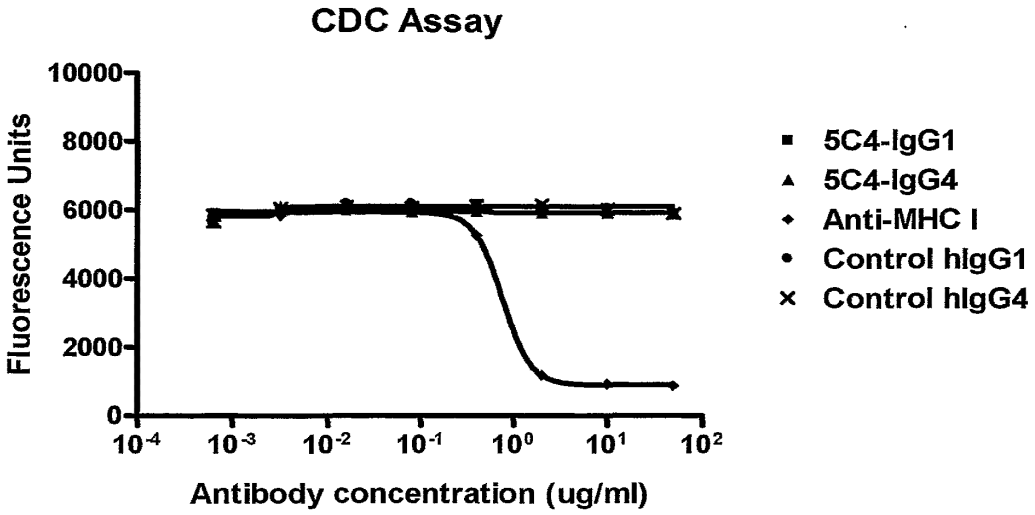

Figure 54

SEQUENCE LISTING

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<110> Ono Pharmaceutical Co., LTD.

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<151> 2005-05-09

<150> US 60/738,434
<151> 2005-11-21

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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Met Leu Tyr
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<400> 3

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Gln Val Tyr Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20                  25                  30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Leu Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80
Leu Gln Met Thr Ser Leu Arg Val Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Ser Asn Val Asp His Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110
Ser
```

<210> 4
<211> 113
<212> PRT
<213> Homo sapiens

<400> 4

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15
Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110
Ser
```

<210> 5
<211> 121
<212> PRT
<213> Homo sapiens

<400> 5
Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Leu Ser Arg Ser
20 25 30
Ser Phe Phe Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45
Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65 70 75 80
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Val Arg Asp Tyr Asp Ile Leu Thr Gly Asp Glu Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 6
<211> 113
<212> PRT
<213> Homo sapiens

<400> 6
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Thr Thr Ser Gly Ile Thr Phe Ser Ser Tyr
20 25 30
Gly Phe His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Val Ile Trp Tyr Asp Gly Ser Lys Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Leu Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Val Thr Gly Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
100 105 110
Ser

<210> 7
<211> 121
<212> PRT
<213> Homo sapiens

<400> 7
Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ser Val Ser Gly Gly Ser Leu Ser Arg Ser

```
            20                  25                  30
Ser Tyr Phe Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35                  40                  45
Trp Ile Ala Ser Ile Phe Tyr Ser Gly Glu Thr Tyr Phe Asn Pro Ser
    50                  55                  60
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Arg Asn Gln Phe
65                  70                  75                  80
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85                  90                  95
Cys Ala Arg Asp Tyr Asp Ile Leu Thr Gly Asp Glu Asp Tyr Trp Gly
            100                 105                 110
Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 8
<211> 107
<212> PRT
<213> Homo sapiens

<400> 8

```
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Ile Ile
        35                  40                  45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> 9
<211> 107
<212> PRT
<213> Homo sapiens

<400> 9

```
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45
Tyr Asp Thr Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> 10
<211> 107
<212> PRT
<213> Homo sapiens

<400> 10
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 11
<211> 107
<212> PRT
<213> Homo sapiens

<400> 11
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 12
<211> 107
<212> PRT
<213> Homo sapiens

<400> 12
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Ser Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile

```
        35                  40                  45
Tyr Ala Ala Ser Asn Leu Arg Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg
                85                  90                  95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> 13
<211> 107
<212> PRT
<213> Homo sapiens

<400> 13

```
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> 14
<211> 107
<212> PRT
<213> Homo sapiens

<400> 14

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
        35                  40                  45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg
                85                  90                  95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> 15
<211> 5

<212> PRT
<213> Homo sapiens

<400> 15
Asn Tyr Gly Met His
1 5

<210> 16
<211> 5
<212> PRT
<213> Homo sapiens

<400> 16
Asn Tyr Gly Phe His
1 5

<210> 17
<211> 5
<212> PRT
<213> Homo sapiens

<400> 17
Asn Tyr Gly Met His
1 5

<210> 18
<211> 5
<212> PRT
<213> Homo sapiens

<400> 18
Asn Ser Gly Met His
1 5

<210> 19
<211> 7
<212> PRT
<213> Homo sapiens

<400> 19
Arg Ser Ser Phe Phe Trp Gly
1 5

<210> 20
<211> 5
<212> PRT
<213> Homo sapiens

<400> 20
Ser Tyr Gly Phe His
1 5

<210> 21
<211> 7
<212> PRT
<213> Homo sapiens

<400> 21
Arg Ser Ser Tyr Phe Trp Gly
1 5

<210> 22
<211> 17
<212> PRT
<213> Homo sapiens

<400> 22
Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15
Gly

<210> 23
<211> 17
<212> PRT
<213> Homo sapiens

<400> 23
Val Ile Trp Tyr Asp Gly Ser Lys Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15
Gly

<210> 24
<211> 17
<212> PRT
<213> Homo sapiens

<400> 24
Leu Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15
Gly

<210> 25
<211> 17
<212> PRT
<213> Homo sapiens

<400> 25
Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15
Gly

<210> 26
<211> 16

<212> PRT
<213> Homo sapiens

<400> 26
Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 27
<211> 17
<212> PRT
<213> Homo sapiens

<400> 27
Val Ile Trp Tyr Asp Gly Ser Lys Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15
Gly

<210> 28
<211> 16
<212> PRT
<213> Homo sapiens

<400> 28
Ser Ile Phe Tyr Ser Gly Glu Thr Tyr Phe Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 29
<211> 4
<212> PRT
<213> Homo sapiens

<400> 29
Asn Asp Asp Tyr
1

<210> 30
<211> 4
<212> PRT
<213> Homo sapiens

<400> 30
Gly Asp Asp Tyr
1

<210> 31
<211> 4
<212> PRT
<213> Homo sapiens

<400> 31
Asn Val Asp His
1

<210> 32
<211> 4
<212> PRT
<213> Homo sapiens

<400> 32
Asn Asp Asp Tyr
1

<210> 33
<211> 11
<212> PRT
<213> Homo sapiens

<400> 33
Asp Tyr Asp Ile Leu Thr Gly Asp Glu Asp Tyr
1 5 10

<210> 34
<211> 4
<212> PRT
<213> Homo sapiens

<400> 34
Gly Asp Asp Tyr
1

<210> 35
<211> 11
<212> PRT
<213> Homo sapiens

<400> 35
Asp Tyr Asp Ile Leu Thr Gly Asp Glu Asp Tyr
1 5 10

<210> 36
<211> 11
<212> PRT
<213> Homo sapiens

<400> 36
Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> 37
<211> 11
<212> PRT
<213> Homo sapiens

<400> 37
Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> 38
<211> 11
<212> PRT
<213> Homo sapiens

<400> 38
Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> 39
<211> 11
<212> PRT
<213> Homo sapiens

<400> 39
Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> 40
<211> 11
<212> PRT
<213> Homo sapiens

<400> 40
Arg Ala Ser Gln Gly Ile Ser Ser Trp Leu Ala
1 5 10

<210> 41
<211> 11
<212> PRT
<213> Homo sapiens

<400> 41
Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> 42
<211> 11
<212> PRT
<213> Homo sapiens

<400> 42
Arg Ala Ser Gln Gly Ile Ser Ser Trp Leu Ala
1 5 10

<210> 43
<211> 7

<212> PRT
<213> Homo sapiens

<400> 43
Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 44
<211> 7
<212> PRT
<213> Homo sapiens

<400> 44
Asp Thr Ser Asn Arg Ala Thr
1 5

<210> 45
<211> 7
<212> PRT
<213> Homo sapiens

<400> 45
Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 46
<211> 7
<212> PRT
<213> Homo sapiens

<400> 46
Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 47
<211> 7
<212> PRT
<213> Homo sapiens

<400> 47
Ala Ala Ser Asn Leu Arg Ser
1 5

<210> 48
<211> 7
<212> PRT
<213> Homo sapiens

<400> 48
Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 49
<211> 7
<212> PRT
<213> Homo sapiens

<400> 49
Ala Ala Ser Ser Leu Gln Ser
1 5

<210> 50
<211> 9
<212> PRT
<213> Homo sapiens

<400> 50
Gln Gln Arg Ser Asn Trp Pro Leu Thr
1 5

<210> 51
<211> 9
<212> PRT
<213> Homo sapiens

<400> 51
Gln Gln Arg Ser Asn Trp Pro Leu Thr
1 5

<210> 52
<211> 9
<212> PRT
<213> Homo sapiens

<400> 52
Gln Gln Ser Ser Asn Trp Pro Arg Thr
1 5

<210> 53
<211> 9
<212> PRT
<213> Homo sapiens

<400> 53
Gln Gln Ser Ser Asn Trp Pro Arg Thr
1 5

<210> 54
<211> 9
<212> PRT
<213> Homo sapiens

<400> 54

Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr
1 5

<210> 55
<211> 9
<212> PRT
<213> Homo sapiens

<400> 55
Gln Gln Arg Ser Asn Trp Pro Leu Thr
1 5

<210> 56
<211> 9
<212> PRT
<213> Homo sapiens

<400> 56
Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr
1 5

<210> 57
<211> 339
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(339)

<400> 57
cag gtg cag ctg gtg gag tct ggg gga gac gtg gtc cag cct ggg ggg 48
Gln Val Gln Leu Val Glu Ser Gly Gly Asp Val Val Gln Pro Gly Gly
1 5 10 15

tcc ctg aga ctc tcc tgt gca gcg tct gga gtc gcc ttc agt aac tat 96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ala Phe Ser Asn Tyr
20 25 30

ggc atg cac tgg gtc cgc cag gct ccc ggc aag ggg ctg gag tgg gtg 144
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

gca gtt atc tgg tat gat gga agt aat aaa tac tat gca gac tcc gtg 192
Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

aag ggc cgg ttc acc atc tcc aga gac aat tcc aag aac atg ctc tat 240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Met Leu Tyr
65 70 75 80

ctg caa atg aac agc ctg aga gcc gag gac acg gct atg tat tac tgt 288
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

```
gcg agg aac gat gac tac tgg ggc cag gga acc ctg gtc acc gtc tcc      336
Ala Arg Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

tca                                                                  339
Ser
```

<210> 58
<211> 339
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(339)

<400> 58

```
cag gtg cag ctg gtg gaa tct ggg gga gac gtg gtc cag cct ggg agg       48
Gln Val Gln Leu Val Glu Ser Gly Gly Asp Val Val Gln Pro Gly Arg
1               5                   10                  15

tcc ctg aga ctc tcc tgt gca gcg tct gga tta acc ttc act aac tat       96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Leu Thr Phe Thr Asn Tyr
            20                  25                  30

ggc ttc cac tgg gtc cgc cag gct cca ggc aag ggg ctg gag tgg gtg      144
Gly Phe His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

gct gtt ata tgg tat gat gga agt aag aaa tat tat gca gac tcc gtg      192
Ala Val Ile Trp Tyr Asp Gly Ser Lys Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

aag ggc cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat      240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

ctg caa atg aac aac ctg aga gcc gag gac acg gct gtg tat tac tgt      288
Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

gcg act ggg gat gac tac tgg ggc cag gga acc ctg gtc acc gtc tcc      336
Ala Thr Gly Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

tca                                                                  339
Ser
```

<210> 59
<211> 339
<212> DNA
<213> Homo sapiens

<220>

<221> CDS
<222> (1)..(339)

<400> 59
```
cag gtg tac ttg gta gag tct ggg gga ggc gtg gtc cag cct ggg agg       48
Gln Val Tyr Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15

tcc ctg aga ctc tcc tgt gca gcg tct gga ttc acc ttc agt aac tat       96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20                  25                  30

ggc atg cac tgg gtc cgc cag gct cca ggc aag ggg ctg gag tgg gtg      144
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

gca ctt ata tgg tat gat gga agt aat aaa tac tat gca gac tcc gtg      192
Ala Leu Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

aag ggc cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat      240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

ctg caa atg acc agt ctg aga gtc gag gac acg gct gtg tat tat tgt      288
Leu Gln Met Thr Ser Leu Arg Val Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

gcg agc aac gtt gac cat tgg ggc cag gga acc ctg gtc acc gtc tcc      336
Ala Ser Asn Val Asp His Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

tca                                                                  339
Ser
```

<210> 60
<211> 339
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(339)

<400> 60
```
cag gtg cag ctg gtg gag tct ggg gga ggc gtg gtc cag cct ggg agg       48
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15

tcc ctg aga ctc gac tgt aaa gcg tct gga atc acc ttc agt aac tct       96
Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
            20                  25                  30

ggc atg cac tgg gtc cgc cag gct cca ggc aag ggg ctg gag tgg gtg      144
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
```

```
gca gtt att tgg tat gat gga agt aaa aga tac tat gca gac tcc gtg      192
Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
    50                  55                  60

aag ggc cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg ttt      240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

ctg caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt      288
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

gcg aca aac gac gac tac tgg ggc cag gga acc ctg gtc acc gtc tcc      336
Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110

tca                                                                 339
Ser
```

<210> 61
<211> 363
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(363)

<400> 61

```
cag ctg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gag       48
Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1               5                   10                  15

acc ctg tcc ctc acc tgc act gtc tct ggt ggc tcc ctc agc agg agt       96
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Leu Ser Arg Ser
            20                  25                  30

agt ttc ttc tgg ggc tgg atc cgt cag ccc cca ggg aag gga ctg gag      144
Ser Phe Phe Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35                  40                  45

tgg att ggg agt atc tat tat agt ggg agc acc tac tac aac ccg tcc      192
Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
    50                  55                  60

ctc aag agt cga gtc acc ata tcc gta gac acg tcc aag aac cag ttc      240
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65                  70                  75                  80

tcc ctg aag ctg agc tct gtg acc gcc gca gac acg gct gtg tat tac      288
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85                  90                  95

tgt gtg aga gat tac gat att ttg act ggc gac gag gac tac tgg ggc      336
Cys Val Arg Asp Tyr Asp Ile Leu Thr Gly Asp Glu Asp Tyr Trp Gly
```

```
          100                 105                 110

cag gga acc ctg gtc acc gtg tcc tca                                  363
Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 62
<211> 339
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(339)

<400> 62

```
cag gtg cag ctg gtg gag tct ggg gga ggc gtg gtc cag cct ggg agg        48
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

tcc ctg aga ctc tcc tgt aca acg tct gga atc acc ttc agt agc tat        96
Ser Leu Arg Leu Ser Cys Thr Thr Ser Gly Ile Thr Phe Ser Ser Tyr
            20                  25                  30

ggc ttt cac tgg gtc cgc cag gct cca ggc aag ggg ctg gag tgg gtg       144
Gly Phe His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

gca gtg ata tgg tat gat gga agt aaa aaa tac tat gca gac tcc gtg       192
Ala Val Ile Trp Tyr Asp Gly Ser Lys Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

aag ggc cga ttc acc ctc tcc aga gac gat tcc aag aac acg ctg tat       240
Lys Gly Arg Phe Thr Leu Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

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Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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gtt act ggg gat gac tac tgg ggc cag gga acc ctg gtc acc gtc tcc       336
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agt tac ttc tgg ggc tgg atc cgc cag ccc cca ggg aag ggg ctg gag 144
Ser Tyr Phe Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45

tgg att gcg agt atc ttt tat agt ggg gaa acc tac ttc aat ccg tcc 192
Trp Ile Ala Ser Ile Phe Tyr Ser Gly Glu Thr Tyr Phe Asn Pro Ser
50 55 60

ctc aag agt cga gtc acc ata tcc gta gac acg tcc agg aac cag ttc 240
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Arg Asn Gln Phe
65 70 75 80

tcc ctg aag ctg agc tct gtg acc gcc gca gac acg gct gtg tat tac 288
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95

tgt gcg aga gat tac gat att ttg act ggc gac gag gac tac tgg ggc 336
Cys Ala Arg Asp Tyr Asp Ile Leu Thr Gly Asp Glu Asp Tyr Trp Gly
100 105 110

cag gga acc ctg gtc acc gtc tcc tca 363
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20 25 30

tta gcc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc atc atc 144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Ile Ile
35 40 45

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tat gat gca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc      192
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60

agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct      240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

gaa gat ttt gca gtt tat tac tgt cag cag cgt agc aac tgg cct ctc      288
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gaa aga gcc acc ctg tcc tgc agg gcc agt cag agt gtt agc agc tac       96
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30

tta gcc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc ctc atc      144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

tat gat aca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc      192
Tyr Asp Thr Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60

agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct      240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

gaa gat ttt gca gtt tat tac tgt cag cag cgt agc aac tgg ccg ctc      288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu
                85                  90                  95

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gaa aga gcc acc ctc tcc tgc agg gcc agt cag agt gtt agt agt tac       96
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30

tta gcc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc ctc atc      144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

tat gat gca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc      192
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60

agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct      240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

gaa gat ttt gca gtt tat tac tgt cag cag agt agc aac tgg cct cgg      288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
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acg ttc ggc caa ggg acc aag gtg gaa atc aaa                         321
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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gaa aga gcc acc ctc tcc tgc agg gcc agt cag agt gtt agt agt tac       96
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30

tta gcc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc ctc atc      144
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Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

tat gat gca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc      192
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60

agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct      240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

gaa gat ttt gca gtt tat tac tgt cag cag agt agc aac tgg cct cgg      288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
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Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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            20                  25                  30

tta gcc tgg tat cag cag aaa cca gag aaa gcc cct aag tcc ctg atc      144
Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
        35                  40                  45

tat gct gca tcc aat tta cga agt ggg gtc cca tca agg ttc agc ggc      192
Tyr Ala Ala Ser Asn Leu Arg Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

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

gaa gat ttt gca act tat tac tgc caa cag tat tat agt tac cct agg      288
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg
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acg ttc ggc caa ggg acc aag gtg gaa atc aaa                         321
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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tta gcc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc ctc atc 144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

tat gat gca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc 192
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct 240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

gaa gat ttt gca gtt tat tac tgt cag cag cgt agc aac tgg cct ctc 288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu
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Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp

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          20                  25                  30

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Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
        35                  40                  45

tat gct gca tcc agt ttg caa agt ggg gtc cca tca agg ttc agc ggc      192
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

agt gga tct ggg aca gat ttc act ctc acc atc agc agc ctg cag cct      240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

gaa gat ttt gca act tat tac tgc caa cag tat tat agt tac cct agg      288
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg
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Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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<210> 72
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<213> Homo sapiens

<400> 72

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            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Ile Ile
        35                  40                  45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
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    50                  55                  60
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<210> 73
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<400> 73

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            20                  25                  30
Ser Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35                  40                  45
Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
    50                  55                  60
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn His Phe
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        35                  40                  45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro
                85                  90                  95
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2006/309606

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. C12N15/09(2006.01)i, C07K16/28(2006.01)i, C12N5/00(2006.01)i, C12P21/08(2006.01)i, C12Q1/02(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. C12N15/09, C07K16/28, C12N5/00, C12P21/08, C12Q1/02

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

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2006
Registered utility model specifications of Japan 1996-2006
Published registered utility model applications of Japan 1994-2006

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS/WPI(DIALOG)
GenBank/EMBL/DDBJ/GeneSeq
JSTPlus(JDream2)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2001/014557 A1 (Dana-Farber Cancer Institute, Inc. et al.) 2001.03.01. & AU 200070727 A & EP 1210428 A1 & NO 200200859 A & NO 200200861 A & BR 200013581 A & US 2002/0160000 A1 & HU 200202449 A2 & JP 2003-507491 A & ZA 200201512 A & MX 2002001817 A1 & NZ 517955 A & US 6808710 B1.	1-7,43-56
X	WO 2002/079499 A1 (Dana-Farber Cancer Institute, Inc. et al.) 2002.10.10. & US 2003/0044768 A1 & NO 200304387 A & EP 1383912 A1 & AU 2002338272 A1 & JP 2004-533226 A & ZA 200307310 A & BR 200208367 A & MX 2003008959 A1 & CA 2442066 C & NZ 528265 A.	1-7,43-56

☑ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01.08.2006	15.08.2006

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Japan Patent Office
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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONALSEARCHREPORT

International application No.
PCT/JP2006/309606

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2003/042402 A1 (Dana-Farber Cancer Institute, Inc. et al.) 2003.05.22. & US 2003/0232323 A1 & EP 1456652 A2 & AU 2002360384 A1 & JP 2005-509421 A.	1-7,43-56
X	Yoshiko Iwai et al., "Microanatomical localization of PD-1 in human tonsils," Immunology Letters, 2002, Vol.83, No.3, pp.215-220.	1-7,43-56
X	WO 2004/72286 A1 (Honjo T. et al.) 2004.08.26 & EP 1591527 A1.	1-7,43-56
A	Nils Lonberg et al., "Antigen-specific human antibodies from mice comprising four distinct genetic modifications," Nature, 1994, Vol.368, No.6474, pp.856-859.	1-7,43-56
A	WO 1997/07671 A1 (KIRIN BEER KK et al.) 1997.03.06 & EP 773288 A2 & AU 9668376 A & EP 773288 A3 & JP 9510126 X & EP 843961 A1 & CN 1200014 A & JP 11313576 A & JP 3030092 B & AU 718138 B & KR 99044300 A & TW 448032 A & KR 308764 B & US 6632976 B1 & US 2004/0073957 A1.	1-7,43-56
A	Lawrence R. Einger et al., "The human PD-1 gene: co,plete cDNA, genomic organization, and developmentally regulated expression in B cell progenitors," Gene, 1997, Vol.197, pp.177-187.	1-7,43-56
A	Takashi Shinohara et al.,"Structure and Chromosomal Localization of the Human PD-1 Gene (PDCD1)," Genomics, 1994, Vol.23, pp.704-706.	1-7,43-56
A	JP 07-291996 A (Honjo T. et al.) 1995.09.06. & EP 670369 A2 & CA 2143491 A & US 5629204 A & CN 1113518 A & US 5698520 A & JP 2003-230395 A & KR 391227 B & CN 1080311 C.	1-7,43-56

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

INTERNATIONALSEARCHREPORT

International application No.
PCT/JP2006/309606

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

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

1. ☑ Claims Nos.: 57-104
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 57 recites [a method of modulating an immune response in a subject]. Claim 58-68 recites [a method of inhibiting growth of tumor cells in a subject]. Since the word [subject] literally includes a human or animal, these methods are related to treatment practiced on a human or animal body.

Moreover, Claim 69-87 recites [a method for treating a hyperproliferative disease]. Claim 88-104 recites [a method for altering an adverse event associated with treatment of a hyperproliferative disease with an immunostimulatory agent]. Theses methods are also construed as substantially related to treatment practiced on a human or animal body.

Therefore, the subject matter of Claim 57-104 relates to [a method for treatment of the human or animal body by therapy], which does not require an international search by the International Searching Authority in accordance with PCT Article 17(2)(a)(i) and Rule 39.1(iv).

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

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

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

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

Invention 1) An isolated human monoclonal antibody, wherein the antibody binds to human with a K_D of 1×10^{-7} M or less;
Invention 2) An isolated human monoclonal antibody, wherein the antibody does not substantially bind to human CD28, CTLA-4 or ICOS;
Invention 3) An isolated human monoclonal antibody, wherein the antibody increases T-cell proliferation in a Mixed Lymphocyte Reaction (MLR) assay;
Invention 4) An isolated human monoclonal antibody, wherein the antibody increases interferon-gamma production in an MLR assay; and
Invention 5) An isolated human monoclonal antibody, wherein the antibody increases interleukin-2 (IL-2) secretion in an MLR assay.
Invention 6) An isolated human monoclonal antibody described in Claim 8(Claim 8-15);
Invention 7) An isolated human monoclonal antibody described in Claim 16(Claim 16);
Invention 8) An isolated human monoclonal antibody described in Claim 17(Claim 17);
Invention 9) An isolated human monoclonal antibody described in Claim 18(Claim 18);
Invention 10) An isolated human monoclonal antibody described in Claim 19(Claim 19);
Invention 11) An isolated human monoclonal antibody described in Claim 20(Claim 20);
Invention 12) An isolated human monoclonal antibody described in Claim 21(Claim 21);
Invention 13) An isolated human monoclonal antibody described in Claim 22(Claim 22-24);
Invention 14) An isolated human monoclonal antibody described in Claim 25(Claim 25-26);
Invention 15) An isolated human monoclonal antibody described in Claim 27(Claim 27-34);
Invention 16) An isolated human monoclonal antibody described in Claim 35(Claim 35-42); and
Invention 17) A method for preparing an anti-PD-1 antibody described in Claim 105(Claim 105)

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

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

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

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7, 43-56(Invention 1)

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

(12)特許協力条約に基づいて公開された国際出願

(19) 世界知的所有権機関
国際事務局

(43) 国際公開日
2004 年1 月15 日 (15.01.2004)

PCT

(10) 国際公開番号
WO 2004/004771 A1

(51) 国際特許分類[7]: A61K 45/00, 38/17, 39/395, 31/7088, A61P 31/00, 35/00, 35/04, 37/04, C12N 5/10, C12Q 1/02, G01N 33/50, A61K 6/027

(21) 国際出願番号: PCT/JP2003/008420

(22) 国際出願日: 2003 年7 月2 日 (02.07.2003)

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(30) 優先権データ:
特願2002-194491 2002 年7 月3 日 (03.07.2002) JP
特願2003-29846 2003 年2 月6 日 (06.02.2003) JP

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(54) Title: IMMUNOPOTENTIATING COMPOSITIONS

(54) 発明の名称: 免疫賦活組成物

(57) Abstract: Compositions for treating cancer or infectious diseases mediated by immunopotentiation which is caused by inhibiting a immunosuppressive signal induced by PD-1, PD-L1 or PD-L2; a therapeutic method using the same; methods of screening an immunopotentiator, an anticancer substance or a substance against infectious diseases contained in these compositions as the active ingredient; a cell line to be used in these screening methods; a method of evaluating an anticancer agent; and a mammal having cancer cells transplanted therein which is to be used in the evaluation. The above compositions inhibiting the function of PD-1, PD-L1 or PD-L2 are useful in treating cancer or infectious diseases.

(57) 要約: PD-1、PD-L1またはPD-L2によって誘導される免疫抑制シグナルを阻害した結果惹起される免疫賦活を介した癌の治療若しくは感染症の治療のための組成物およびそれらを用いた治療方法、その有効成分として含まれる免疫賦活物質、癌治療物質若しくは感染症治療物質のスクリーニング方法、それらスクリーニング方法に使用される細胞株、癌治療物質を選別する評価法、およびその評価に用いられる癌細胞移植哺乳動物に関する。PD-1、PD-L1、またはPD-L2の機能を阻害する本発明の組成物は、癌または感染症に対する治療に有用である。

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明　細　書

免疫賦活組成物

技術分野

本発明は、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２によって誘導される免疫抑制シグナルを阻害することを特徴とする免疫賦活、癌の治療若しくは感染症の治療のための組成物、およびそれらを用いる治療方法に関する。

さらに詳しく言えば、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２によって誘導される免疫抑制シグナルを阻害した結果惹起される免疫賦活を介した癌の治療若しくは感染症の治療ための組成物、それらを用いる治療方法、その組成物に有効成分として含まれる免疫賦活物質、癌治療物質若しくは感染症治療物質のスクリーニング方法、それらスクリーニング方法に使用される細胞株、癌治療物質を選別する評価法、およびその評価に用いられる癌細胞移植哺乳動物に関する。

背景技術

免疫療法は、ほとんどの薬物療法において避け難い副作用が軽減され、極めて特異性の高い治療方法として期待されている。特に、癌治療や感染症治療における薬物療法が、患者に対して大きな負担を課す治療方法であるために、患者のＱＯＬ（クオリティ・オブ・ライフ）の回復が重要視されているが、免疫療法は、ヒトがもともと備え持っている免疫反応を外因的な方法によって賦活化させ、薬物投与による負担の一部を肩代わりさせることによって、患者のＱＯＬを回復させる目的のもとに行なうことができる。

免疫の賦活化は、Ｔリンパ球細胞の免疫反応を活性化させる方法で行なうことができる。Ｔ細胞の活性化には、抗原レセプター（ＴＣＲ）を介した刺

激だけでなく、共役刺激分子群（例えば、ＣＤ２８）を介した付加的な刺激誘導が必要であるといわれている。一方、最近、共役刺激分子群と相同的な構造を有する分子群、ＣＴＬＡ－４とＰＤ－１が発見され、抗原レセプター（ＴＣＲ）シグナルを抑制するシグナルを発していることが報告されている。Ｔ細胞の活性化の方法として、この共役抑制分子の機能を抑制することも有効な１つの手段であると考えられている。

ＰＤ－１は免疫グロブリンファミリーに属する５５ｋＤのＩ型膜タンパクとしてクローニングされた（The EMBO Journal，1992年，第11巻，第11号，p.3887～3895、特開平5-336973号公報、特開平7-291996号公報）。ヒトＰＤ－１ｃＤＮＡは、EMBL / GenBank Acc. No. NM_005018に示される塩基配列で構成され、マウスＰＤ－１ｃＤＮＡは、Acc. No. X67914に示される塩基配列で構成され、それら発現は、胸腺細胞においてはＣＤ４－ＣＤ８－からＣＤ４＋ＣＤ８＋細胞に分化する際に認められる（International Immunology，1996年，第18巻，第5号，p.773～780、Journal of Experimental Medicine，2000年，第191巻，第5号，p.891～898）。また、末梢におけるＰＤ－１の発現は、抗原レセプターからの刺激により活性化したＴ細胞、Ｂ細胞（International Immunology，1996年，第18巻，第5号，p.765～772参照）または活性化マクロファージを含む骨髄細胞に認められることが報告されている。

ＰＤ－１の細胞内領域には、ＩＴＩＭモチーフ（Immunoreceptor Tyrosine - based Inhibitory Motif）があり、免疫反応に対する抑制ドメインと考えられている。さらに、ＰＤ－１欠損マウスが、糸球体腎炎、関節炎といったループス様自己免疫病（Ｃ５７ＢＬ／６遺伝子背景の場合）（International Immunology，1998年，第10巻，第10号，p.1563～1572、Immunity，1999年，第11巻，第２号，p.141～151）や拡張性心筋症様疾患（ＢＡＬＢ／ｃ遺伝子背景の場合）（Science，2001年，第291巻，第5502号，p.319～332）を発症することから、ＰＤ－１が自己免疫疾患発症、特に、末梢自己免疫寛容の制

御因子であることも示唆されている。

ＰＤ－１のリガンドであるＰＤ－Ｌ１（ヒトＰＤ－Ｌ１ｃＤＮＡはEMBL/GenBank Acc. No. AF233516、マウスＰＤ－Ｌ１ｃＤＮＡはNM_021893で示される塩基配列で構成される。）は、活性化した単球や樹状細胞などのいわゆる抗原提示細胞に発現している（Journal of Experimental Medicine，2000年，第19巻，第7号，p.1027～1034）。これら細胞は、Ｔリンパ球細胞に対して、さまざまな免疫誘導シグナルを誘導する相互作用分子を提示しており、ＰＤ－Ｌ１は、ＰＤ－１による抑制シグナルを誘導する分子の１つである。ＰＤ－Ｌ１リガンド刺激は、ＰＤ－１を発現しているＴリンパ球細胞の活性化（細胞増殖、各種サイトカイン産生誘導）を抑制することが示されている。さらに、ＰＤ－Ｌ１の発現は、免疫担当細胞のみでなく、ある種の腫瘍細胞株（単球性白血病由来細胞株、肥満細胞種由来細胞株、肝癌由来細胞株、神経芽細胞種由来細胞株、乳癌由来各種細胞株）でも確認されている（Nature Immunology，2001年，第2巻，第3号，p.261～267参照）。

ＰＤ－Ｌ２（ヒトＰＤ－Ｌ２ｃＤＮＡは EMBL / GenBank Acc. No. NM_025239、マウスＰＤ－Ｌ２ｃＤＮＡはNM_021896で示される塩基配列で構成される（Nature Immunology，2001年，第2巻，第3号，p.261～267）。）は、ＰＤ－１の第２番目リガンドとして同定されたが、その発現と機能はＰＤ－Ｌ１とほぼ同じであることが報告されている。

ＰＤ－１に代表される共役抑制分子からの抑制シグナルは、抗原レセプター（ＴＣＲ）および共役刺激分子によるポジティブなシグナルを適性に制御するメカニズムによって、リンパ球発生または成熟過程での免疫寛容や自己抗原に対する異常な免疫反応を制御していると考えられている。また、ある種の腫瘍やウイルスは、直接的もしくは間接的なメカニズムによって、Ｔ細胞の活性化および増殖を遮断し、これら共役抑制分子を自らに対する宿主免疫反応を衰弱させるのに利用していると考えられている（Cell，1992年，第

71巻，第7号，p.1093～1102、Science，1993年，第259巻，第5093号，p.368～370参照）。さらに、Ｔ細胞の機能障害に起因すると考えられている疾患の一部では、これら共役抑制分子の異常がＴ細胞の機能障害を起していると考えられている。

発明の開示

本発明の課題は、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２による抑制シグナルを阻害して、免疫賦活させる組成物およびこの機構を介した癌治療または感染症治療のための組成物の提供にある。

本発明者らは、癌治療または感染症治療における新たな標的として、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２に注目し、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２による抑制シグナルを阻害する物質が、免疫機能の回復、さらには賦活機構を介して癌の増殖を阻害することを見出した。さらに、感染したウイルスの排除にＰＤ－１シグナル、具体的にはＰＤ－１とＰＤ－Ｌ１またはＰＤ－１とＰＤ－Ｌ２の相互作用が関与していることを見出した。これら事実に基いて、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２による抑制シグナルを阻害する物質が、癌または感染症に対して治療効果を有することを見出し、本発明を完成した。

すなわち、本発明は、

１．ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を含有してなる免疫賦活組成物、

２．ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を含有してなる癌治療組成物、

３．癌転移を抑制する組成物である前項２記載の癌治療組成物、

４．ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を含有してなる感染症治療組成物、

５．免疫賦活を介して作用することを特徴とする前項２または３記載の癌治療組成物、

６．免疫賦活を介して作用することを特徴とする前項４記載の感染症治療組成物、

７．ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、およびＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質から選択される一以上の免疫抑制シグナル阻害物質である前項１乃至６のいずれかに記載の組成物、

８．ＰＤ－１抗体、ＰＤ－Ｌ１抗体、可溶化ＰＤ－１、および可溶化ＰＤ－Ｌ１から選択される一以上のＰＤ－１とＰＤ－Ｌ１の相互作用阻害物質である前項７記載の組成物、

９．国際受託番号 FERM BP-8392 で識別されるハイブリドーマが産生する抗ヒトＰＤ－１抗体、非ヒト抗体をヒト化させた抗ＰＤ－１抗体、および完全ヒト型抗ヒトＰＤ－１抗体から選択されるＰＤ－１抗体である前項８記載の組成物、

１０．遺伝子改変によりＰＤ－１発現が阻害されたリンパ球細胞が、免疫抑制シグナル阻害物質である前項１乃至６のいずれかに記載の組成物、

１１．ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、またはＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質が、タンパク質、ポリペプチド若しくはペプチド、ポリヌクレオチド若しくはポリヌクレオシド、抗体若しくはそれらの誘導体、有機合成化合物、無機化合物、および天然物から選択される一以上の物質である前項７記載の組成物、

１２．ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を投与することからなる免疫賦活方法、

１３．ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物

質を投与することからなる癌治療方法、

１４．癌転移を抑制する前項１３記載の癌治療方法、

１５．ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を投与することからなる感染症治療方法、

１６．免疫賦活を介して作用することを特徴とする前項１３または１４記載の癌治療方法、

１７．免疫賦活を介して作用することを特徴とする前項１５記載の感染症治療方法、

１８．ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、およびＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質から選択される一以上の免疫抑制シグナル阻害物質である前項１２乃至１７のいずれかに記載の方法、

１９．ＰＤ－１抗体、ＰＤ－Ｌ１抗体、可溶化ＰＤ－１、および可溶化ＰＤ－Ｌ１から選択される一以上のＰＤ－１とＰＤ－Ｌ１の相互作用阻害物質である前項１８記載の方法、

２０．国際受託番号 FERM BP-8392 で識別されるハイブリドーマが産生する抗ヒトＰＤ－１抗体、非ヒト抗体をヒト化させたＰＤ－１抗体、および完全ヒト型抗ヒトＰＤ－１抗体から選択されるＰＤ－１抗体である前項１９記載の方法、

２１．遺伝子改変によりＰＤ－１発現が阻害されたリンパ球細胞が、免疫抑制シグナル阻害物質である前項１２乃至１７のいずれかに記載の方法、

２２．ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、またはＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質が、タンパク質、ポリペプチド若しくはペプチド、ポリヌクレオチド若しくはポリヌクレオシド、抗体若しくはそれらの誘導体、有機合成化合物、無機化合物、および天然物から選択される１つ

以上の物質である前項１８記載の方法、

２３．免疫賦活組成物を製造するためのＰＤ－1、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質の使用、

２４．癌治療組成物を製造するためのＰＤ－1、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質の使用、

２５．癌治療組成物が、癌転移抑制組成物である前項２４記載の物質の使用、

２６．感染症治療組成物を製造するためのＰＤ－1、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質の使用、

２７．ＰＤ－Ｌ１またはＰＤ－Ｌ２を発現するように形質転換されたスクリーニング用癌細胞株、

２８．前項２７記載の細胞、リンパ球細胞および被験物質を接触させて、前項２７記載の細胞へのリンパ球細胞の免疫反応に対する被験物質の増強作用を評価することを特徴とする免疫賦活物質のスクリーニング方法、

２９．癌細胞である前項２７記載の細胞、リンパ球細胞および被験物質を接触させて、その癌細胞へのリンパ球細胞の免疫反応に対する被験物質の増強作用またはその腫瘍細胞の増殖に対する阻害作用を評価することを特徴とする癌治療物質のスクリーニング方法、

３０．病原体を感染させた前項２７記載の細胞またはＰＤ－Ｌ１若しくはＰＤ－Ｌ２を発現する細胞、リンパ球細胞および被験物質を接触させて、その感染細胞へのリンパ球細胞の免疫反応に対する被験物質の増強作用または病原体増殖に対する阻害作用を評価することを特徴とする感染症治療物質のスクリーニング方法、

３１．前項２７記載の癌細胞株を移植して作出した哺乳動物、

３２．前項３１記載の哺乳動物に被験物質を投与し、被験物質による移植癌細胞の増殖に対する抑制率またはその被移植哺乳動物の生存率を評価することを特徴とする癌治療物質の選別方法に関する。

本発明中のＰＤ－１、ＰＤ－Ｌ１またはＰＤ－Ｌ２は、それぞれマウス、ラット、ハムスター、モルモット、イヌ、ブタ、サルまたはヒトを含む霊長類の哺乳動物を由来とするものを含む。好ましくは、ヒトＰＤ－１、ヒトＰＤ－Ｌ１およびヒトＰＤ－Ｌ２である。

本発明中のＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２による免疫抑制シグナルは、少なくともＰＤ－１とＰＤ－Ｌ１またはＰＤ－１とＰＤ－Ｌ２との相互作用、ＰＤ－１の細胞内シグナルから構成される。さらに、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２分子自身の産生もこれに含まれる。

本発明中のＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２による免疫抑制シグナルは、ＰＤ－１とＰＤ－Ｌ１またはＰＤ－１とＰＤ－Ｌ２との相互作用またはＰＤ－１の細胞内シグナルの直接的あるいは間接的な阻害によって阻害される。これらの阻害活性を有する物質として、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２にそれぞれ選択的に結合する物質が挙げられる。好ましくは、例えば、タンパク質、ポリペプチド若しくはペプチド、ポリヌクレオチド若しくはポリヌクレオシド、抗体若しくはそれら誘導体、有機合成化合物、無機化合物、または天然物が挙げられる。特に、特異性に優れた物質として、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２に対する抗体が挙げられる。

また、当該免疫抑制シグナルは、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２分子自身の産生の阻害によっても阻害される。

ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２に対する抗体は、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２による免疫抑制シグナルを阻害するものであれば、ヒト由来抗体、マウス由来抗体、ラット由来抗体、ウサギ由来抗体またはヤギ由来抗体のいずれの抗体でもよく、さらにそれらのポリクローナル若しくはモノクローナル抗体、完全型若しくは短縮型（例えば、Ｆ（ａｂ'）$_2$、Ｆａｂ'、ＦａｂまたはＦｖ断片）抗体、キメラ化抗体、ヒト化抗体または完全ヒト型抗体のいずれのものでもよい。

そのような抗体は、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の細胞外領域の部分タンパク質を抗原として、公知の抗体または抗血清の製造法に従って製造することができる。細胞外領域の部分タンパク質は、公知のタンパク質発現ならびに精製法によって調製することができる。

ポリクローナル抗体は、公知の方法によって製造することができる。例えば、抗原タンパク質あるいはそれとキャリアー蛋白質との混合物で、適当な動物に免疫を行ない、その免疫動物から抗原タンパク質に対する抗体含有物を採取して、抗体の分離精製を行なうことにより製造することができる。用いられる動物としては、マウス、ラット、ヒツジ、ヤギ、ウサギ、モルモットが一般的に挙げられる。抗体産生能を高めるため、完全フロイントアジュバントや不完全フロイントアジュバントを抗原タンパク質と共に投与することができる。投与は、通常約２週毎に１回ずつ、計約３～１０回程度行なうのが一般的である。ポリクローナル抗体は、上記の方法で免疫された動物の血液、腹水などから採取することができる。抗血清中のポリクローナル抗体価の測定は、ＥＬＩＳＡ法によって測定することができる。ポリクローナル抗体の分離精製は、例えば、抗原結合固相あるいはプロテインＡあるいはプロテインＧなどの活性吸着剤を用いた精製法、塩析法、アルコール沈殿法、等電点沈殿法、電気泳動法、イオン交換体による吸脱着法、超遠心法、ゲルろ過法などの免疫グロブリンの分離精製法に従って行なうことができる。

抗体製剤としては、モノクローナル抗体あるいはその修飾体がより好ましい。

モノクローナル抗体産生細胞の作製は、抗原で免疫された動物から抗体価の認められた個体を選択し、最終免疫の２～５日後に脾臓またはリンパ節を採取し、それらに含まれる抗体産生細胞を同種または異種動物の骨髄腫細胞と融合させることにより、継代培養可能なモノクローナル抗体産生ハイブリドーマを作製することにより行なうことができる。抗原タンパク質の投与は、

抗体産生が可能な部位にそれ自体あるいは担体、希釈剤と共に行なう。投与には、抗体産生能を高めるため、完全フロイントアジュバントや不完全フロイントアジュバントを投与するのが一般的である。また、“ＤＮＡ免疫”と呼ばれる方法によっても、動物を免疫することができる。この方法は、免疫動物の後足前脛骨筋にカルジオトキシン（Cardiotoxin）を処置し、さらに抗原タンパク質を発現するベクターを導入した後、組織修復の過程でベクターが筋細胞に取りこまれ、タンパク質を発現する現象を利用した方法である（Nature Immunology，2001年，第2巻，第3号，p.261～267）。

免疫される動物としては、マウス、ラット、ヒツジ、ヤギ、ウサギまたはモルモットが可能であるが、好ましくはマウスまたはラットが用いられる。融合操作は、コーラー（Kohler）とミルシュタイン（Milstein）の方法（Nature，1975年，第256巻，第5517号，p.495～497）で実施することができ、融合促進剤としては、ポリエチレングリコール（ＰＥＧ）やセンダイウィルスなどが用いられる。骨髄腫細胞としては、Ｐ３Ｕ１、ＮＳ１、ＳＰ２／０、ＡＰ１などの骨髄腫細胞が挙げられるが、通常Ｐ３Ｕ１がよく利用される。モノクローナル抗体産生細胞の選別は、例えば、抗原タンパク質を直接あるいは担体と共に吸着させた固相にハイブリドーマ培養上清を添加することによるＥＬＩＳＡ法などにより検出して行なうことができる。さらに、ハイブリドーマ培養上清の抗体価は、ＥＬＩＳＡ法によって測定できる。モノクローナル抗体の分離精製は、上記のポリクローナル抗体の分離精製と同様の免疫グロブリンの分離精製法に従って行なうことができる。具体的には、国際受託番号FERM BP-8392で識別されるハイブリドーマが産生する抗ヒトＰＤ－１抗体あるいは国際受託番号FERM BP-8396で識別されるハイブリドーマが産生する抗マウスＰＤ－Ｌ１抗体である。

国際受託番号FERM BP-8392で識別されるハイブリドーマは、2002年12月19日付で日本国茨城県つくば市東１丁目１番地１　中央第６（郵便番号

305-8566）、独立行政法人産業技術総合研究所特許生物寄託センターに受託番号FERM P-19162で寄託され、2003年6月5日付で国際寄託に移管されている。また、国際受託番号FERM BP-8396で識別されるハイブリドーマは、2002年6月25日付で同センターに受託番号FERM P-18908で寄託され、2003年6月11日付で国際寄託に移管されている。

抗体断片とは、F（ａｂ'）$_2$、Ｆａｂ'、Ｆａｂまたはｓｃ Ｆｖ抗体フラグメントであり、プロテアーゼ酵素により処理し、場合により還元して得ることができる。

F（ａｂ'）$_2$抗体フラグメントは、精製されたモノクローナル抗体をペプシンで完全に消化し、イオン交換クロマトグラフィー、ゲルろ過、プロテインＡあるいはプロテインＧカラムなどのアフィニティークロマトグラフィーのいずれかの方法により精製することができる。ペプシンの消化時間は、Ｉｇサブタイプにより異なるため、適当に調製することが必要である。Ｆａｂ'抗体フラグメントは、調製したF（ａｂ'）$_2$を２－メルカプトエチルアミンで部分還元することによって作製することができる。また、Ｆａｂ抗体フラグメントは、システイン存在下で消化酵素パパインで直接消化し、精製して作製することができる。

また、モノクローナル抗体は、抗体のアミノ酸配列を決定したり、ハイブリドーマから単離できる抗体をコードするＤＮＡ配列を利用して、遺伝子組換え技術により改変し、改変抗体やハイブリッド抗体を産生させることも可能である。例えば、通常の完全型抗体ではなく単鎖抗体として作製することもできる。ｓｃＦｖ抗体（Single Chain Fv）は、ジョストらの方法（Journal of Biological Chemistry，1994年，第269巻，第42号，p.26267～26273）により行なうことができる。重鎖および軽鎖の可変領域をそれぞれコードするＤＮＡ断片を中性アミノ酸（グリシンあるいはセリン）をコードするスペーサーで連結し、この融合ＤＮＡを含む発現ベクターを適当な宿主細胞で発現させ

ることによって、本来の抗体の特性と親和性を保持する単鎖の抗体を作製することができる。

非ヒト抗体をヒトの治療に用いる場合、その抗体の抗原性を減少させることが不可欠である。患者の抗体に対する免疫反応は、有効な治療期間を短縮させる場合が多く、抗体をヒト化あるいは完全ヒト型化させ、抗体の抗原性を減らす工程が必要である。ヒトへの投与が可能となるよう改変されたヒト化抗体とは、同抗体をヒトに投与したときに薬理学的に許容し得る程度に、抗原性が減少あるいは血中動態が改善するよう改変された抗体である。

本発明の明細書中のヒトＰＤ－１抗体またはヒトＰＤ－Ｌ１抗体は、これをヒト化あるいは完全ヒト型化した抗体をも含む。

ヒト化抗体は、ヒト以外の哺乳動物に免疫して作製された非ヒト抗体の一部をヒト抗体の一部に置換することによって作製できる。具体的には、ヒト抗体の定常領域をコードする遺伝子とのキメラを構築することによって作製できることが知られている（Proc. Natl. Acad. Sci. （USA），1987 年，第 84 巻，p.3439～3443、Journal of Immunology, 1987 年，第 139 巻，第 1 号，p.3521）。ヒトの定常領域のＤＮＡ配列は文献に記載されており、その定常領域遺伝子は既知のクローンから容易に入手できる。続いて、抗体の可変領域をコードするＤＮＡ配列をヒトの定常領域の配列に融合させる。ヒトの定常領域のアイソタイプは所望のエフェクター機能または抗体依存細胞性細胞毒性における活性によって選択できる。好ましいアイソタイプはＩｇＧ１、ＩｇＧ３およびＩｇＧ４である。また、ヒト軽鎖定常領域、κ鎖またはλ鎖のいずれも用いることができる。このヒト化キメラ抗体は通常の方法によって発現させることができる。

完全ヒト型抗体は、ヒト免疫グロブリンの定常領域遺伝子を導入されたマウス（ゼノマウス（Chemical Biology, 2000 年，第 7 巻，第 8 号，p.R185-6）、ヒューマブマウス（infection and immunity, 2002 年，第 70 巻，第 2 号，p.612-9）、

ＴＣマウス（Biotechnology and Genetics Enginnering Revew, 2002年, 第19巻, p.73～82）、ＫＭマウス（Cloning Stem Cells, 2002年, 第4巻, 第1号, p.91-102））を利用して作製することができ、さらにそれらマウスから単離した抗体産生リンパ球をハイブリドーマにして、目的の抗体を量産させることができる。また、ファージ・ディスプレー法（FEBS Letter, 1998年, 第441巻, p.20-24）によっても作製することができる。この方法は、環状一本鎖ＤＮＡにヒト抗体遺伝子を組み込んだファージを利用して、ファージを構成する外殻タンパク質と融合した形で、ヒト型抗体をファージの表面に発現されることができる。

ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２に結合するポリペプチドまたはその誘導体は、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２のそれぞれの部分タンパク質であって、免疫抑制シグナルを誘導しないものが挙げられる。ＰＤ－１の免疫抑制シグナルの誘導には、ＰＤ－１が抗原レセプターの近傍に存在することが不可欠であり、そのためには抗原提示細胞、腫瘍あるいは癌細胞に存在するＰＤ－Ｌ１またはＰＤ－Ｌ２との相互作用による拘束が必要である。したがって、細胞外ドメインのみであってＰＤ－１と相互作用する部分を有する可溶化ＰＤ－Ｌ１または可溶化ＰＤ－Ｌ２は、ＰＤ－１の免疫抑制シグナルを阻害することができる。逆に、同様の構造を有し、ＰＤ－Ｌ１またはＰＤ－Ｌ２に結合できる可溶化ＰＤ－１も、免疫抑制シグナルを阻害することができる。これら可溶化タンパク質は、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２に結合するのに必要十分な細胞外領域を含むものであればよく、公知のタンパク質発現法および精製法によって調製することができる。

ＰＤ－１とＰＤ－Ｌ１またはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質がタンパク質またはポリペプチドであり、それらの相互作用に必須の領域が、連続するポリペプチドのみによって構成されている場合、そのようなポリペプチド断片は、互いの拮抗物質になり得る。さらに、このポリペプチド断片

を化学的に修飾されたあるいはポリペプチド断片の立体構造をもとにコンピュータによって設計された分子群から、より強力な活性を有する拮抗物質を同定することが可能となる。また、相互作用領域のタンパク質立体構造解析データをもとにコンピュータによって設計された分子群の中から、最適の拮抗分子をより効率的に選択することもできる。

また、ＰＤ－１とＰＤ－Ｌ１またはＰＤ－１とＰＤ－Ｌ２のの相互作用を阻害する物質を直接スクリーニングすることができる。そのような物質は、タンパク質、ポリペプチド若しくはペプチド、ポリヌクレオチド若しくはポリヌクレオシド、非ペプチド性化合物、有機合成化合物、または天然物（例えば、発酵生産物、細胞抽出物、植物抽出物、動物組織抽出物）のライブラリーから同定することが可能である。

ＰＤ－１細胞内ドメインの抑制シグナルは、ＰＤ－１細胞内ドメインであるＩＴＩＭに結合した脱リン酸化酵素（例えば、ＳＨＰ－１、２（Sathish JG, Journal of Immunology），2001 年，第 166 巻，第 3 号，p.1763-70）が、抗原受容体複合体の細胞内複合体に接触することによって生じることから、総じて抗原受容体複合体とＰＤ－１の接触の阻害によって阻害される。抑制シグナルを阻害する物質として、ＩＴＩＭのチロシン残基のリン酸化を阻害する物質、ＩＴＩＭへの脱リン酸化酵素の結合を阻害する物質、その脱リン酸化酵素の活性を直接阻害する物質などが挙げられる。抗原受容体複合体としては、Ｔ細胞受容体複合体、Ｂ細胞受容体複合体が挙げられる。

ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の産生は、特定のポリヌクレオチド若しくはポリヌクレオシド、有機合成化合物、無機化合物、または天然物などによって阻害できる。特に、好適なポリヌクレオチドまたはポリヌクレオシドとして、リボザイムと呼ばれるアンチセンスヌクレオチド誘導体が挙げられる。これは、ＰＤ－１、ＰＤ－Ｌ１または、ＰＤ－Ｌ２のｍＲＮＡに相補的なポリヌクレオチド誘導体を、対象の発現細胞に導入することによ

って、発現するｍＲＮＡが破壊される機構を利用したものである。さらに、ベクターは、患者から採取したリンパ球前駆細胞に対して、ＰＤ－１の発現を阻害するような遺伝子操作に使用でき、その操作された細胞は、増殖、分化、そして、活性化させて、再び患者に投与する細胞医療に利用することができる。特に、癌に対する免疫療法においては、リンパ球前駆細胞の成熟化および活性化の際に、標的細胞の特異抗原を添加することによって、標的細胞に特異的で、よりクローナルなリンパ球細胞を調製することができる。

本発明のスクリーニング法は、細胞機能を測定する方法で行なうことができる。同方法で使用されるＰＤ－Ｌ１またはＰＤ－Ｌ２を発現するように形質転換したスクリーニング用癌細胞株とは、ＰＤ－Ｌ１またはＰＤ－Ｌ２を発現するように構築された発現ベクターを公知の方法によって細胞に導入し、一過性あるいは安定的に形質転換された癌細胞株を含む。使用される癌細胞株としては、サルＣＯＳ－１細胞、ＣＯＳ－７細胞、Ｖｅｒｏ、チャイニーズハムスター細胞ＣＨＯ（以下、ＣＨＯ細胞と略記する。）、ｄｈｆｒ遺伝子欠損チャイニーズハムスター細胞ＣＨＯ（以下、ＣＨＯ（ｄｈｆｒ－）細胞と略記する。）、マウスＬ細胞、マウスＡｔＴ－２０、マウスミエローマ細胞、ラットＧＨ３、ＨＥＫ２９３Ｔ細胞、ヒトＦＬ細胞などが用いられる。特に、動物細胞を形質転換する場合には、例えば、「細胞工学別冊８新細胞工学実験プロトコール」（秀潤社、1995年、第263）や「Virology」（1973年、第52巻、第456号）に記載の方法に従って行なうことができる。

また、自然にＰＤ－Ｌ１またはＰＤ－Ｌ２を発現する細胞を用いることもできる。そのような細胞として、白血球細胞、好ましくは単球、マクロファージ若しくは抗原提示細胞、上皮系細胞、腫瘍細胞、癌細胞またはそれらの細胞株などである。腫瘍細胞または癌細胞として使用できる細胞として、例えば、Ｐ３８Ｄ１細胞、Ｐ８１５細胞、ＮＢ４１Ａ３細胞、ＭＤＡ－２３１細胞、ＳＫＢＲ－３細胞、ＭＣＦ－７細胞、ＢＴ４７４細胞、Ｊ５５８Ｌ細

胞、Ｐ３Ｕ１細胞、ＰＡＩ細胞、Ｘ６３細胞またはＳＰ２／０細胞を用いることができる。

ＰＤ－Ｌ１若しくはＰＤ－Ｌ２を自然に発現する細胞または強制的に発現させた細胞であって、病原体を感染させた細胞を用いることもできる。感染させる病原体としては、ヒト肝炎ウイルス（Ｂ型肝炎、Ｃ型肝炎、Ａ型肝炎）またはＥ型肝炎）、ヒトレトロウイルス、ヒト免疫不全ウイルス（ＨＩＶ１、ＨＩＶ２）、ヒトＴ細胞白血病ウイルス、ヒトＴリンパ向性ウイルス（ＨＴＬＶ１、ＨＴＬＶ２）、単純ヘルペスウイルス１型若しくは２型、エプスタイン・バーウイルス、サイトメガロウイルス、水痘－帯状疱疹ウイルス、ヒトヘルペスウイルス６を含むヒトヘルペスウイルス、ポリオウイルス、麻疹ウイルス、風疹ウイルス、日本脳炎ウイルス、おたふくウイルス、インフルエンザウイルス、アデノウイルス、エンテロウイルス、ライノウイルス、重症急性呼吸器症候群（ＳＡＲＳ）を発症するウイルス、エボラウイルス、西ナイルウイルス、またはそれらを人為的に改変されたウイルスが挙げられる。その他の病原体として、例えば、病原性原生動物（例えば、トリパノソーマ、マラリアおよびトキソプラズマ）、細菌（例えば、マイコバクテリウム、サルモネラおよびリステリア）、または真菌（例えば、カンジダ）が挙げられる。

本発明のスクリーニング法で使用されるリンパ球細胞としては、Ｔ細胞またはＢ細胞であり、好ましくは、細胞傷害性Ｔリンパ球細胞（ＣＴＬ）である。また、本発明のスクリーニング法におけるリンパ球細胞の免疫反応は、細胞傷害反応（例えば、腫瘍免疫反応）、混合リンパ球反応、サイトカイン、抗体、補体若しくはその他細胞表面抗原の産生、または細胞増殖が挙げられる。

本発明の免疫賦活または癌治療組成物の有効成分のスクリーニング法は、具体的には、細胞傷害性Ｔリンパ球の対象細胞に対する細胞傷害活性を測定

し、その活性に対する被験物質の効果を定量することによって行なうことができる。この方法は、ＰＤ－１を自然に発現する細胞傷害性Ｔリンパ球細胞（ＣＴＬ）あるいは細胞株（例えば、２Ｃ細胞）および同系マウス由来であってＰＤ－Ｌ１またはＰＤ－Ｌ２を自然に発現するもしくは強制的に発現させた細胞の混合培養に対して、被験物質を添加することによる細胞傷害活性の回復あるいは増強を定量するものである。本法の特徴は、ＰＤ－Ｌ１またはＰＤ－Ｌ２を発現していない細胞に対する細胞傷害活性に比べ、ＰＤ－Ｌ１またはＰＤ－Ｌ２を発現している細胞に対する細胞傷害活性が低く、被験物質による細胞傷害活性の回復（上昇幅）をより明確に測定できるところにある。被験物質による細胞傷害性の回復は、本発明の特徴とするＰＤ－Ｌ１またはＰＤ－Ｌ２による細胞傷害性の抑制の阻害に相当するものとして評価することができる。さらに、被験物質による細胞毒性を任意に測定することがより望ましい。これに使用される細胞としては、自然にＰＤ－Ｌ１またはＰＤ－Ｌ２を発現する腫瘍細胞株または癌細胞株（Nature Immunology, 2001年, 第2巻, 第3号, p.261～267）、例えば、Ｐ３８Ｄ１細胞、Ｐ８１５細胞、ＮＢ４１Ａ３細胞、ＭＤＡ－２３１細胞、ＳＫＢＲ－３細胞、ＭＣＦ－７細胞、ＢＴ４７４細胞、Ｊ５５８Ｌ細胞、Ｐ３Ｕ１細胞、ＰＡＩ細胞、Ｘ６３細胞、またはＳＰ２／０細胞を用いることができるが、ＰＤ－Ｌ１またはＰＤ－Ｌ２を安定的にあるいは一過的に発現するように形質転換させた腫瘍細胞株または癌細胞株も使用することができる。

一方、使用できる細胞傷害性リンパ球は、ＰＤ－１を発現しているものであって、対象とする細胞に対して同系の動物由来の細胞であることが好ましい。

本発明の感染症治療組成物の有効成分のスクリーニングは、ＰＤ－１を自然に発現する細胞傷害性Ｔリンパ球細胞（ＣＴＬ）あるいは細胞株（例えば、２Ｃ細胞）、病原菌またはウイルスを感染させた同系由来のＰＤ－Ｌ１また

はＰＤ－Ｌ２を自然に発現するもしくは強制的に発現させた細胞の混合培養に対して、被験物質を添加することによるその感染細胞へのリンパ球細胞の免疫反応の増強作用または病原菌若しくはウイルスの増殖活性に対する阻害作用を定量するものである。

さらに、同様の原理を利用した評価法では、上記のＰＤ－Ｌ１またはＰＤ－Ｌ２を発現するように形質転換させたスクリーニング用癌細胞株または自然にＰＤ－Ｌ１またはＰＤ－Ｌ２を発現する細胞を、同系哺乳動物に移植して作出した哺乳動物を使用することができる。作出する工程としては、細胞を移植する工程、評価対象が適切となるまで同哺乳動物を飼育する工程が不可欠である。この評価法は、移植細胞の増殖、各種サイトカイン若しくは細胞表面抗原の産生量、特に、細胞が癌細胞の場合は、同細胞の増殖、浸潤若しくは転移の組織学的解析または被移植哺乳動物の生存率を評価することを特徴とする。細胞増殖は、腹水癌または血液癌の場合はその単位容量当りの癌細胞数、固形癌の場合はその大きさまたは摘出後の重量によって評価することができる。本法における被験物質による癌治療効果は、ＰＤ－Ｌ１またはＰＤ－Ｌ２による細胞傷害性抑制の阻害に由来する効果に相当するものとして評価することができる。移植する癌細胞株は、インビトロでのスクリーニング法で使用できる癌細胞のいずれも使用できるが、移植哺乳動物と同系由来のもので、増殖性が良いものがより好ましい。哺乳動物としては、ヒトを除く霊長類、マウス、ラット、ハムスター、モルモット、イヌ、ブタ、サルが挙げられる。

産業上の利用可能性

本発明者らは、癌細胞移植動物モデルへの投与によって、癌の増殖を顕著に抑制し個体の延命効果を発揮する物質として、ＰＤ－１、ＰＤ－Ｌ１の機能を阻害するそれぞれの特異的抗体（抗ＰＤ－１抗体、抗ＰＤ－Ｌ１抗体）

を発明した。これら抗体は、ＰＤ－１を発現しているＣＴＬ（細胞傷害性Ｔリンパ球細胞）へのＰＤ－Ｌ１リガンドの提示によって相対的に低下する細胞傷害活性を回復あるいは増強させる作用を示した（実施例１，第１図参照）。これは、ＣＴＬによる癌細胞に対する細胞傷害活性が、これら抗体の投与によって増強しうることを示唆するものである。さらに、抗ＰＤ－Ｌ１抗体の投与は、ＰＤ－Ｌ１を人為的に発現させた肥満細胞腫由来細胞株を移入した同系マウスを用いた癌細胞移植動物モデル（蛋白質・核酸・酵素、1981 年、26 巻、3 号、p.208～224）において、癌細胞の増殖、浸潤および転移を抑制し、個体の延命効果を示した（第２図、第３図参照）。さらに、この抗体によるＰＤ－Ｌ１機能の阻害による効果と同様の効果が、ＰＤ－１の機能あるいは産生を阻害することによって得られることが示唆された。これは、ＰＤ－１欠損マウスを用いた癌移入モデルでは、移入癌細胞の増殖が全く認められなかったからであり、ＰＤ－１の機能阻害あるいは産生阻害も有効な癌治療方法になることを示すものである（実施例５，第５図参照）。

実際に、癌細胞移植動物モデルにおいて、抗ＰＤ－１抗体の投与が移入癌細胞の肝臓への転移を有意に抑制することが証明されていた（実施例１３参照）。

さらに、発明者らは、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２によって誘導される免疫抑制シグナルを阻害する物質が、感染症治療に有用であることを示した（実施例１１，第１５図，第１６図参照）。

本発明者らが実験的に示したこれら結果は、ＰＤ－１抗体またはＰＤ－Ｌ１抗体のみが既述の効果を呈することを示すものではなく、ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２からの免疫抑制シグナルを阻害し得るいずれの物質もほぼ同様の効果を呈することを証明するものである。そのような効果を有する物質として、例えば、抗ＰＤ－Ｌ２抗体、可溶化ＰＤ－１、可溶化ＰＤ－Ｌ１、可溶化ＰＤ－Ｌ２、ＰＤ－１拮抗物質、ＰＤ－Ｌ１拮抗物質、Ｐ

Ｄ－Ｌ２拮抗物質、ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用を阻害する物質、ＰＤ－１産生阻害物質、ＰＤ－Ｌ１産生阻害物質、ＰＤ－Ｌ２産生阻害物質、またはＰＤ－１による細胞内抑制シグナル阻害物質が挙げられる。

本発明の癌治療組成物の投与によって、その効果が期待される癌または腫瘍として、例えば、癌腫、扁平上皮癌（例えば、子宮頚管、瞼、結膜、膣肺、口腔、、皮膚、膀胱、舌、喉頭、食道）、腺癌（例えば、前立腺、小腸、子宮内膜、子宮頚管、大腸、肺、膵、食道、直腸、子宮、胃、乳房、卵巣）が挙げられる。さらに、肉腫（例えば、筋原性肉腫）、白血病、神経腫、メラノーマ、リンパ腫も含まれる。

これら癌または腫瘍のうち、特にＰＤ－Ｌ１またはＰＤ－Ｌ２を顕著に発現しているものに対してその効果が顕著である。ＰＤ－Ｌ１またはＰＤ－Ｌ２の発現を、外科的に切除した癌または腫瘍塊あるいは体外に採取した病変部をサンプルとする検査により同定でき、本発明の組成物の投与は、ＰＤ－Ｌ１またはＰＤ－Ｌ２を顕著に発現している腫瘍または癌を有する患者に対する外科的処置後の治療として、効率的で有効な方法となる。ＰＤ－Ｌ１またはＰＤ－Ｌ２の発現の同定は、例えば、ＰＤ－Ｌ１抗体若しくはＰＤ－Ｌ２抗体を用いた免疫化学的方法、ＲＴ－ＰＣＲ法またはＤＮＡアレイ法によって検査することができる。

癌に対する化学療法および放射線療法は、リンパ球の増殖を激しく減少させるという副作用が不可避である。本発明の組成物の投与は、減少したリンパ球細胞を刺激、増殖させる効果を示すと共に、通常の化学療法に付随する激烈な副作用を最小限に抑制することができる。また、放射線療法についても同様である。また、本発明の組成物との併用によって、化学療法剤の用量または照射放射線量を通常使用される用量あるいは照射量から大幅に減少させることができる。

本発明の癌治療組成物は、既存の化学療法剤と併用あるいは合剤化することができる。そのような化学療法剤として、例えば、アルキル化剤、ニトロソウレア剤、代謝拮抗剤、抗癌性抗生物質、植物由来アルカロイド、トポイソメラーゼ阻害剤、ホルモン療法剤、ホルモン拮抗剤、アロマターゼ阻害剤、Ｐ糖蛋白阻害剤、白金錯体誘導体、その他免疫療法剤またはその他の抗癌剤が挙げられる。さらに、患者のＱＯＬ回復のための癌治療補助剤である白血球（好中球）減少症治療薬、血小板減少症治療薬、制吐剤、癌性疼痛治療薬と併用あるいは合剤化ができる。

本発明の癌治療組成物は、その他の免疫賦活物質と併用あるいは合剤化することができる。そのような免疫賦活物質としては、例えば、各種サイトカインや腫瘍抗原などが挙げられる。免疫反応を刺激するサイトカインとしては、例えば、ＧＭ－ＣＳＦ、Ｍ－ＣＳＦ、Ｇ－ＣＳＦ、インターフェロン－α、β、γ、ＩＬ－１、ＩＬ－２、ＩＬ－３、ＩＬ－１２などが挙げられる。また、Ｂ７リガンド誘導体、抗ＣＤ３抗体および抗ＣＤ２８抗体、抗ＣＴＬＡ－４抗体も免疫反応を高めることができる。

癌抗原の投与も、癌細胞に対するＴリンパ球細胞の特異的免疫反応を高めることができ、本発明の癌治療組成物との併用によって、付加的あるいは相乗的な増強を与えることができる。癌抗原は、遺伝子が明らかなものについては精製タンパク質として、また不明なものについては、癌細胞自体の溶解物として調製することができる。

そのような癌抗原として、例えば、悪性黒色腫のＭＡＧＥ－１、ＭＡＧＥ－３由来のＨＬＡ－Ａ１およびＨＬＡ－Ａ２拘束ペプチドやＭＡＲＴ－１、ｇｐ１００が挙げられる。また、乳癌や卵巣癌のＨＥＲ２／ｎｅｕペプチドや腺癌のＭＵＣ－１ペプチド、さらに、転移性癌のＮＹ－ＥＳＯ－１も挙げることができる。

ウイルスは、感染宿主の免疫防御から逃れるための１つの方法として、Ｔ

リンパ球細胞の共役抑制因子を利用していると考えられている（Journal Experimental Medicine，2000年，第191巻，第11号，p.1987～1997）。ウイルス感染は、このようなウイルスのエスケープ機能に一部起因しており、本発明の組成物の投与によってＴリンパ球細胞のウイルスに対する免疫反応を高めることができると考えられる。

本発明の感染症治療組成物の投与は、例えば、ヒト肝炎ウイルス（Ｂ型肝炎、Ｃ型肝炎、Ａ型肝炎またはＥ型肝炎）、ヒトレトロウイルス、ヒト免疫不全ウイルス（ＨＩＶ１、ＨＩＶ２）、ヒトＴ細胞白血病ウイルスまたはヒトＴリンパ向性ウイルス（ＨＴＬＶ１、ＨＴＬＶ２）の感染治療に有効である。また、単純ヘルペスウイルス１型および２型、エプスタイン・バーウイルス、サイトメガロウイルス、水痘－帯状疱疹ウイルス、ヒトヘルペスウイルス６を含むヒトヘルペスウイルス、ポリオウイルス、麻疹ウイルス、風疹ウイルス、日本脳炎ウイルス、おたふくウイルス、インフルエンザウイルスまたは風邪ウイルスとして挙げられるアデノウイルス、エンテロウイルス若しくはライノウイルス、重症急性呼吸器症候群（ＳＡＲＳ）を発症するウイルス、エボラウイルス、または西ナイルウイルスの感染治療にも有効であると考えられる。

その他の病原体として、例えば、病原性原生動物（例えば、トリパノソーマ、マラリアおよびトキソプラズマ）、細菌（例えば、マイコバクテリウム、サルモネラおよびリステリア）または真菌（例えば、カンジダ）による感染に対しても有効であると考えられる。

本発明の感染症治療組成物は、既存の抗ＨＩＶ剤、抗ウイルス剤、抗生物質製剤、抗菌剤、内臓真菌症治療薬と併用あるいは合剤化することができる。

抗ＨＩＶ剤として、例えば、逆転写酵素阻害剤（例えば、ＡＺＴ，ｄｄＩ，３ＴＣ，ｄ４Ｔ）、プロテアーゼ阻害剤（例えば、メシル酸サキナビル、リトナビル、メシル酸ネルフィナビル、アンプレナビル、メシル酸デラビルジ

ン、サキナビル、ロピナビル／リトナビル）、またはＣＣＲ５受容体拮抗剤が挙げられる。抗ウイルス剤としては、例えば、抗ヘルペスウイルス剤、抗インフルエンザウイルス剤、インターフェロンーαおよびβ、または各種免疫グロブリンが挙げられる。

本発明の感染症治療組成物は、ウイルスもしくは病原体のワクチンと併用あるいは共に製剤化することができる。そのようなワクチンとしては、例えば、ポリオワクチン、麻疹ワクチン、日本脳炎ワクチン、ＢＣＧワクチン、３種混合ワクチン、おたふくワクチン、水痘ワクチン、インフルエンザワクチン、Ａ型肝炎ワクチン、Ｂ型肝炎ワクチン、またはコレラワクチンが挙げられる。

本発明の組成物は、通常、全身的または局所的に、経口または非経口の形で投与される。

投与量は、本発明に用いる薬物により異なると同時に、年齢、体重、症状、治療効果、投与方法、処理時間等により異なるが、経口投与の場合は、通常、成人一人あたり、１回につき、１μｇから１００ｍｇの範囲で、１日１回から数回投与される。非経口投与の場合は、成人一人あたり、１回につき、0.1ｎｇから１０ｍｇの範囲で、１日１回から数回投与され、その非経口投与形態は、好ましくは、静脈内投与であり、１日１時間から２４時間の範囲で静脈内に持続投与される。

もちろん前記したように、投与量は種々の条件により変動するので、上記投与量より少ない量で十分な場合もあるし、また範囲を越えて投与の必要な場合もある。

本発明の組成物と他の薬剤の併用剤を投与する際には、経口投与のための内服用固形剤、内服用液剤および、非経口投与のための注射剤、外用剤、坐剤、吸入剤、経鼻剤等として用いられる。

経口投与のための内服用固形剤には、錠剤、丸剤、カプセル剤、散剤、顆

粒剤等が含まれる。カプセル剤には、ハードカプセルおよびソフトカプセルが含まれる。また錠剤には舌下錠、口腔内貼付錠、口腔内速崩壊錠などが含まれる。

このような内服用固形剤においては、ひとつまたはそれ以上の活性物質はそのままか、または賦形剤（ラクトース、マンニトール、グルコース、微結晶セルロース、デンプン等）、結合剤（ヒドロキシプロピルセルロース、ポリビニルピロリドン、メタケイ酸アルミン酸マグネシウム等）、崩壊剤（繊維素グリコール酸カルシウム等）、滑沢剤（ステアリン酸マグネシウム等）、安定剤、溶解補助剤（グルタミン酸、アスパラギン酸等）等と混合され、常法に従って製剤化して用いられる。また、必要によりコーティング剤（白糖、ゼラチン、ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロースフタレート等）で被覆していてもよいし、また２以上の層で被覆していてもよい。さらにゼラチンのような吸収されうる物質のカプセルも包含される。

舌下錠は公知の方法に準じて製造される。例えば、ひとつまたはそれ以上の活性物質に賦形剤（ラクトース、マンニトール、グルコース、微結晶セルロース、コロイダルシリカ、デンプン等）、結合剤（ヒドロキシプロピルセルロース、ポリビニルピロリドン、メタケイ酸アルミン酸マグネシウム等）、崩壊剤（デンプン、Ｌ－ヒドロキシプロピルセルロース、カルボキシメチルセルロース、クロスカルメロースナトリウム、繊維素グリコール酸カルシウム等）、滑沢剤（ステアリン酸マグネシウム等）、膨潤剤（ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロース、カーボポール、カルボキシメチルセルロース、ポリビニルアルコール、キサンタンガム、グアーガム等）、膨潤補助剤（グルコース、フルクトース、マンニトール、キシリトール、エリスリトール、マルトース、トレハロース、リン酸塩、クエン酸塩、ケイ酸塩、グリシン、グルタミン酸、アルギニン等）安定剤、溶解補

助剤（ポリエチレングリコール、プロピレングリコール、グルタミン酸、アスパラギン酸等）、香味料（オレンジ、ストロベリー、ミント、レモン、バニラ等）等と混合され、常法に従って製剤化して用いられる。また、必要によりコーティング剤（白糖、ゼラチン、ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロースフタレート等）で被覆していてもよいし、また２以上の層で被覆していてもよい。また、必要に応じて常用される防腐剤、抗酸化剤、着色剤、甘味剤等の添加物を加えることもできる。口腔内貼付錠は公知の方法に準じて製造される。例えば、ひとつまたはそれ以上の活性物質に賦形剤（ラクトース、マンニトール、グルコース、微結晶セルロース、コロイダルシリカ、デンプン等）、結合剤（ヒドロキシプロピルセルロース、ポリビニルピロリドン、メタケイ酸アルミン酸マグネシウム等）、崩壊剤（デンプン、Ｌ－ヒドロキシプロピルセルロース、カルボキシメチルセルロース、クロスカルメロースナトリウム、繊維素グリコール酸カルシウム等）、滑沢剤（ステアリン酸マグネシウム等）、付着剤（ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロース、カーボポール、カルボキシメチルセルロース、ポリビニルアルコール、キサンタンガム、グアーガム等）、付着補助剤（グルコース、フルクトース、マンニトール、キシリトール、エリスリトール、マルトース、トレハロース、リン酸塩、クエン酸塩、ケイ酸塩、グリシン、グルタミン酸、アルギニン等）安定剤、溶解補助剤（ポリエチレングリコール、プロピレングリコール、グルタミン酸、アスパラギン酸等）、香味料（オレンジ、ストロベリー、ミント、レモン、バニラ等）等と混合され、常法に従って製剤化して用いられる。また、必要によりコーティング剤（白糖、ゼラチン、ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロースフタレート等）で被覆していてもよいし、また２以上の層で被覆していてもよい。また、必要に応じて常用される防腐剤、抗酸化剤、着色剤、甘味剤等の添加物を加えることもできる。

口腔内速崩壊錠は公知の方法に準じて製造される。例えば、ひとつまたはそれ以上の活性物質をそのまま、あるいは原末もしくは造粒原末粒子に適当なコーティング剤（エチルセルロース、ヒドキシプロピルセルロース、ヒドロキシプロピルメチルセルロース、アクリル酸メタクリル酸コポリマー等）、可塑剤（ポリエチレングリコール、クエン酸トリエチル等）を用いて被覆を施した活性物質に賦形剤（ラクトース、マンニトール、グルコース、微結晶セルロース、コロイダルシリカ、デンプン等）、結合剤（ヒドロキシプロピルセルロース、ポリビニルピロリドン、メタケイ酸アルミン酸マグネシウム等）、崩壊剤（デンプン、Ｌ－ヒドロキシプロピルセルロース、カルボキシメチルセルロース、クロスカルメロースナトリウム、繊維素グリコール酸カルシウム等）、滑沢剤（ステアリン酸マグネシウム等）、分散補助剤（グルコース、フルクトース、マンニトール、キシリトール、エリスリトール、マルトース、トレハロース、リン酸塩、クエン酸塩、ケイ酸塩、グリシン、グルタミン酸、アルギニン等）安定剤、溶解補助剤（ポリエチレングリコール、プロピレングリコール、グルタミン酸、アスパラギン酸等）、香味料（オレンジ、ストロベリー、ミント、レモン、バニラ等）等と混合され、常法に従って製剤化して用いられる。また、必要によりコーティング剤（白糖、ゼラチン、ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロースフタレート等）で被覆していてもよいし、また２以上の層で被覆していてもよい。また、必要に応じて常用される防腐剤、抗酸化剤、着色剤、甘味剤等の添加物を加えることもできる。

経口投与のための内服用液剤は、薬剤的に許容される水剤、懸濁剤、乳剤、シロップ剤、エリキシル剤等を含む。このような液剤においては、ひとつまたはそれ以上の活性物質が、一般的に用いられる希釈剤（精製水、エタノールまたはそれらの混液等）に溶解、懸濁または乳化される。さらにこの液剤は、湿潤剤、懸濁化剤、乳化剤、甘味剤、風味剤、芳香剤、保存剤、緩衝剤

等を含有していてもよい。

非経口投与のための外用剤の剤形には、例えば、軟膏剤、ゲル剤、クリーム剤、湿布剤、貼付剤、リニメント剤、噴霧剤、吸入剤、スプレー剤、エアゾル剤、点眼剤、および点鼻剤等が含まれる。これらはひとつまたはそれ以上の活性物質を含み、公知の方法または通常使用されている処方により製造される。

軟膏剤は公知または通常使用されている処方により製造される。例えば、ひとつまたはそれ以上の活性物質を基剤に研和、または溶融させて調製される。軟膏基剤は公知あるいは通常使用されているものから選ばれる。例えば、高級脂肪酸または高級脂肪酸エステル（アジピン酸、ミリスチン酸、パルミチン酸、ステアリン酸、オレイン酸、アジピン酸エステル、ミリスチン酸エステル、パルミチン酸エステル、ステアリン酸エステル、オレイン酸エステル等）、ロウ類（ミツロウ、鯨ロウ、セレシン等）、界面活性剤（ポリオキシエチレンアルキルエーテルリン酸エステル等）、高級アルコール（セタノール、ステアリルアルコール、セトステアリルアルコール等）、シリコン油（ジメチルポリシロキサン等）、炭化水素類（親水ワセリン、白色ワセリン、精製ラノリン、流動パラフィン等）、グリコール類（エチレングリコール、ジエチレングリコール、プロピレングリコール、ポリエチレングリコール、マクロゴール等）、植物油（ヒマシ油、オリーブ油、ごま油、テレピン油等）、動物油（ミンク油、卵黄油、スクワラン、スクワレン等）、水、吸収促進剤、かぶれ防止剤から選ばれるもの単独または２種以上を混合して用いられる。さらに、保湿剤、保存剤、安定化剤、抗酸化剤、着香剤等を含んでいてもよい。

ゲル剤は公知または通常使用されている処方により製造される。例えば、ひとつまたはそれ以上の活性物質を基剤に溶融させて調製される。ゲル基剤は公知あるいは通常使用されているものから選ばれる。例えば、低級アルコ

ール（エタノール、イソプロピルアルコール等）、ゲル化剤（カルボキシメチルセルロース、ヒドロキシエチルセルロース、ヒドロキシプロピルセルロース、エチルセルロース等）、中和剤（トリエタノールアミン、ジイソプロパノールアミン等）、界面活性剤（モノステアリン酸ポリエチレングリコール等）、ガム類、水、吸収促進剤、かぶれ防止剤から選ばれるもの単独または２種以上を混合して用いられる。さらに、保存剤、抗酸化剤、着香剤等を含んでいてもよい。

クリーム剤は公知または通常使用されている処方により製造される。例えば、ひとつまたはそれ以上の活性物質を基剤に溶融または乳化させて調製される。クリーム基剤は公知あるいは通常使用されているものから選ばれる。例えば、高級脂肪酸エステル、低級アルコール、炭化水素類、多価アルコール（プロピレングリコール、１，３－ブチレングリコール等）、高級アルコール（２－ヘキシルデカノール、セタノール等）、乳化剤（ポリオキシエチレンアルキルエーテル類、脂肪酸エステル類等）、水、吸収促進剤、かぶれ防止剤から選ばれるもの単独または２種以上を混合して用いられる。さらに、保存剤、抗酸化剤、着香剤等を含んでいてもよい。

湿布剤は公知または通常使用されている処方により製造される。例えば、ひとつまたはそれ以上の活性物質を基剤に溶融させ、練合物とし支持体上に展延塗布して製造される。湿布基剤は公知あるいは通常使用されているものから選ばれる。例えば、増粘剤（ポリアクリル酸、ポリビニルピロリドン、アラビアゴム、デンプン、ゼラチン、メチルセルロース等）、湿潤剤（尿素、グリセリン、プロピレングリコール等）、充填剤（カオリン、酸化亜鉛、タルク、カルシウム、マグネシウム等）、水、溶解補助剤、粘着付与剤、かぶれ防止剤から選ばれるもの単独または２種以上を混合して用いられる。さらに、保存剤、抗酸化剤、着香剤等を含んでいてもよい。

貼付剤は公知または通常使用されている処方により製造される。例えば、

ひとつまたはそれ以上の活性物質を基剤に溶融させ、支持体上に展延塗布して製造される。貼付剤用基剤は公知あるいは通常使用されているものから選ばれる。例えば、高分子基剤、油脂、高級脂肪酸、粘着付与剤、かぶれ防止剤から選ばれるもの単独または２種以上を混合して用いられる。さらに、保存剤、抗酸化剤、着香剤等を含んでいてもよい。

リニメント剤は公知または通常使用されている処方により製造される。例えば、ひとつまたはそれ以上の活性物を水、アルコール（エタノール、ポリエチレングリコール等）、高級脂肪酸、グリセリン、セッケン、乳化剤、懸濁化剤等から選ばれるもの単独または２種以上に溶解、懸濁または乳化させて調製される。さらに、保存剤、抗酸化剤、着香剤等を含んでいてもよい。

噴霧剤、吸入剤、およびスプレー剤は、一般的に用いられる希釈剤以外に亜硫酸水素ナトリウムのような安定剤と等張性を与えるような緩衝剤、例えば塩化ナトリウム、クエン酸ナトリウムあるいはクエン酸のような等張剤を含有していてもよい。

非経口投与のための注射剤としては、溶液、懸濁液、乳濁液および用時溶剤に溶解または懸濁して用いる固形の注射剤を包含する。注射剤は、ひとつまたはそれ以上の活性物質を溶剤に溶解、懸濁または乳化させて用いられる。溶剤として、例えば注射用蒸留水、生理食塩水、植物油、プロピレングリコール、ポリエチレングリコール、エタノールのようなアルコール類等およびそれらの組み合わせが用いられる。さらにこの注射剤は、安定剤、溶解補助剤（グルタミン酸、アスパラギン酸、ポリソルベート８０（登録商標）等）、懸濁化剤、乳化剤、無痛化剤、緩衝剤、保存剤等を含んでいてもよい。これらは最終工程において滅菌するか無菌操作法によって製造される。また無菌の固形剤、例えば凍結乾燥品を製造し、その使用前に無菌化または無菌の注射用蒸留水または他の溶剤に溶解して使用することもできる。

非経口投与のための吸入剤としては、エアロゾル剤、吸入用粉末剤又は吸

入用液剤が含まれ、当該吸入用液剤は用時に水又は他の適当な媒体に溶解又は懸濁させて使用する形態であってもよい。

これらの吸入剤は公知の方法に準じて製造される。

例えば、吸入用液剤の場合には、防腐剤（塩化ベンザルコニウム、パラベン等）、着色剤、緩衝化剤（リン酸ナトリウム、酢酸ナトリウム等）、等張化剤（塩化ナトリウム、濃グリセリン等）、増粘剤（カリボキシビニルポリマー等）、吸収促進剤などを必要に応じて適宜選択して調製される。

吸入用粉末剤の場合には、滑沢剤（ステアリン酸およびその塩等）、結合剤（デンプン、デキストリン等）、賦形剤（乳糖、セルロース等）、着色剤、防腐剤（塩化ベンザルコニウム、パラベン等）、吸収促進剤などを必要に応じて適宜選択して調製される。

吸入用液剤を投与する際には通常噴霧器（アトマイザー、ネブライザー）が使用され、吸入用粉末剤を投与する際には通常粉末薬剤用吸入投与器が使用される。

非経口投与のためその他の組成物としては、ひとつまたはそれ以上の活性物質を含み、常法により処方される直腸内投与のための坐剤および膣内投与のためのペッサリー等が含まれる。

スプレー剤は、一般的に用いられる希釈剤以外に亜硫酸水素ナトリウムのような安定剤と等張性を与えるような緩衝剤、例えば塩化ナトリウム、クエン酸ナトリウムあるいはクエン酸のような等張剤を含有していてもよい。スプレー剤の製造方法は、例えば、米国特許第2,868,691号明細書および米国特許第3,095,355号明細書に詳しく記載されている。

図面の簡単な説明

第１図（Ａ）は、Ｈ－２Ｌ^{d}特異的2C CTLクローンのＰＤ－１発現と、Ｐ８１５（肥満細胞種由来細胞株）のＰＤ－Ｌ１発現安定形質転換株でのＰＤ

－Ｌ１発現を示すフローサイトメトリー、（Ｂ）は、ＰＤ－Ｌ１発現Ｐ８１５細胞株に対する2C CTL細胞株の細胞傷害活性と抗ＰＤ－Ｌ１抗体（anti－ＰＤ－Ｌ１Ｆ（ａｂ')$_2$ＩｇＧ）の細胞傷害活性に対する効果を示す。

第２図は、同系マウスにおける移植ＰＤ－Ｌ１発現Ｐ８１５細胞株の腫瘍増殖性と浸潤性を示す。（Ａ）は移植ＰＤ－Ｌ１発現Ｐ８１５腫瘍の腫瘍容積（上）、移植後の生存率（下）であり、（Ｂ）は同系ＤＢＡ／２マウスにおける移植ＰＤ－Ｌ１発現Ｐ８１５腫瘍塊の組織染色像である（ａは腹壁および腹膜への腫瘍細胞の浸潤を示す４０倍像、ｂは同じく４００倍像、ｃは脾臓への転移、ｄは肝臓への転移を示す。）。

第３図は、同系マウスにおける移植ＰＤ－Ｌ１発現Ｐ８１５細胞株の腫瘍増殖性に対する抗ＰＤ－Ｌ１抗体のin vivo効果を示す。（Ａ）は当該マウスにおける腫瘍特異的ＣＤ８＋Ｔ細胞からのＩＦＮ－γ産生に対する抗ＰＤ－Ｌ１抗体のin vivo効果、（Ｂ）は当該マウスにおける移植ＰＤ－Ｌ１発現Ｐ８１５腫瘍の腫瘍容積(上)と生存率(下)に対する抗ＰＤ－Ｌ１抗体のin vivo効果を示す（図中、□は対照群（ラットＩｇＧ投与群）、○は抗ＰＤ－Ｌ１抗体（anti－ＰＤ－Ｌ１Ｆ（ａｂ')$_2$ＩｇＧ）投与群）。

第４図は、ＰＤ－１遺伝子ホモ欠損同系マウス（ＰＤ－１$^{-/-}$）でのＰＤ－Ｌ１発現Ｂ１６メラノーマの増殖抑制を示す。

第５図は、同系マウス（ＢＡＬＢ／ｃ）における移植ミエローマ細胞株の腫瘍増殖性に対する抗ＰＤ－Ｌ１抗体のin vivo効果とＰＤ－１の関与を示す。（Ａ）は各種ミエローマ細胞株でのＰＤ－Ｌ１発現を示すフローサイトメトリー、（Ｂ）は前記マウスにおける移植Ｊ５５８Ｌ腫瘍の腫瘍容積に対する抗ＰＤ－Ｌ１抗体のin vivo効果、（Ｃ）は野生型およびＰＤ－１遺伝子欠損ＰＤ－１$^{-/-}$の同系マウスにおける移植Ｊ５５８Ｌ腫瘍の腫瘍増殖の比較を示す。

第６図は、血管内皮でのＰＤ－Ｌ１発現を示す。（Ａ）はマウス心臓の血

管内皮細胞でのＰＤ－Ｌ１，ＰＤ－Ｌ２の発現を示す。（Ｂ）はマウス各組織でのＰＤ－Ｌ１発現の確認を組織染色を示す。（ａ）：眼球、（ｂ）：顎下腺、（ｃ）：心臓、（ｄ）：肺、（ｅ）：肝臓、（ｆ）：腎臓でのＰＤ－Ｌ１の発現を示す。図中、Ｃｈは脈絡膜、ＣＶは中心静脈、Ｇｌは糸球体、Ｒｅは網膜を示す。各矢印は、血管内皮細胞を示す。各染色像は４０倍拡大像である。

第７図は、肝臓非実質細胞でのＰＤ－Ｌ１発現を示す。（Ａ）はマウス肝臓でのＩＣＡＭ－１、（Ｂ）はＰＤ－Ｌ１の発現を示す（ＣＶ：中心静脈）。

第８図は、クップファー細胞（Kupffer Cell）および肝臓類洞周囲腔内皮細胞（ＬＳＥＣ）での細胞表面分子のフェノタイプを示す。

第９図は、ＰＤ－１遺伝子ホモ欠損マウス（$PD-1^{-/-}$）あるいは野生型マウス（ｗｔ）のＣＤ４陽性Ｔ細胞での細胞表面分子のフェノタイプを示す。

第１０図は、ＰＤ－１遺伝子ホモ欠損マウス（$PD-1^{-/-}$）あるいは野生型マウス（ｗｔ）のＣＤ８陽性Ｔ細胞での細胞表面分子のフェノタイプを示す。

第１１図は、Ｔ細胞増殖に対するＬＮＰＣのＰＤ－Ｌ１の効果を示す。（Ａ）は$PD-1^{-/-}$マウスおよびｗｔマウスのそれぞれのナイーブＴ細胞刺激時の細胞増殖を示す。（Ｂ）は$PD-1^{-/-}$マウスおよびｗｔマウス由来で既に活性化されたＴ細胞とＬＮＰＣとの共培養における細胞増殖に対する抗ＰＤ－Ｌ１抗体の効果を示す。

第１２図は、サイトカイン産生に対するＬＮＰＣのＰＤ－Ｌ１の効果を示す。（Ａ）は$PD-1^{-/-}$マウスおよびｗｔマウスのそれぞれのナイーブＴ細胞刺激時のサイトカイン産生を示す。（Ｂ）は、$PD-1^{-/-}$マウスおよびｗｔマウス由来で既に活性化されたＴ細胞とＬＮＰＣとの共培養におけるサイトカイン産生に対する抗ＰＤ－Ｌ１抗体の効果を示す。（Ｃ）はＬＮＰＣとの共培養における$PD-1^{-/-}$マウスおよびｗｔマウスからの活性化Ｔ細胞の細胞分裂を示す。

第１３図は、ウイルス感染したマウス肝臓におけるＴリンパ球細胞の増殖に対するＰＤ－１の関与を示す。（A）はアデノウイルス感染後０日目、（B）は同７日目でのＰＤ－１$^{-/-}$マウスおよびｗｔマウスの肝臓および脾臓でのＣＤ１９陽性およびＣＤ３陽性リンパ球細胞の増殖を示す。

第１４図は、ウイルス感染したマウス肝臓におけるＴリンパ球細胞の増殖に対するＰＤ－１の関与を示す。（A）はアデノウイルス感染後７日目でのＰＤ－１$^{-/-}$マウスおよびｗｔマウスの肝臓でのＣＤ４陽性およびＣＤ８陽性リンパ球細胞の増殖を示す。（B）は、感染後７日目での各種増殖性リンパ球の割合を示す。

第１５図は、ウイルス感染に対するＰＤ－１の関与を示す。図中（ａ）～（ｄ）は、アデノウイルス感染後０日および７日目でのＰＤ－１$^{-/-}$マウスおよびｗｔマウスの肝臓でのそれぞれの細胞増殖を示す組織染色像である。（ｅ）、（ｆ）はアデノウイルス感染後７日目でのＰＤ－１$^{-/-}$マウスでのＣＤ４陽性、ＣＤ８陽性Ｔ細胞の細胞増殖を示す。

第１６図は、ウイルス感染に対するＰＤ－１の関与を示す。図中（ｇ）～（ｎ）は、アデノウイルス感染後０日および７日目でのＰＤ－１$^{-/-}$マウスおよびｗｔマウスの肝臓のヘマトキシリン＆エオジン組織染色像である。図中（ｏ）～（ｒ）は、アデノウイルス感染後０日および７日目でのＰＤ－１$^{-/-}$マウスおよびｗｔマウスの肝臓のＸ－Ｇａｌ染色像である。

第１７図は、細胞傷害活性に対する各物質の増強作用を示す。図中（ａ）抗マウスＰＤ－１抗体の増強作用、（ｂ）抗マウスＰＤ－Ｌ１抗体の増強作用、（ｃ）マウスＰＤ－１Ｆｃの増強作用（ｄ）ヒトＰＤ－１Ｆｃの増強作用を示す。

発明を実施するための最良の形態

以下に実施例を挙げて本発明を、より具体的に説明するが、これらは本発

明の範囲を制限するものではない。

実施例１

マウスＰＤ－Ｌ１発現ベクターの作製は、マウス PD-L1cDNA（Journal of Experimental Medicine，2000年，第19巻，第7号，p.1027～1034）を制限酵素EcoRIで消化して、発現ベクターpApuroXS（The EMBO Journal，1994年，第13巻，第６号，p.1341～1349）に挿入し、連結させることによって行った。作製した発現ベクターpApuroXS-PD-L1のＰ８１５細胞への導入は、エレクトロポーレーション法（３６０Ｖ、５００μＦ）で行った。Ｐ８１５細胞の培養は、ＦＣＳ（１０%）、２－メルカプトエタノール（10^{-5}M）、各種抗生物質含 RPMI-1640 培地で培養できるが、さらに、抗生物質ピューロマイシン（Puromycin；３μｇ／ｍｌ）を含んだ培地の培養に対して耐性の同細胞株を継代培養することによって、マウスＰＤ－Ｌ１を安定的に発現する形質転換Ｐ８１５細胞株を取得した。ＰＤ－Ｌ１の発現は、フローサイトメトリー解析にて確認した。第１図（A）にＨ－２Ｌ^{d}特異的2C CTLクローンのＰＤ－１発現（ｉ）と、Ｐ８１５（肥満細胞種由来細胞株）のＰＤ－Ｌ１発現安定形質転換株でのＰＤ－Ｌ１発現（ii）を示すフローサイトメトリーを示す。

同様の方法で、ＰＤ－Ｌ１を安定的に発現する形質転換Ｂ１６細胞株（Ｂ１６／ＰＤ－Ｌ１）を取得した（第１図（A）（iii）～（ｖ）参照）。ここでは発現ベクターとして同様の方法で作製したpEFBOSneo-PD-L1（Nucleic Acid Research，1990年，第18巻，第17号，p.5322）を用い、細胞株の選択培養にはＧ４１８（0.5ｍｇ／ｍｌ）を使用した。

全長マウス PD-L1cDNA の3'末端側に６個のヒスチジンがタンデムに並んだペプチドタグ（His-Tag）を連結させたタンパク質をコードするｃＤＮＡを制限酵素 EcoRI および NotI で消化して、発現ベクターpVL1393（商品名：Clontechより購入）に挿入させた。続いて、この発現ベクターをＳＦ９昆虫細

胞（Invitrogen より購入）に導入して、封入体を回収した。この封入体ウイルスを HiFive 昆虫細胞（Invitrogen より購入）に２日間、２７℃下で培養することによって感染させた。溶解緩衝液（Tris-ＨＣｌ（５０mM，ｐＨ７，含１％ TritonX-100）、ＥＤＴＡ（１０mM）、ＮａＣｌ（１５０mM）、各種プロテアーゼ阻害剤）で溶解させた細胞溶解液を、Ｎｉ－セファロースカラムクロマトグラフィーで処理することによって、抗原となる精製ＰＤ－Ｌ１タンパク質を取得した。

透析した同ＰＤ－Ｌ１タンパク質を完全フロイントアジュバンドと共に８週令メス Whister ラット（SLC Japan より購入）に免疫して、数日後、末梢リンパ節から回収した２×１０^{8}細胞を、PEG1500（Amersham より購入）を用いて同数のＳＰ２／０細胞と細胞融合させた。さらに、RPMI1640 培地（ＨＡＴ（Sigma より購入）、Origen（１０%，Igen より購入）、ＦＣＳ（１０%）、２－メルカプトエタノール（１０$^{-5}$M）、各種抗生物質）で培養することによって選択し、産生抗体の存在をフローサイトメトリー解析にて確認した。これによって樹立されたハイブリドーマ（国際受託番号：FERM BP-8396 で認識されるハイブリドーマ）を Balb/C nu/nu マウスに移入して、後に腹水からの回収液をプロテインＧセファロースカラムクロマトグラフィーで精製することによって、ＰＤ－Ｌ１に対するモノクローナル抗体（１－１１１）を取得した。フローサイトメトリー等で使用される抗体は、Sulfo-NHS-LC-biotin（商品名：Pierce より購入）を用いてビオチン化したものを用いた。

また、同様の方法に従い、抗ヒトＰＤ－１抗体（国際受託番号：FERM BP-8392 で認識されるハイブリドーマから産生されるモノクローナル抗体）を作製した。

細胞傷害性アッセイは、^{51}Ｃｒ（クロム）遊離アッセイによって行った。

２Ｃ細胞（Journal of Immunology，1996 年，第 157 巻，第 2 号，p.670～678）は、２Ｃトランスジェニック Ｂ６マウス由来の（Ｈ－２Ｌ）^{d}アロ反応性の細

胞傷害性Ｔ細胞である。第１図（Ｂ）に、２Ｃ細胞（Ｅ：エフェクター）を^{51}Ｃｒラベル化したＰ８１５細胞（Ｔ：ターゲット）と共に（○）または３つのＰＤ－Ｌ１発現Ｐ８１５細胞株（Ｐ８１５／ＰＤ－Ｌ１）（□、◇、△）と共にあるいはさらに１０ｍｇ／ｍｌラット anti-PD-L1F(ab')$_2$IgG 存在下（▲）を、さまざまなＥ／Ｔ比で混合して、４時間で遊離される^{51}Ｃｒを測定した結果を示す。

抗ＰＤ－Ｌ１抗体（anti-PD-L1F(ab')$_2$）は、細胞傷害性Ｔリンパ球細胞の低下した細胞傷害活性を回復させた。これらの結果から、ＰＤ－Ｌ１の機能を阻害することによるＰＤ－１およびＰＤ－Ｌ１シグナルの阻害は、癌細胞に対する細胞傷害活性を増強させることができると考えられる。

実施例２

１×１０^{6}細胞のＰ８１５細胞（ｎ＝６）またはＰ８１５／ＰＤ－Ｌ１細胞（ｎ＝６）を同系ＤＢＡ／２マウスの皮下にそれぞれ移入し、腫瘍増殖とマウスの生存率を評価した。その結果を第２図（Ａ）に示す。図中、○はＰ８１５細胞株移植群、□、△はＰＤ－Ｌ１発現Ｐ８１５細胞株移植群である。さらにＰ８１５／ＰＤ－Ｌ１細胞を移入した群の組織学的解析を行った。移入２０日後のマウスの腹壁ならびに腹膜腔の組織切片を、１０％ホルムアルデヒドで固定、パラフィンで砲架して、ヘマトキシリン、エオジンで染色して得られた染色像を第２図（Ｂ）に示す。図中、ａは腹壁および腹膜への腫瘍細胞の浸潤を示す４０倍像、ｂは同じく４００倍像であり、ｃは脾臓への転移、ｄは肝臓への転移を示す。

Ｐ８１５細胞を移植した群では、Ｐ８１５細胞の増殖は抑えられており、６～７週目では３０％が生存したのに対して、ＰＤ－Ｌ１を発現するＰ８１５細胞（Ｐ８１５／ＰＤ－Ｌ１）を移植した群では、癌細胞の増殖は著しく、２～４週目までに全例が死亡した（第２図（Ａ））。Ｐ８１５／ＰＤ－Ｌ１

は、腹膜腔、さらに、腹腔へ浸潤しており、また、肝および脾臓への転移が認められた（第２図（Ｂ）ａ～ｄ参照）。

実施例３

Ｐ８１５細胞を移入し免疫したマウスから細胞傷害性Ｔ細胞ＣＴＬを調整し、２×１０^{6}個数のＣＴＬ細胞と、５×１０^{6}個数のＰ８１５細胞またはＰ８１５／ＰＤ－Ｌ１細胞のみ、あるいは anti-PD-L1F(ab')$_2$IgG（１０ｍｇ／ｍｌ）存在下でＰ８１５／ＰＤ－Ｌ１細胞をそれぞれ混合培養し、２４時間後の培養上清中のＩＦＮ－γをＥＬＩＳＡキット（Bioscience から購入）で測定した。その結果を第３図（Ａ）に示す。

また、第３図（Ｂ）に、３×１０^{6}個数のＰ８１５／ＰＤ－Ｌ１細胞を皮下移入した同系ＤＢＡ／２マウス（ｎ＝１０）に、ラットＩｇＧ（□）あるいは anti-PD-L1F(ab')$_2$IgG（0.1ｍｇ／一匹）（○）を、細胞移入後１、３、５、７日後にそれぞれ腹腔内投与し、腫瘍増殖とマウスの生存率を評価した結果を示す。

抗ＰＤ－Ｌ１抗体は、Ｐ８１５／ＰＤ－Ｌ１により抑制された細胞傷害性Ｔリンパ球細胞からのＩＦＮ－γ産生を回復させた（第３図（Ａ））。抗ＰＤ－Ｌ１抗体の投与は、癌細胞増殖を抑制し、明確な生存効果を示した（第３図（Ｂ））。この結果は、抗ＰＤ－Ｌ１抗体の投与が癌治療に有効であることを示している。

実施例４

１×１０^{6}個数のＢ１６メラノーマ（ｎ＝６）またはＢ１６／ＰＤ－Ｌ１細胞（ｎ＝６）をＢ６マウスにはぞれぞれ皮下移入し、同数のＢ１６／ＰＤ－Ｌ１細胞をＰＤ－１トランスジェニックＢ６マウス（ｎ＝５）およびＰＤ－１遺伝子ホモ欠損Ｂ６マウス（ＰＤ－１$^{-/-}$（ｎ＝４））（Science, 2001 年,

291巻，5502号，p.319～332）に移入し、以後２５日までそれぞれの腫瘍増殖を測定した。その結果を第４図に示す。

実施例５

2.5×１０^{8}個数のＪ５５８Ｌミエローマ細胞を皮下移入した同系Ｂａｌｂ／Ｃマウス（ｎ＝９）に、ラットＩｇＧあるいは anti-PD-L1F(ab')$_2$IgG（0.1ｍｇ／一匹）を細胞移入後３、５、７日後にそれぞれ腹腔内投与し、腫瘍増殖を評価した（第５図（Ｂ））。また、同様にＪ５５８Ｌミエローマ細胞を皮下移入したＰＤ－１ホモ欠損マウスとＢａｌｂ／Ｃ（ｎ＝４）での腫瘍増殖を比較した（第５図（Ｃ））。

抗ＰＤ－Ｌ１抗体の投与は、ＰＤ－Ｌ１を発現しているＪ５５８癌細胞（第５図（Ａ）に各種ミエローマ細胞株でのＰＤ－Ｌ１発現を示すフローサイトメトリーを示す。）の増殖を抑制した（第５図（Ｂ））。また、Ｊ５５８細胞を移植したＰＤ－１欠損マウスでは移植癌細胞の増殖は完全に阻害された（第５図（Ｃ））。これらの結果は、ＰＤ－Ｌ１もしくはＰＤ－１の阻害が癌治療に有効であることを示している。

実施例６

血管内皮細胞（以下、ＥＣｓと略す。）をマレリーバーグの方法（Journal Immunology Methods，2000年，第244巻，第1-2号，p.205～215）により、マウス心臓から取得した。具体的には、心臓組織をコラゲナーゼで消化した後、マウスＩｇとともに前培養、さらに、ＦＩＴＣ修飾した抗ＣＤ３１抗体、同修飾抗ＣＤ１０５抗体、同修飾抗 isolectinB4 抗体、および、抗ＦＩＴＣビーズを添加して培養した。この血管内皮細胞を Magnetic-activated cell-sorting separation columns（商品名：Miltenyi Biotec より購入）を用いて、ポジティブ選択によって精製した。

取得した血管内皮細胞でのＰＤ－Ｌ１、ＰＤ－Ｌ２の発現を、フローサイトメトリーで確認した。同細胞の標識には、抗ＰＤ－Ｌ１抗体（抗体名：１－１１１）、抗ＰＤ－Ｌ２抗体（抗体名：＃１２２）および蛍光標識した２次抗体を用いて行った(第６図(Ａ))。解析はCellQuestソフトウェア(Dickinsonより購入）を使用したFacscalibur（機器名：Becton Dickinsonより購入）で、１万回イベントで解析した。ＰＤ－Ｌ１あるいはＰＤ－Ｌ２の発現は、開口曲線で示され、コントロールＩｇは充填曲線で示される。

マウス各組織でのＰＤ－Ｌ１発現の確認を組織染色によって行った。各組織サンプリング１時間前に１００μｇビオチン標識化抗ＰＤ－Ｌ１抗体（１－１１１）を溶解した１００μｌＰＢＳをマウスに静脈投与した。続いて、５μｍ凍結切片を４％パラホルムアルデヒド（ＰＦＡ）で固定して、Streptavidin-FITCで染色した。さらに、各切片をPhalloidinで対比染色した（第６図（Ｂ）、（ａ）眼球、（ｂ）顎下腺、（ｃ）心臓、（ｄ）肺、（ｅ）肝臓、（ｆ）腎臓でのＰＤ－Ｌ１の発現を示す。図中、Ｃｈは脈絡膜、ＣＶは中心静脈、Ｇｌは糸球体、Ｒｅは網膜を示す。各矢印は、血管内皮細胞を示す。各染色像は４０倍拡大像である。）。ＰＤ－Ｌ１は、心臓、肺、腎臓、胃、小腸、顎下腺、眼球、肝臓の血管内皮で認められた。肝臓での発現は、肝臓洞様毛細血管に局在していた。

実施例７

肝臓非実質細胞（以下、ＬＮＰＣｓと略記する。）でのＰＤ－Ｌ１発現を組織染色（第７図（Ａ））ならびにフローサイトメトリー（第７図（Ｂ））で確認した。組織染色は、３％ＰＦＡで固定した５μｍ肝臓凍結切片をラット血清で前処理し、ビオチン標識化抗ＰＤ－Ｌ１抗体（１－１１１）あるいはビオチン標識化抗ＩＣＡＭ－１抗体（商品名：BD Pharmingenより購入）にて１時間、室温下で抗体反応を行ない、ビオチン抗体をtyramide signal

amplification（ＴＳＡ）fluorescence system（機器名：PerkinElmer Life Sciences より購入）にて視覚化した（第７図（Ａ）：ＩＣＡＭ－１の発現、同図（Ｂ）：ＰＤ－Ｌ１の発現、ＣＶ：中心静脈を示す。各染色像は４０倍拡大像である。）。

ＬＮＰＣｓは、pronaseE 法（Experimental Cell Research, 1976 年, 第 99 巻, p.444～449）によってマウス肝臓より単離した。具体的には、ＬＮＰＣｓは、肝臓を pronaseE 溶液（Merck）にて還流、培養し、密度勾配遠心法にて分離した。その細胞懸濁液中のクップファー細胞（Kupffer Cells）（ＣＤ５４$^{+}$、ＣＤ１１ｂ^{high}）の相対的分布は２０～２５％であり、肝臓類洞周囲腔内皮細胞（以下、ＬＳＥＣｓと略記する。）（ＣＤ５４＋、ＣＤ１１ｂ^{high}）は、７５～８０％である。

クップファー細胞とＬＳＥＣｓを、ＦＩＴＣ標識化抗ＣＤ１１ｂ抗体およびＩＣＡＭ－１、ＰＤ－Ｌ１、Ｂ７－１、Ｂ７－２に対するそれぞれのビオチン化モノクローナル抗体、それに続くＰＥ標識化 Streptavidin にて、それぞれ二重染色した。クップファー細胞とＬＳＥＣｓは、それぞれＣＤ１１ｂ^{high}とＣＤ１１ｂ^{low}細胞としてゲートされる（第８図）。

ＰＤ－Ｌ１は、クップファー細胞では、ＩＣＡＭ－１、Ｂ７－１、Ｂ７－２を共発現しているが、ＬＳＥＣｓではその発現は弱いものであった（第８図）。

実施例８

ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型Ｃ５７ＢＬ／６マウス（ｗｔ）の脾臓ならびにリンパ組織から、T-cell enrichment column（商品名：Genzyme より購入）を用いたネガティブ選別によって、ナイーブＴ細胞（精製度９０％以上）を精製した。同細胞を１０μｇ／ｍｌ抗ＣＤ３モノクローナル抗体（２Ｃ１１）とともに４８時間培養して活性化させた。

上記方法によって活性化したナイーブＴ細胞を、ＦＩＴＣ標識化抗ＣＤ４

抗体あるいはＡＰＣ標識化抗ＣＤ８抗体ならびにＰＥ標識化抗ＣＤ２５抗体、ＰＥ標識化抗ＣＤ４４抗体、ＰＥ標識化抗ＣＤ６９抗体、ＰＥ標識化抗ＣＴＬＡ－４抗体、ビオチン標識化抗Ｂ７－１（ＣＤ８０）抗体、ビオチン標識化抗Ｂ７－２（ＣＤ８６）抗体、抗ＰＤ－１抗体（抗体名：Ｊ４３、国際受託番号 FERM BP-8118 で認識されるハイブリドーマから産生されるモノクローナル抗体）、抗ＰＤ－Ｌ１抗体（１－１１１）で二重染色して、フローサイトメトリーにてそれぞれの分子の発現を解析した（第９図、第１０図）。

なお、国際受託番号 FERM BP-8118 で識別されるハイブリドーマは、2001年 5 月 30 日付で日本国茨城県つくば市東１丁目１番地１ 中央第６（郵便番号 305-8566）、独立行政法人産業技術総合研究所特許生物寄託センターに受託番号 FERM P-18356 で寄託され、2002 年 7 月 16 日付で国際寄託に移管されている。

実施例９

ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型マウス（ｗｔ）のナイーブＴ細胞の活性化は、上記実施例８に記載の方法で行った。活性化後の同細胞の増殖をＢｒｄＵ取りこみ法によって測定した（第１１図（Ａ））。ＢｒｄＵを４８時間の最後の６時間に添加して細胞を標識し、これを Proliferation ELISA kit（商品名：Roche より購入）を用いる測定によって細胞増殖を決定した。また、この時のＩＦＮ－γ産生量を ELISA Kit（商品名：Genzyme より購入）にて測定した（第１２図（Ａ））。

ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型マウス（ｗｔ）由来のＴ細胞を上記実施例８で記載の方法であらかじめ活性化させた。続いて、既に活性化しているＴ細胞に対して、野生型マウスからのマイトマイシンＣ処理ＬＮＰＣｓの存在あるいは非存在下で、さらに、３０μｇ／ｍｌ抗ＰＤ－Ｌ１抗体（１－１１１）（コントロールとしたラットＩｇＧ）、２０

μｇ／ｍｌCTLA4-Ig（Genzyme）（コントロールとしたヒトＩｇＧ）の存在あるいは非存在下でそれぞれ６０時間培養して、最後の１２時間の同細胞の増殖をＢｒｄＵ取りこみ法によって測定した（第１１図（Ｂ））。また、４８時間時点でのＩＦＮ－γ産生量も測定した（第１２図（Ｂ））。

ナイーブＴ細胞の活性化時におけるＩＦＮ－γ産生量は、ＰＤ－１$^{-/-}$と野生型マウスの違いによる有意な差異は認められなかった。一方、既に活性化されたＴ細胞においては、野生型マウス由来のＴ細胞のＩＦＮ－γ産生量は、ＰＤ－１$^{-/-}$由来のものに比べ有意に低かった（第１２図）。このことから、既に活性化しているＴ細胞に対するＰＤ－１の抑制作用の効果は、ナイーブＴ細胞の活性化に対する効果よりも高いことが示唆された。

野生型マウス由来の活性化したＴ細胞は、ＬＮＰＣｓとの共培養では、Ｔ細胞の細胞増殖およびＩＦＮ－γ産生量に有意な変化は認められないが、ＰＤ－１$^{-/-}$由来の活性化したＴ細胞は、ＬＮＰＣｓとの共培養によって、その細胞増殖に有意な増加が認められた（第１１図（Ｂ）、第１２図（Ｂ））。また、野生型マウス由来の活性化したＴ細胞とＬＮＰＣｓとの共培養への抗ＰＤ－Ｌ１抗体の添加によって、Ｔ細胞の細胞増殖の増加が認められた（第１１図（Ｂ））。これらの結果は、ＬＮＰＣｓのＰＤ－１あるいはＰＤ－Ｌ１が、活性化Ｔ細胞の抑制に関与しており、ＰＤ－１の欠如あるいはＰＤ－１とＰＤ－Ｌ１間の相互作用の阻害が、Ｔ細胞を活性化することを示すものである。

ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型マウス（ｗｔ）の活性化Ｔ細胞を、５μＭ CFSE(5-(6)-carboxy-fluorescein diacetate succinimidyl diester)（商品名：Molecular probes より購入）で標識して、ＬＮＰＣｓとともに４８時間共培養した。この時の細胞分裂を、ＦＡＣＳを用いたＣＦＳＥ活性測定によって決定した（第１２図（Ｃ））。

活性化Ｔ細胞の細胞増殖抑制は、細胞分裂の停止に起因しており、ＰＤ－

１シグナルがＴ細胞の細胞分裂を抑制することが示唆された（第１２図（Ｃ））。

実施例１０

ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型マウス（ｗｔ）（３匹／群）に10^9-10^{10}PFU (plaque-forming units) Ad-lacZを静脈内投与して、マウスをアデノウイルスに感染させた。ここで使用したAd-lacZは、Ｅ１およびＥ３領域に欠損を有し、さらに、１ａｃＺ遺伝子を持った５型アデノウイルスであり、２９３細胞で増殖させた後、塩化セシウム密度勾配遠心分離（Nucleic Acid Research，1995年，第234巻，第19号，p.3816～3821）の記載によって精製したものである。

感染後０日目あるいは７日目に、同マウスに対して屠殺１時間前に0.5ｍｇＢｒｄＵ（商品名：Sigmaより購入）を静脈内投与して、採取した脾臓細胞および肝臓内リンパ球を抗ＢｒｄＵ抗体および抗ＣＤ１９抗体あるいは抗ＣＤ３抗体で二重標識した（第１３図）。

さらに、感染後７日目の同細胞に対しては、抗ＢｒｄＵ抗体、抗ＣＤ１９抗体、抗ＣＤ３抗体、抗ＣＤ４抗体、抗ＣＤ８抗体で二重標識した（第１４図（Ｂ）、それぞれの棒グラフはＢｒｄＵ陽性細胞の割合を示す。）。

アデノウイルス感染したＰＤ－１$^{-/-}$マウスの肝臓では、同様に感染した野生型マウスの肝臓に比較して、増殖性（ＢｒｄＵ陽性）の各リンパ球（ＣＤ１９陽性、ＣＤ３陽性、ＣＤ４陽性、ＣＤ８陽性）の割合が増加していた。一方、このような現象が脾臓では認められなかったことから、ＰＤ－１は、炎症を起した組織でのＴ細胞の増殖を阻害することが示唆された（第１４図（Ｂ））。

実施例１１

ＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－１$^{-/-}$）あるいは野生型マウス（ｗｔ）

（３匹／群）に 10^9-10^{10}PFU Ad-lacZ を静脈内投与して、マウスをアデノウイルスに感染させ、さらに、感染後０日目あるいは７日目の屠殺１時間前に 0.5 ｍｇ ＢｒｄＵ（商品名：Sigma より購入）を静脈内投与して、採取した肝臓切片を抗ＢｒｄＵ抗体で標識した（第１５図（ａ）～（ｄ））、２０倍拡大像）。感染後７日目のＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－$1^{-/-}$）の肝臓切片に対しては、抗ＢｒｄＵ抗体および抗ＣＤ４抗体あるいは抗ＣＤ８抗体で二重標識した（第１５図（ｅ）、（ｆ）、４０倍拡大像）。

感染後３０日目の野生型マウスの肝臓では、洞様毛細血管および非実質領域への中程度の局所的な細胞浸潤が認められたが、ＰＤ－$1^{-/-}$マウスでは、肝炎を示す症状は認められなかった（第１６図（ｈ）、（ｉ）および（ｊ）、（ｎ））。

感染後７日目あるいは３０日目のＰＤ－１遺伝子ホモ欠損マウス（ＰＤ－$1^{-/-}$）あるいは野生型マウス（ｗｔ）の肝臓切片に対して、ヘマトキシリン＆エオジン染色（第１６図（ｇ）～（ｊ），２０倍拡大像、（ｋ）～（ｎ），４０倍拡大像）およびＸ－Ｇａｌ染色を行った（第１６図（ｏ）～（ｒ），４０倍拡大像）。

感染後７日目および３０日目の野生型マウスの肝臓では、Ｘ－Ｇａｌ染色像が示すアデノウイルスの感染が認められたが、ＰＤ－$1^{-/-}$マウスでは、その感染は、３０日目にはほぼ排除されていた（第１６図（ｏ）、（ｐ）および（ｑ）、（ｒ））。これらの結果から、ＰＤ－１シグナルが、ウイルス感染による炎症組織におけるエフェクターＴ細胞の細胞増殖を誘導することによるウイルスの排除に関与することが示された。

実施例１２

マウスＰＤ－Ｌ１を強制発現させたＰ－８１５／ＰＤ－Ｌ１細胞は、５μｇ／ｍＬ puromycin（Sigma より購入）を含む通常培地（以下、選択培地と略

す。）を用いて、培養フラスコに播種し、３７℃、５％CO_2／９５％airの条件で５０％～９０％コンフルエントになるまで培養した。マウス cytotoxic T lymphocyte 2C 細胞は、ＭＭＣ（Mitomycin C）処理したＰ－８１５細胞およびＣｏｎＡ刺激ラット脾細胞培養上清とともに通常培地中で数日継代培養した。回収したＰ－８１５／ＰＤ－Ｌ１細胞に DELFIA EuTDA Cytotoxicity Reagents（PerkinElmer より購入）の BATDA Reagent を３μＬ加え、１５分間培養した。さらに、ＰＢＳで洗浄した。２Ｃ細胞は、Ｐ－８１５細胞を加えて継代した５～８日目の細胞を用いた。

被検物質である抗マウスＰＤ－１抗体（第１７図中、anti-mPD-1Ab（Ｊ４３））、抗マウスＰＤ－Ｌ１抗体（同図中、anti-mPD-L1Ab（１－１１１））、マウスＰＤ－１Ｆｃ（同図中、ｍＰＤ－１Ｆｃ）、ヒトＰＤ－１Ｆｃ（同図中、ｈＰＤ－１Ｆｃ）、マウスＩｇＧ２ａκ（同図中、ControlIg）またはＰＢＳを９６well plate に２０μＬ（１０ｎｇ／ｍＬ）ずつ分注し、Ｐ－８１５／ＰＤ－Ｌ１細胞または通常培地を５０μＬずつ加えた。さらに２Ｃ細胞、通常培地または１％TritonX100 を含む通常培地を５０μＬずつ加えた。通常培地を添加したウェルの上清５０μＬをバックグラウンド用として回収し、その他の上清を回収するまで、３７℃で保存した。残りの細胞は４時間培養した。続いて、同９６well plate を遠心し、上清を回収した。回収した上清に Cytotoxicity Reagents（PerkinElmer より購入）の DELFIA Europium Solution を２００μＬ添加して１５分間振とうした。振とう後、ARVOsx マルチラベルカウンター（WALLAC）にて時間分解蛍光測定を行った。なお、１％TritonX100 を含む通常培地を添加したウェルの上清を高コントロール、通常培地を添加したウェルの上清を低コントロールとした。

評価群の組成は、被検物質、Ｐ－８１５／ＰＤ－Ｌ１細胞及び２Ｃ細胞、高コントロール群の組成は、ＰＢＳ、Ｐ－８１５／ＰＤ－Ｌ１細胞および１％TritonX100 を含む通常培地、低コントロール群の組成は、ＰＢＳ、Ｐ－８１５

／ＰＤ－Ｌ１細胞および通常培地、２Ｃ細胞コントロール群の組成は、ＰＢＳ、通常培地および２Ｃ細胞、バックグラウンド群の組成は、ＰＢＳ、Ｐ－８１５／ＰＤ－Ｌ１細胞及び通常培地である。ＣＴＬ活性（％）は、以下の式にて算出した。すべての値は、バックグランドで得た平均値を引いたものを使用した。

CTL活性(%)＝([評価群測定値]－[2C細胞コントロール群測定値]－[低コントロール群測定値])÷([高コントロール群測定値]－[低コントロール群測定値])×100

抗ＰＤ－１抗体、抗ＰＤ－Ｌ１抗体およびＰＤ－１Ｆｃは、ＣＴＬ活性を有意に増強した（第１７図（ａ）～（ｄ）、図中、Ｅ：Tratio は、２Ｃ細胞とＰＤ－Ｌ１／Ｐ８１５細胞の混合比を示す。）。

実施例１３

Ｂ１６メラノーマ細胞を脾臓に移入したＣ５７ＢＬ／６マウスに、抗マウスＰＤ－１モノクローナル抗体を２日おきに腹腔内に投与して、移入後１８日目の肝臓重量を測定することによって、癌転移に対する抗ＰＤ－１抗体の抑制効果を評価した。

コントロールＩｇＧのみを投与したコントロール群に比べ、抗ＰＤ－１抗体投与群では、肝臓重量増加が有意に抑制された（肝臓重量／癌細胞非移入群：1.3ｇ、コントロール群：6.8ｇから抗ＰＤ－１抗体投与群：3.5ｇへ減少）。この重量増加の抑制は、Ｂ１６メラノーマ細胞の転移を抑制することを示すものである。

請 求 の 範 囲

1． ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を含有してなる免疫賦活組成物。

2． ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を含有してなる癌治療組成物。

3． 癌転移を抑制する組成物である請求の範囲２記載の癌治療組成物。

4． ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を含有してなる感染症治療組成物。

5． 免疫賦活を介して作用することを特徴とする請求の範囲２または３記載の癌治療組成物。

6． 免疫賦活を介して作用することを特徴とする請求の範囲４記載の感染症治療組成物。

7． ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、およびＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質から選択される一以上の免疫抑制シグナル阻害物質である請求の範囲１乃至６のいずれかに記載の組成物。

8． ＰＤ－１抗体、ＰＤ－Ｌ１抗体、可溶化ＰＤ－１、および可溶化ＰＤ－Ｌ１から選択される一以上のＰＤ－１とＰＤ－Ｌ１の相互作用阻害物質で

ある請求の範囲７記載の組成物。

９． 国際受託番号 FERM BP-8392 で識別されるハイブリドーマが産生する抗ヒトＰＤ－１抗体、非ヒト抗体をヒト化させた抗ＰＤ－１抗体、および完全ヒト型抗ヒトＰＤ－１抗体から選択されるＰＤ－１抗体である請求の範囲８記載の組成物。

１０． 遺伝子改変によりＰＤ－１発現が阻害されたリンパ球細胞が、免疫抑制シグナル阻害物質である請求の範囲１乃至６のいずれかに記載の組成物。

１１． ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、またはＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質が、タンパク質、ポリペプチド若しくはペプチド、ポリヌクレオチド若しくはポリヌクレオシド、抗体若しくはそれらの誘導体、有機合成化合物、無機化合物、および天然物から選択される一以上の物質である請求の範囲７記載の組成物。

１２． ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を投与することからなる免疫賦活方法。

１３． ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質を投与することからなる癌治療方法。

１４． 癌転移を抑制する請求の範囲１３記載の癌治療方法。

１５． ＰＤ－１、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害

物質を投与することからなる感染症治療方法。

１６．　免疫賦活を介して作用することを特徴とする請求の範囲１３または１４記載の癌治療方法。

１７．　免疫賦活を介して作用することを特徴とする請求の範囲１５記載の感染症治療方法。

１８．　ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、およびＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質から選択される一以上の免疫抑制シグナル阻害物質である請求の範囲１２乃至１７のいずれかに記載の方法。

１９．　ＰＤ－１抗体、ＰＤ－Ｌ１抗体、可溶化ＰＤ－１、および可溶化ＰＤ－Ｌ１から選択される一以上のＰＤ－１とＰＤ－Ｌ１の相互作用阻害物質である請求の範囲１８記載の方法。

２０．　国際受託番号 FERM BP-8392 で識別されるハイブリドーマが産生する抗ヒトＰＤ－１抗体、非ヒト抗体をヒト化させたＰＤ－１抗体、および完全ヒト型抗ヒトＰＤ－１抗体から選択されるＰＤ－１抗体である請求の範囲１９記載の方法。

２１．　遺伝子改変によりＰＤ－１発現が阻害されたリンパ球細胞が、免疫抑制シグナル阻害物質である請求の範囲１２乃至１７のいずれかに記載の方法。

２２．　ＰＤ－１とＰＤ－Ｌ１若しくはＰＤ－１とＰＤ－Ｌ２の相互作用阻害物質、ＰＤ－１の細胞内シグナル阻害物質、またはＰＤ－１、ＰＤ－Ｌ１若しくはＰＤ－Ｌ２の産生阻害物質が、タンパク質、ポリペプチド若しくはペプチド、ポリヌクレオチド若しくはポリヌクレオシド、抗体若しくはそれらの誘導体、有機合成化合物、無機化合物、および天然物から選択される１つ以上の物質である請求の範囲１８記載の方法。

２３．　免疫賦活組成物を製造するためのＰＤ－1、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質の使用。

２４．　癌治療組成物を製造するためのＰＤ－1、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質の使用。

２５．　癌治療組成物が、癌転移抑制組成物である請求の範囲２４記載の物質の使用。

２６．　感染症治療組成物を製造するためのＰＤ－1、ＰＤ－Ｌ１、またはＰＤ－Ｌ２の免疫抑制シグナル阻害物質の使用。

２７．　ＰＤ－Ｌ１またはＰＤ－Ｌ２を発現するように形質転換されたスクリーニング用癌細胞株。

２８．　請求の範囲２７記載の細胞、リンパ球細胞および被験物質を接触させて、請求の範囲２７記載の細胞へのリンパ球細胞の免疫反応に対する被験物質の増強作用を評価することを特徴とする免疫賦活物質のスクリーニング方法。

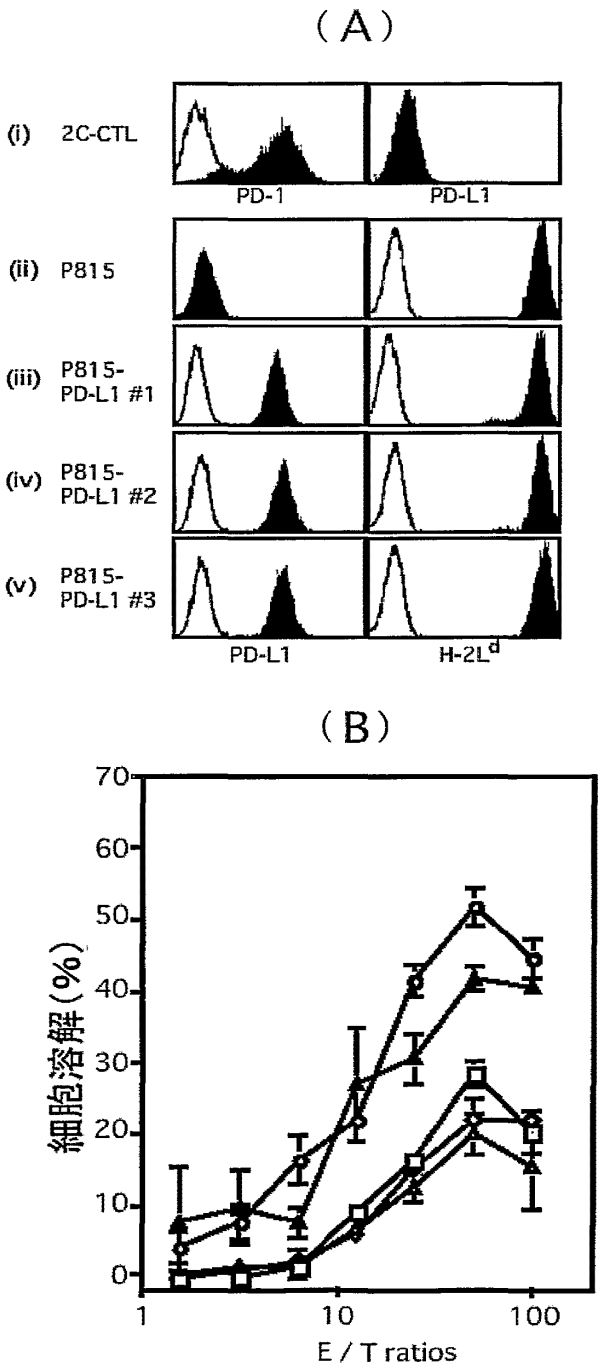
２９．　癌細胞である請求の範囲２７記載の細胞、リンパ球細胞および被験物質を接触させて、その癌細胞へのリンパ球細胞の免疫反応に対する被験物質の増強作用またはその腫瘍細胞の増殖に対する阻害作用を評価することを特徴とする癌治療物質のスクリーニング方法。

３０．　病原体を感染させた請求の範囲２７記載の細胞またはＰＤ－Ｌ１若しくはＰＤ－Ｌ２を発現する細胞、リンパ球細胞および被験物質を接触させて、その感染細胞へのリンパ球細胞の免疫反応に対する被験物質の増強作用または病原体増殖に対する阻害作用を評価することを特徴とする感染症治療物質のスクリーニング方法。

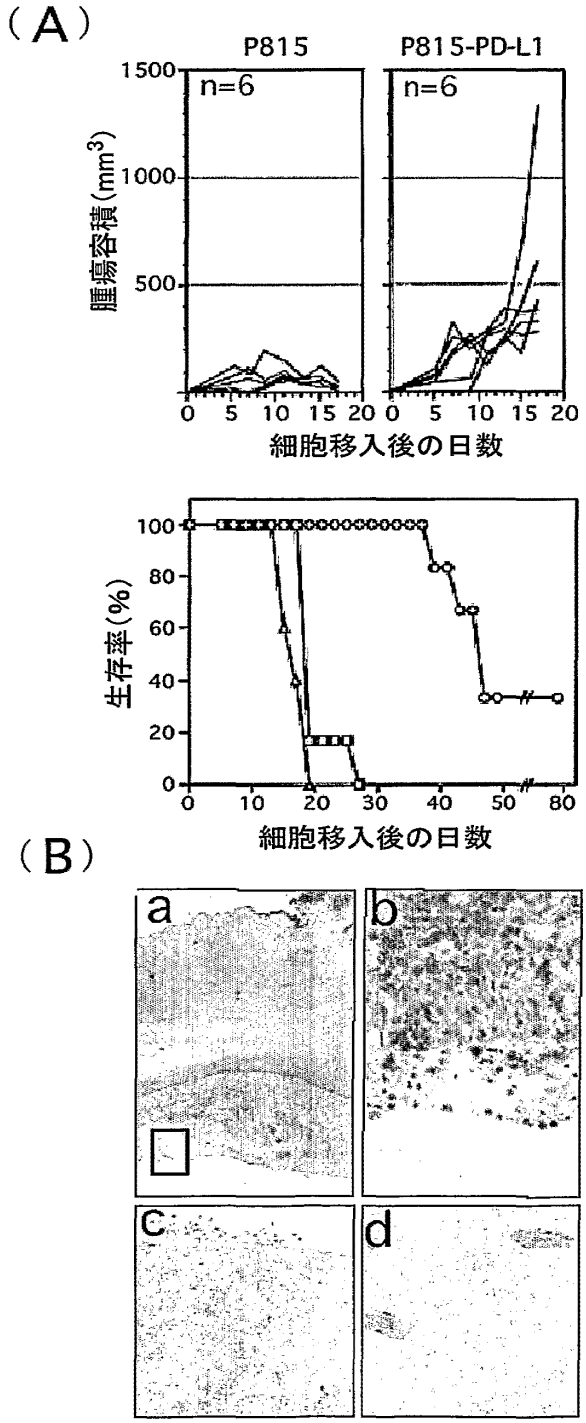
３１．　請求の範囲２７記載の癌細胞株を移植して作出した哺乳動物。

３２．　請求の範囲３１記載の哺乳動物に被験物質を投与し、被験物質による移植癌細胞の増殖に対する抑制率またはその被移植哺乳動物の生存率を評価することを特徴とする癌治療物質の選別方法。

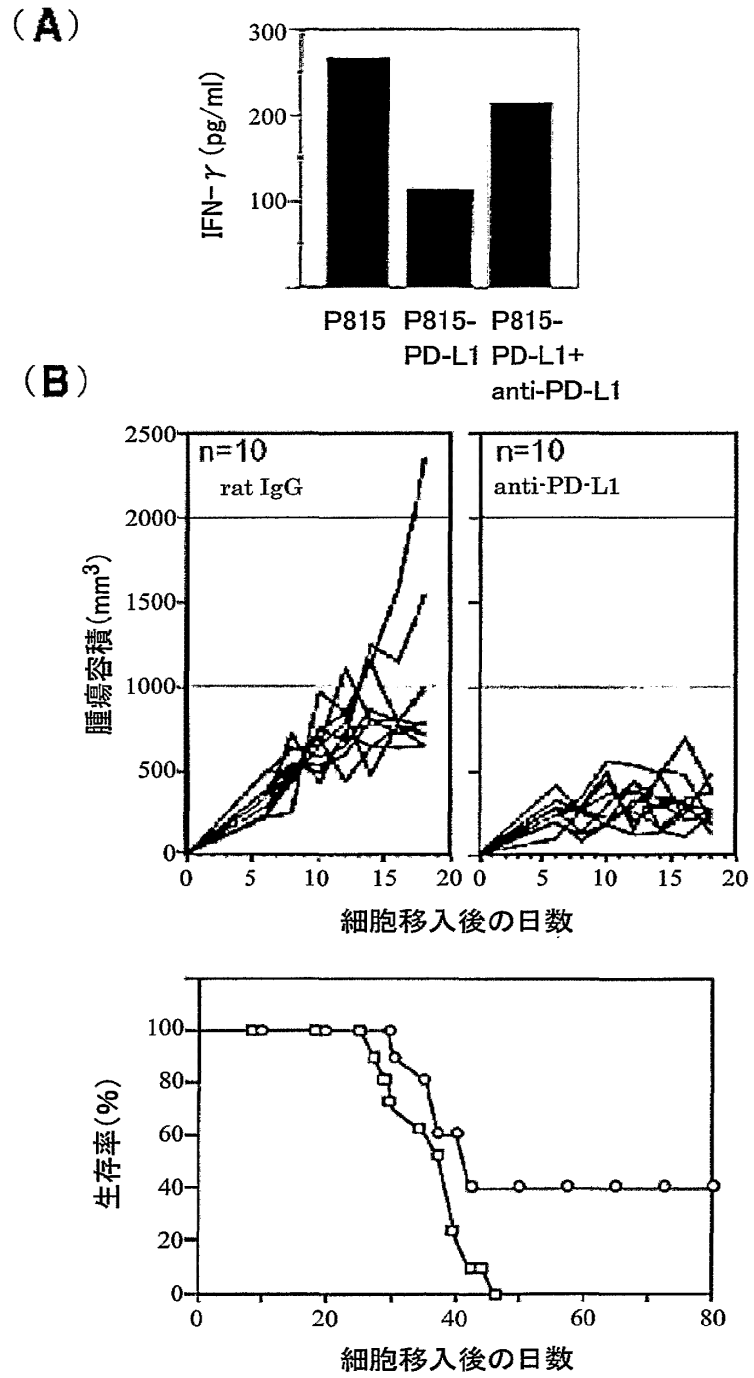
第１図


(A)
(i) 2C-CTL
PD-1
PD-L1
(ii) P815
(iii) P815-PD-L1 #1
(iv) P815-PD-L1 #2
(v) P815-PD-L1 #3
PD-L1
H-2L^d
(B)
細胞溶解(%)
70
60
50
40
30
20
10
0
1
10
100
E / T ratios

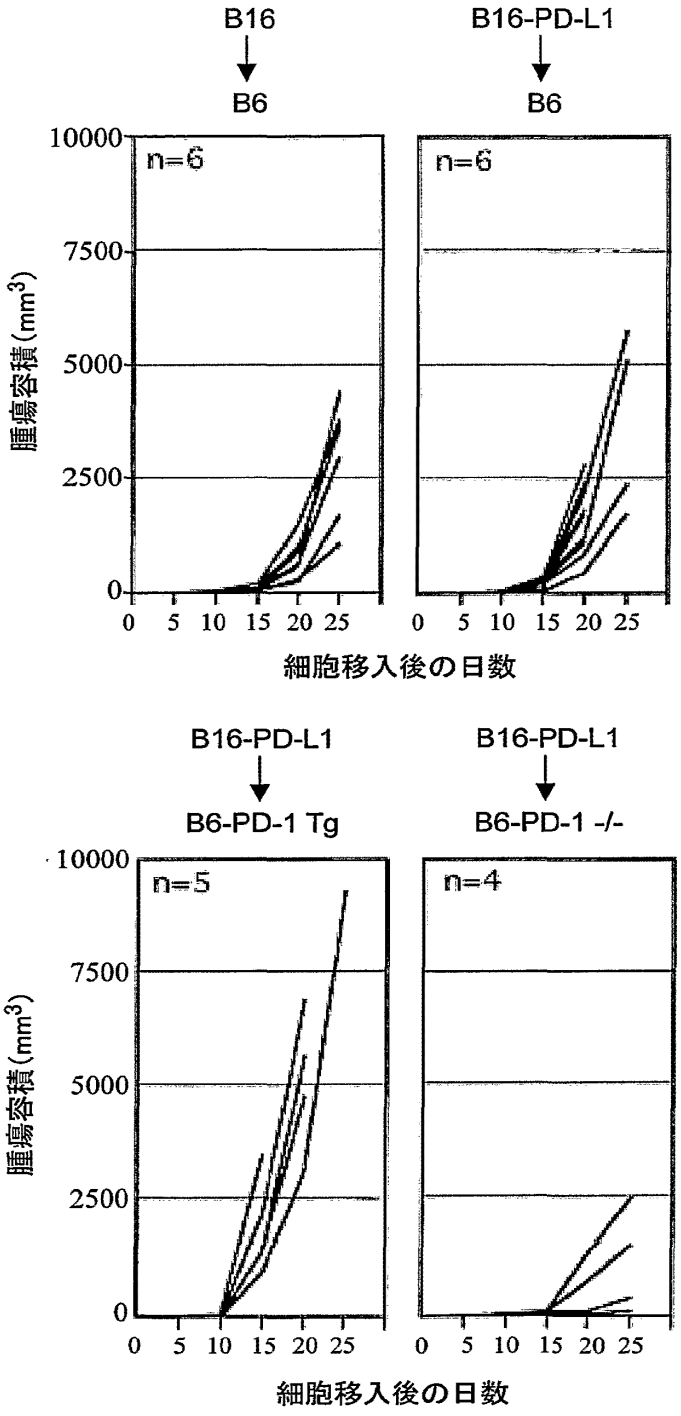
第２図


（A）
P815
P815-PD-L1
n=6
n=6
腫瘍容積(mm3)
1500
1000
500
0 5 10 15 20
0 5 10 15 20
細胞移入後の日数
生存率(%)
100
80
60
40
20
0
0 10 20 30 40 50 80
細胞移入後の日数
（B）
a
b
c
d

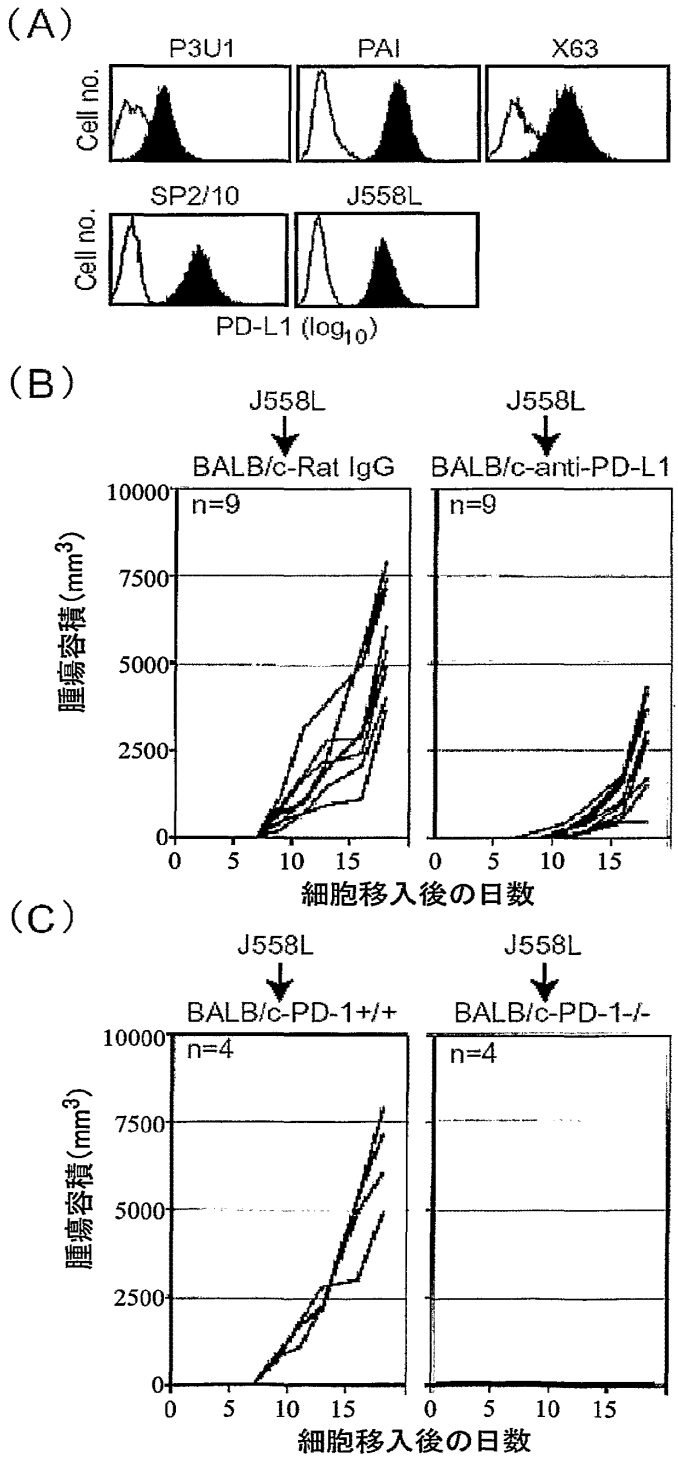
第３図


(A)
IFN-γ (pg/ml)
300
200
100
P815
P815-PD-L1
P815-PD-L1+anti-PD-L1
(B)
n=10
rat IgG
n=10
anti-PD-L1
腫瘍容積 (mm3)
2500
2000
1500
1000
500
0
0 5 10 15 20
細胞移入後の日数
生存率 (%)
100
80
60
40
20
0
0 20 40 60 80
細胞移入後の日数

第４図

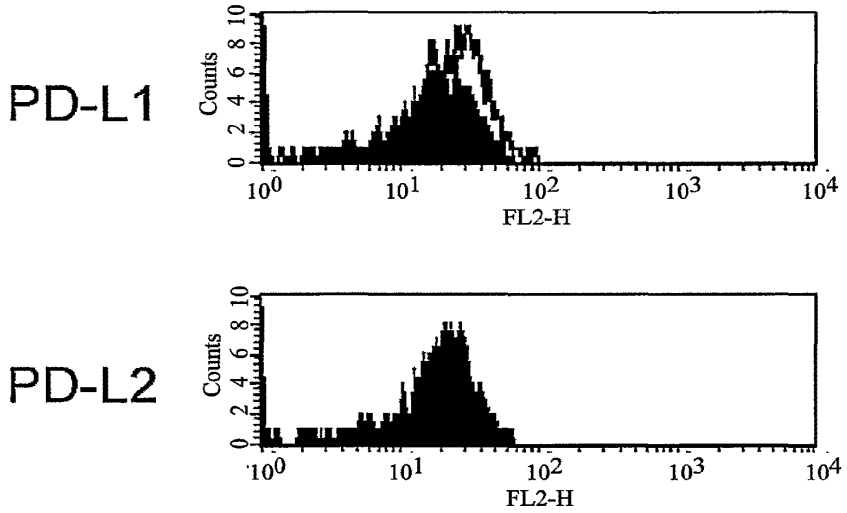

B16
B6
B16-PD-L1
B6
n=6
n=6
10000
7500
5000
2500
0
腫瘍容積(mm3)
0 5 10 15 20 25
0 5 10 15 20 25
細胞移入後の日数
B16-PD-L1
B6-PD-1 Tg
B16-PD-L1
B6-PD-1 -/-
n=5
n=4
10000
7500
5000
2500
0
腫瘍容積(mm3)
0 5 10 15 20 25
0 5 10 15 20 25
細胞移入後の日数

第５図

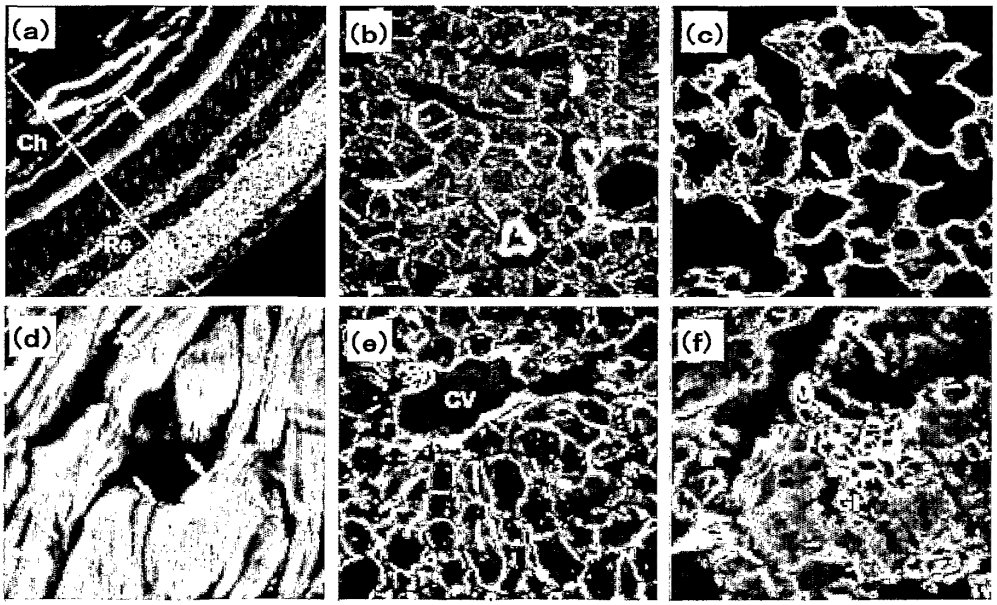

(A)
P3U1
PAI
X63
Cell no.
SP2/10
J558L
Cell no.
PD-L1 (log10)
(B)
J558L
J558L
BALB/c-Rat IgG
BALB/c-anti-PD-L1
n=9
n=9
腫瘍容積(mm3)
10000
7500
5000
2500
0
0 5 10 15
0 5 10 15
細胞移入後の日数
(C)
J558L
J558L
BALB/c-PD-1+/+
BALB/c-PD-1-/-
n=4
n=4
腫瘍容積(mm3)
10000
7500
5000
2500
0
0 5 10 15
0 5 10 15
細胞移入後の日数

第６図

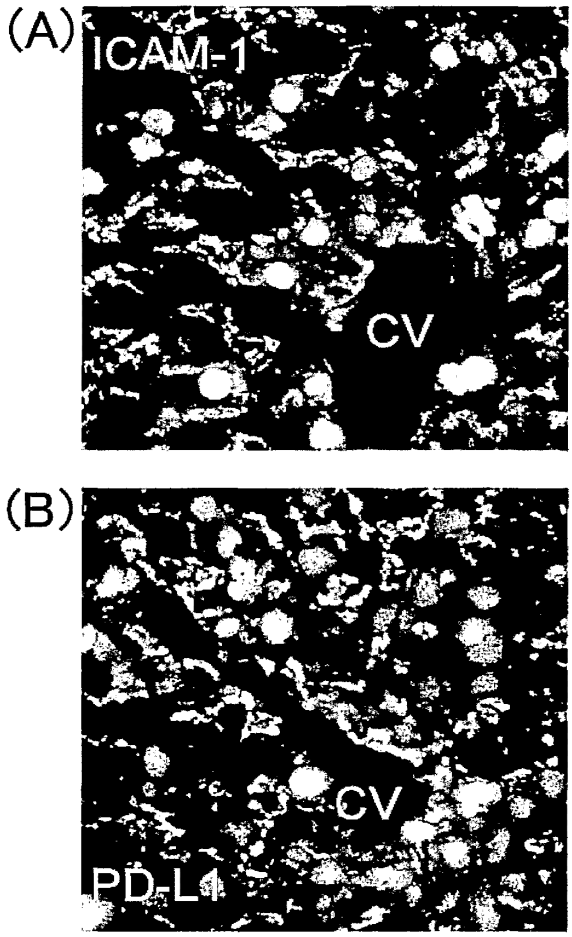
（A）


PD-L1
Counts
0 2 4 6 8 10
10^0 10^1 10^2 10^3 10^4
FL2-H
PD-L2
Counts
0 2 4 6 8 10
10^0 10^1 10^2 10^3 10^4
FL2-H

（B）

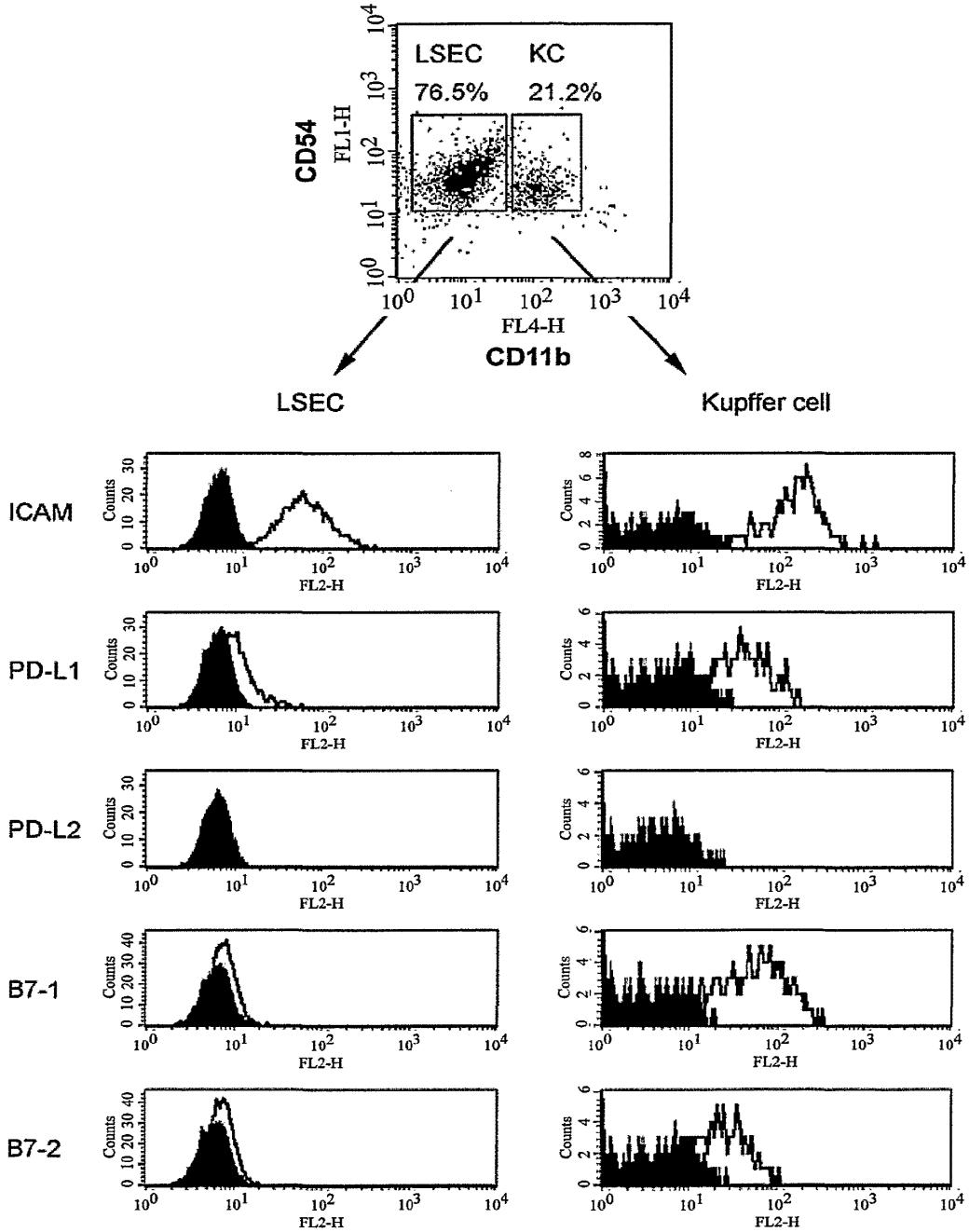

(a)
Ch
Re
(b)
A
(c)
(d)
(e)
CV
(f)

第７図

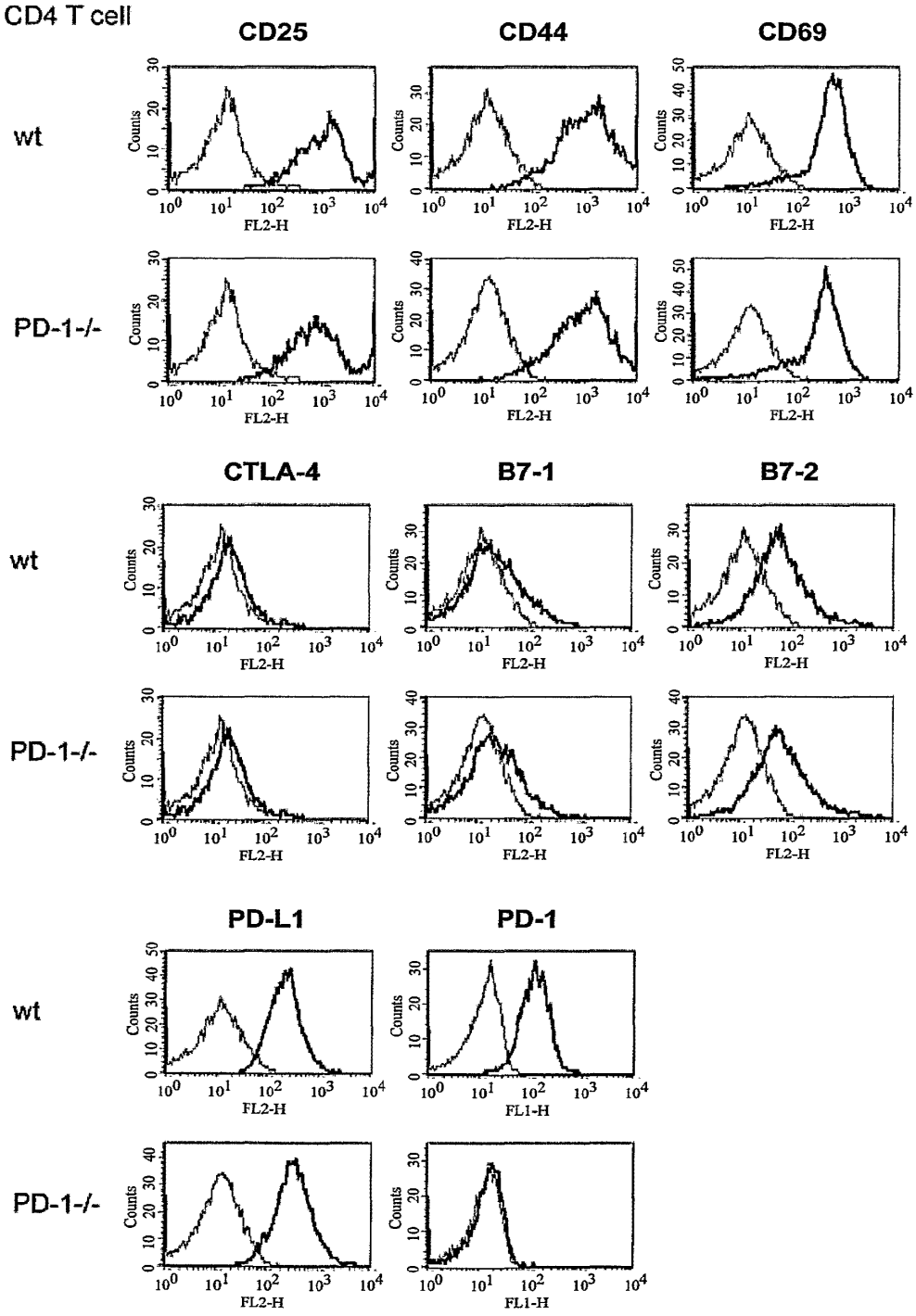


x40

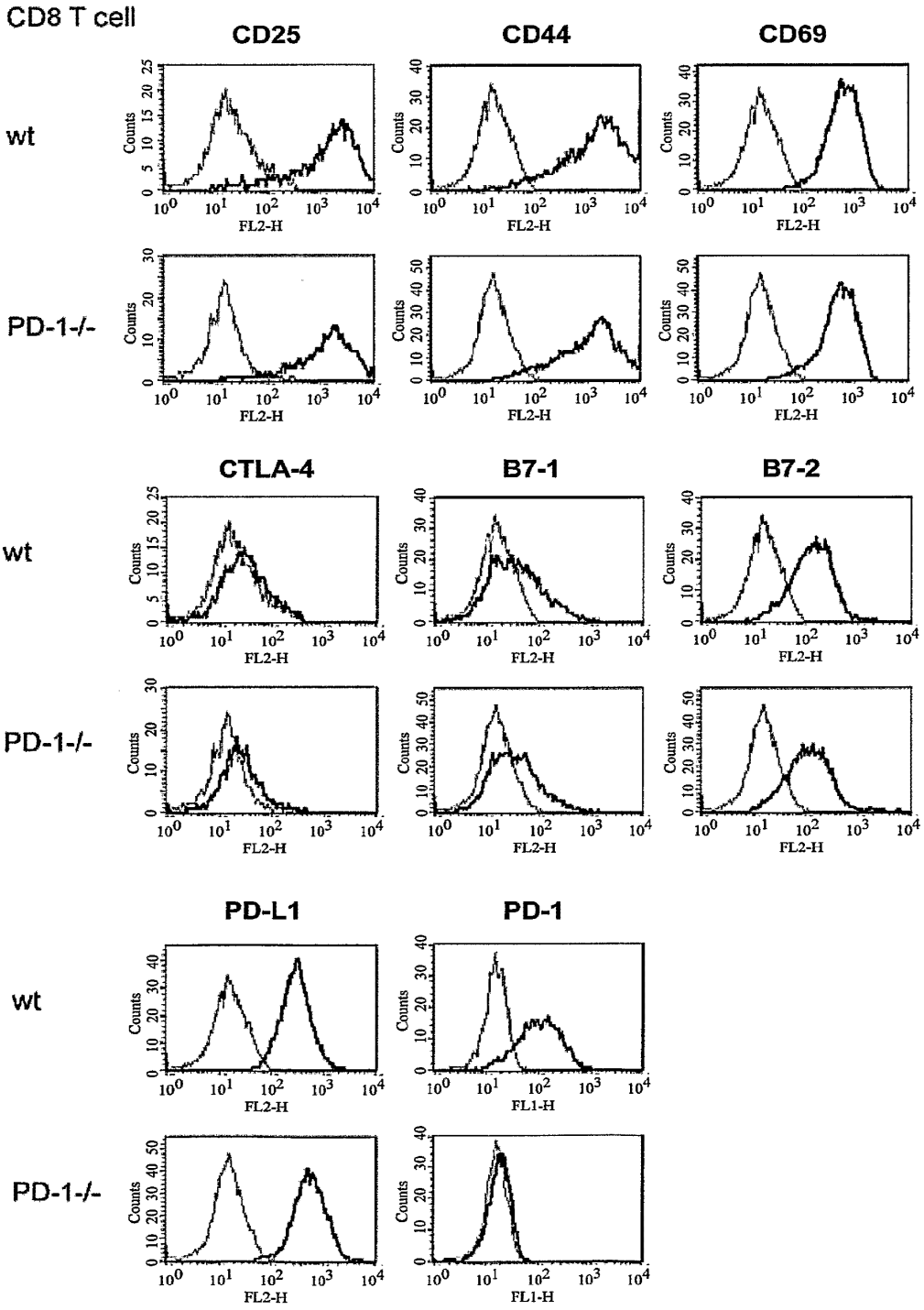
第８図


LSEC
KC
76.5%
21.2%
CD54
FL1-H
FL4-H
CD11b
LSEC
Kupffer cell
ICAM
PD-L1
PD-L2
B7-1
B7-2
Counts
FL2-H

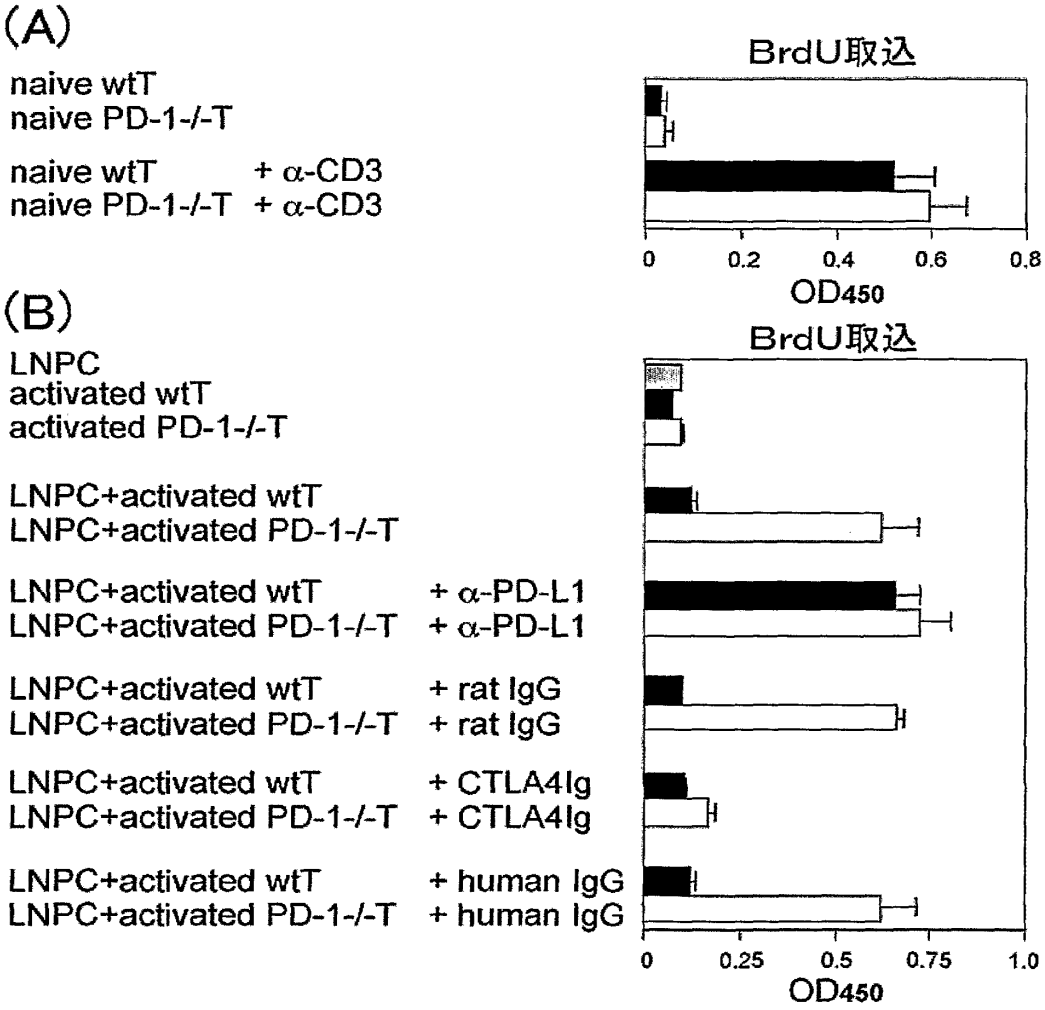
第９図


CD4 T cell
CD25
CD44
CD69
wt
PD-1-/-
CTLA-4
B7-1
B7-2
wt
PD-1-/-
PD-L1
PD-1
wt
PD-1-/-
Counts
FL2-H
FL1-H

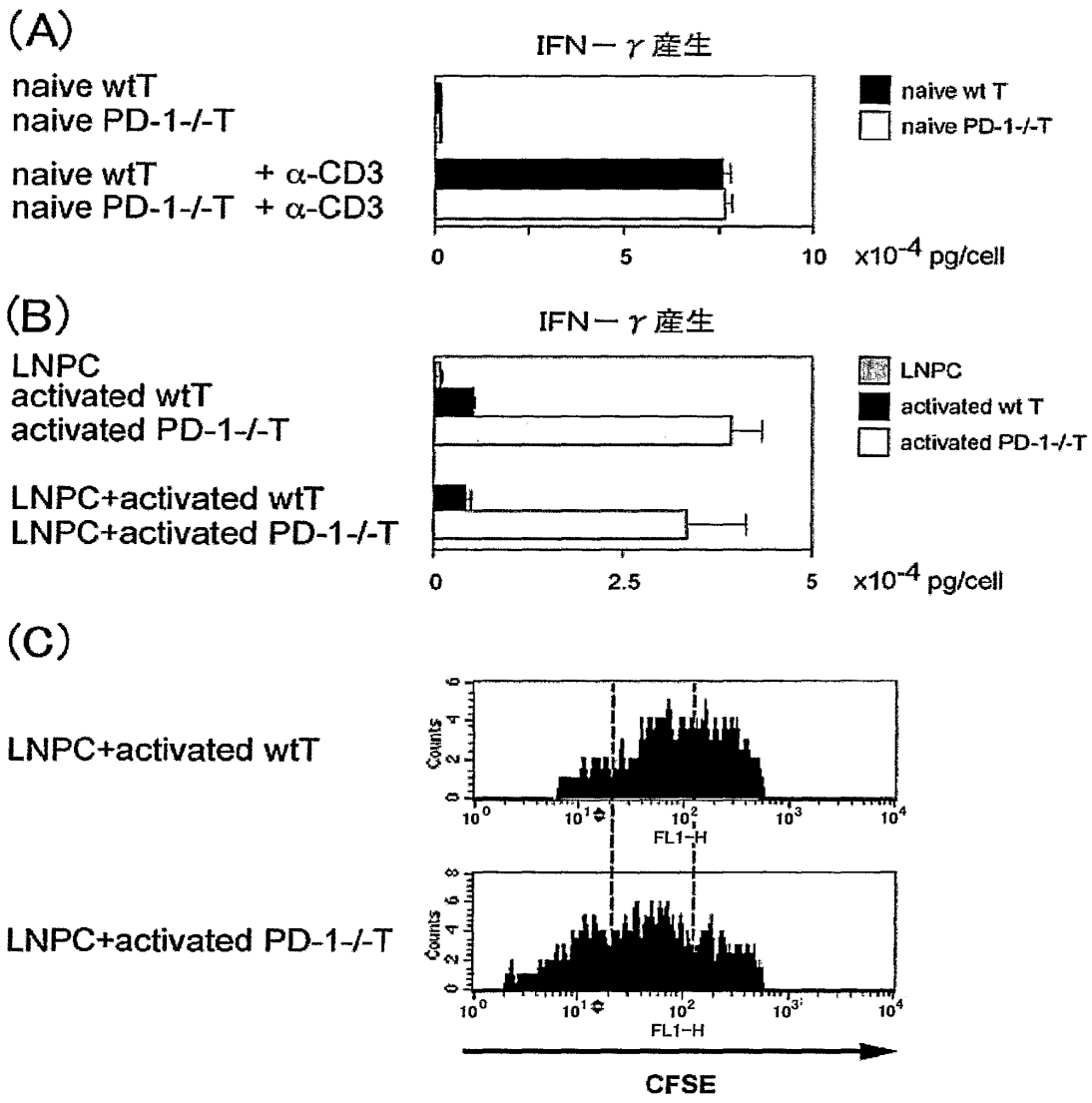
第１０図


CD8 T cell
CD25
CD44
CD69
wt
PD-1-/-
CTLA-4
B7-1
B7-2
PD-L1
PD-1
Counts
FL2-H
FL1-H

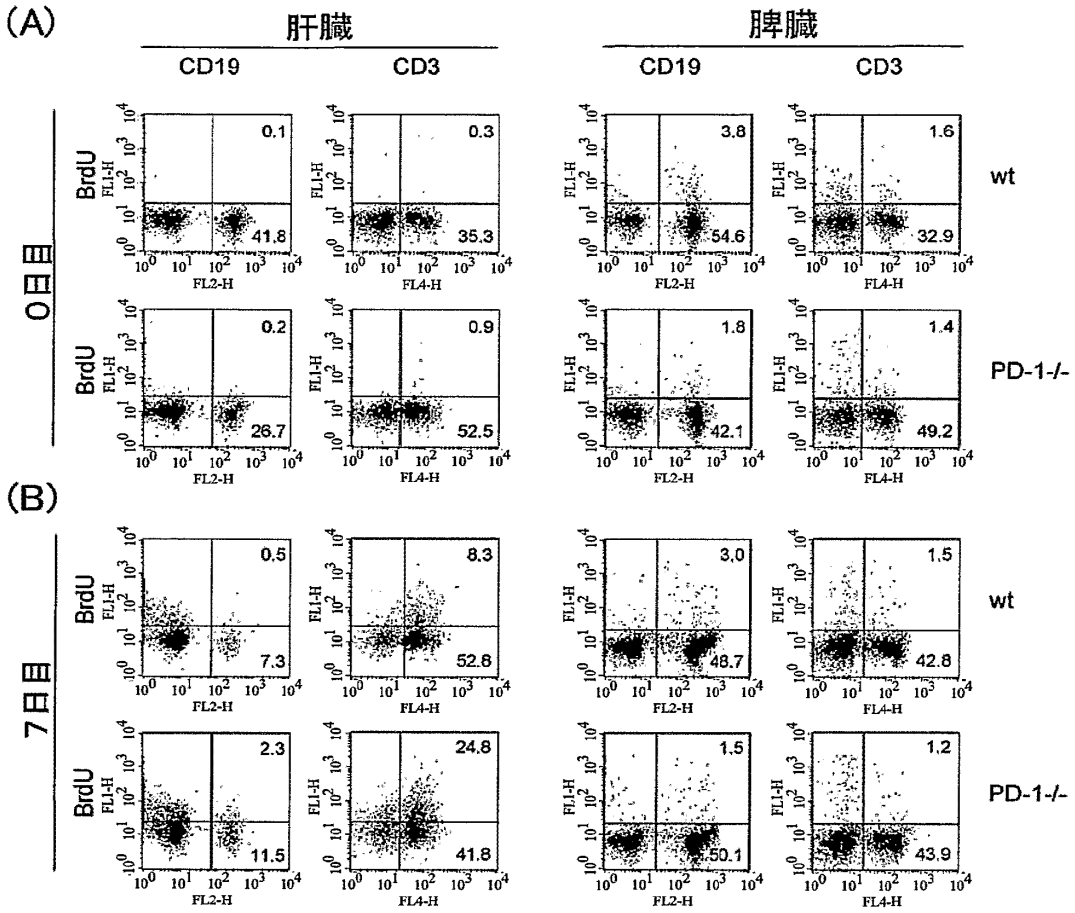
第１１図


(A)
naive wtT
naive PD-1-/-T
naive wtT + α-CD3
naive PD-1-/-T + α-CD3
BrdU取込
0
0.2
0.4
0.6
0.8
OD450
(B)
LNPC
activated wtT
activated PD-1-/-T
LNPC+activated wtT
LNPC+activated PD-1-/-T
LNPC+activated wtT + α-PD-L1
LNPC+activated PD-1-/-T + α-PD-L1
LNPC+activated wtT + rat IgG
LNPC+activated PD-1-/-T + rat IgG
LNPC+activated wtT + CTLA4Ig
LNPC+activated PD-1-/-T + CTLA4Ig
LNPC+activated wtT + human IgG
LNPC+activated PD-1-/-T + human IgG
BrdU取込
0
0.25
0.5
0.75
1.0
OD450

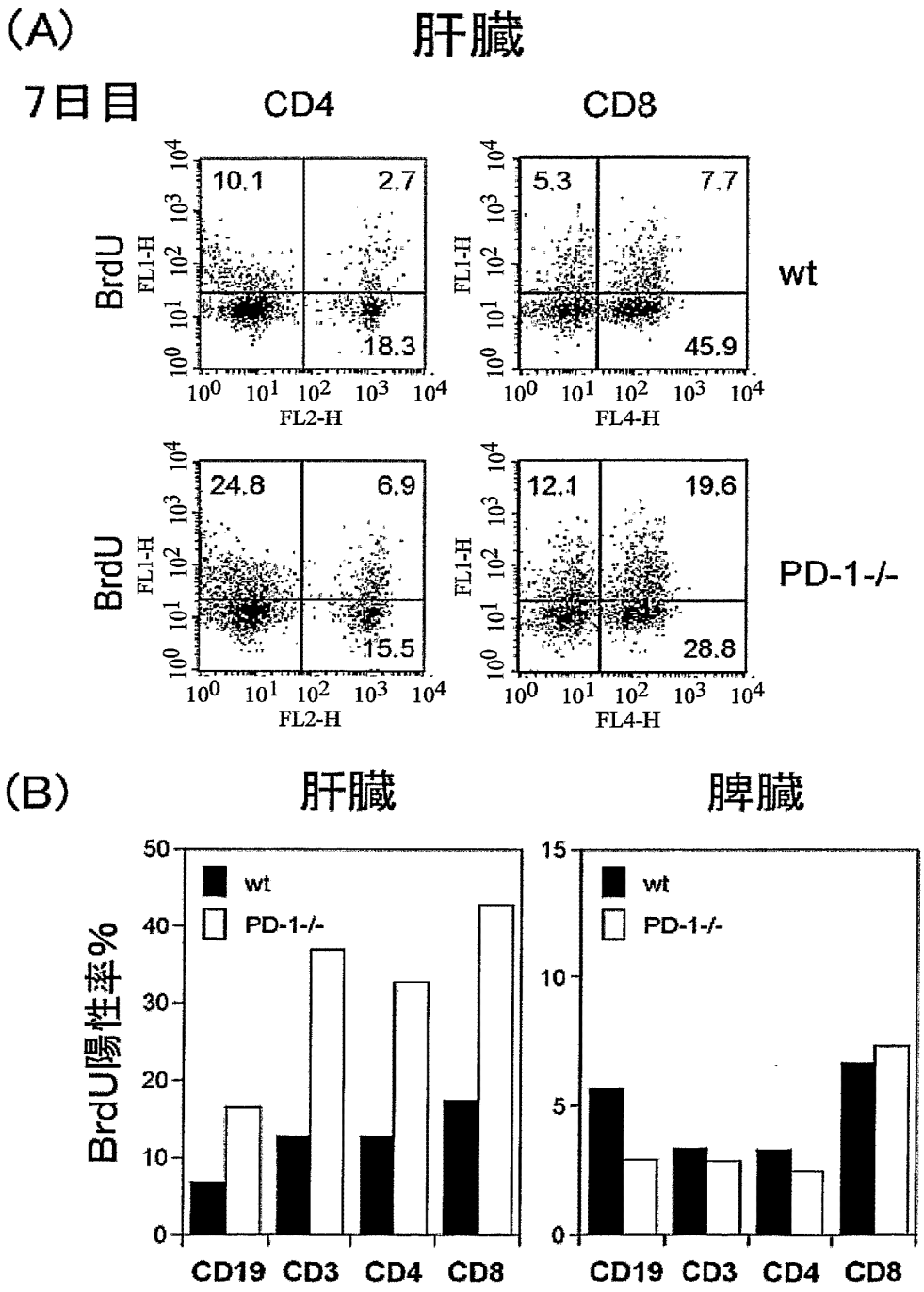
第１２図


(A)
naive wtT
naive PD-1-/-T
naive wtT + α-CD3
naive PD-1-/-T + α-CD3
IFNーγ産生
naive wt T
naive PD-1-/-T
0
5
10
x10-4 pg/cell
(B)
LNPC
activated wtT
activated PD-1-/-T
LNPC+activated wtT
LNPC+activated PD-1-/-T
IFNーγ産生
LNPC
activated wt T
activated PD-1-/-T
0
2.5
5
x10-4 pg/cell
(C)
LNPC+activated wtT
LNPC+activated PD-1-/-T
Counts
FL1-H
CFSE

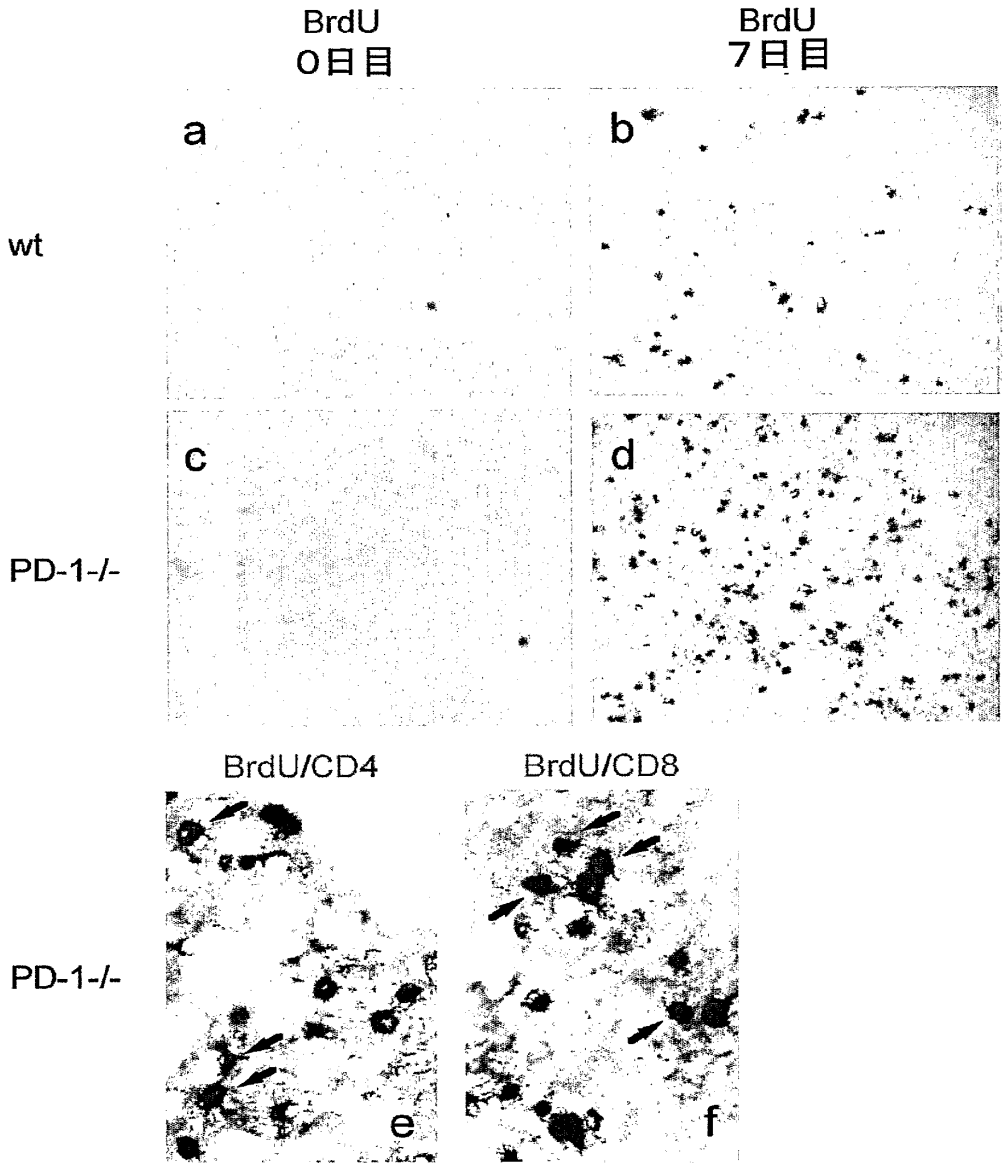
第１３図


(A)
肝臓
脾臓
CD19
CD3
CD19
CD3
0日目
BrdU
FL1-H
FL2-H
FL4-H
0.1
41.8
0.3
35.3
3.8
54.6
1.6
32.9
wt
0.2
26.7
0.9
52.5
1.8
42.1
1.4
49.2
PD-1-/-
(B)
7日目
0.5
7.3
8.3
52.8
3.0
48.7
1.5
42.8
wt
2.3
11.5
24.8
41.8
1.5
50.1
1.2
43.9
PD-1-/-

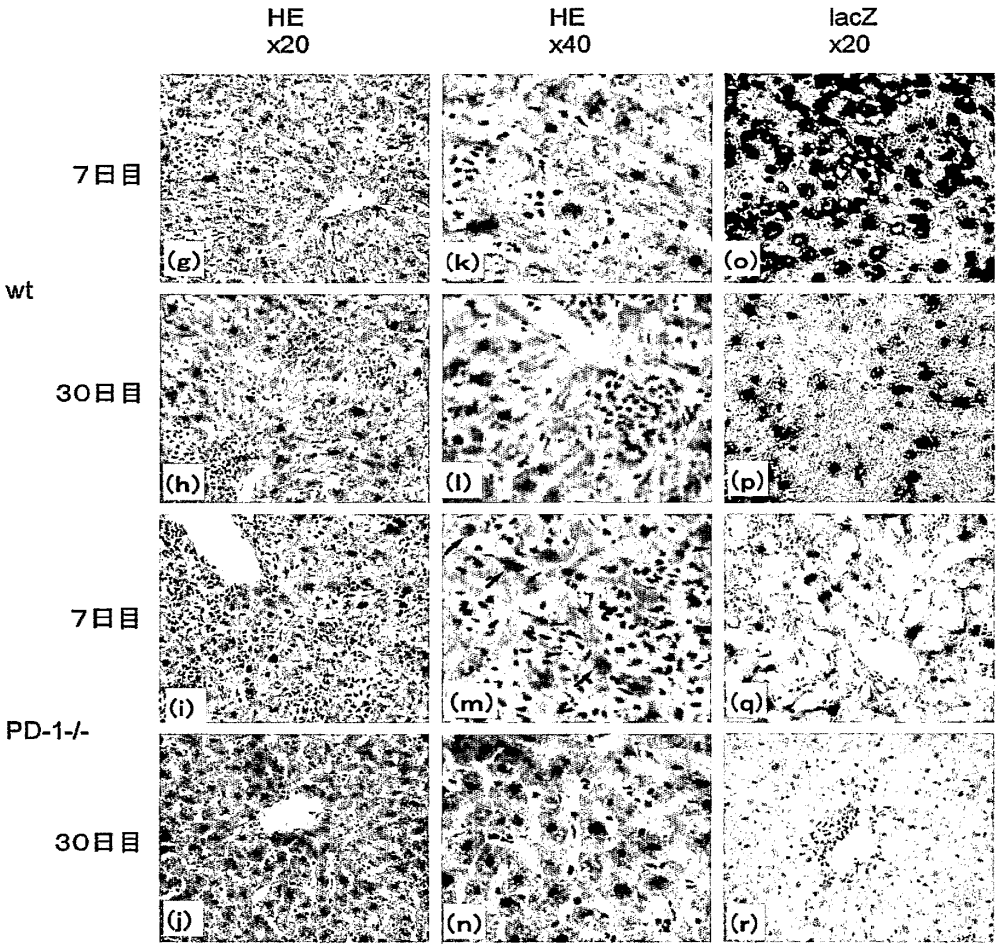
第１４図


(A)
肝臓
7日目
CD4
CD8
BrdU
FL1-H
FL2-H
FL4-H
10.1
2.7
18.3
5.3
7.7
45.9
wt
24.8
6.9
15.5
12.1
19.6
28.8
PD-1-/-
(B)
肝臓
脾臓
BrdU陽性率%
wt
PD-1-/-
CD19
CD3
CD4
CD8

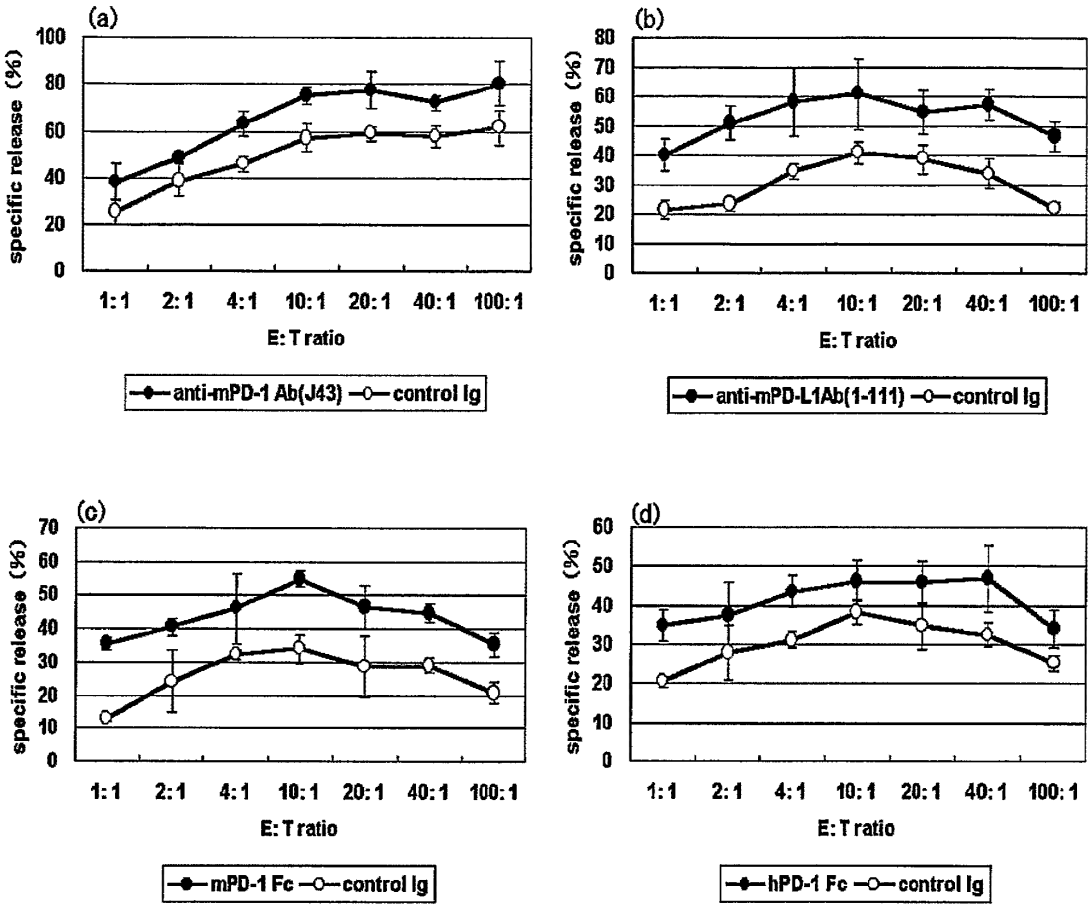
第１５図



第１６図



第１７図


(a)
specific release (%)
100
80
60
40
20
0
1:1 2:1 4:1 10:1 20:1 40:1 100:1
E:T ratio
anti-mPD-1 Ab(J43)
control Ig
(b)
specific release (%)
80
70
60
50
40
30
20
10
0
1:1 2:1 4:1 10:1 20:1 40:1 100:1
E:T ratio
anti-mPD-L1Ab(1-111)
control Ig
(c)
specific release (%)
70
60
50
40
30
20
10
0
1:1 2:1 4:1 10:1 20:1 40:1 100:1
E:T ratio
mPD-1 Fc
control Ig
(d)
specific release (%)
60
50
40
30
20
10
0
1:1 2:1 4:1 10:1 20:1 40:1 100:1
E:T ratio
hPD-1 Fc
control Ig

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP03/08420

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] A61K45/00, 38/17, 39/395, 31/7088, A61P31/00, 35/00, 35/04, 37/04, C12N5/10, C12Q1/02, G01N33/50, A01K6//027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] A61K45/00, 38/17, 39/395, 31/7088, A61P31/00, 35/00, 35/04, 37/04, C12N5/10, C12Q1/02, G01N33/50, A01K6//027

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAPLUS(STN), MEDLINE(STN), EMBASE(STN), BIOSIS(STN), JICST(JOIS)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/14557 A1 (DANA-FARBER CANCER INSTITUTE, INC.), 01 March, 2001 (01.03.01), Particularly, abstract; Claims; page 82, line 8 to page 86, line 10 & EP 1210428 A1 & US 2002/102651 A1 & US 2002/160000 A1 & BR 2000013581 A & NO 2002000859 A	1-11,23-33
X	WO 02/00730 A2 (GENETICS INSTITUTE, INC.), 03 January, 2002 (03.01.02), Particularly, abstract; Claims; page 104, line 9 to page 108, line 13 & WO 02/00730 A3 & WO 02/00730 C2 & EP 1297135 A2 & US 2002/110836 A1 & US 2002/164600 A1 & AU 2001071731 A5	1-11,23-33

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 October, 2003 (04.10.03)	21 October, 2003 (21.10.03)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP03/08420

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/00692 A2 (GENETICS INSTITUTE, INC.), 03 January, 2002 (03.01.02), Particularly, abstract; Claims; page 104, line 9 to page 108, line 13 & WO 02/00692 A3 & WO 02/00692 C2 & EP 1320599 A2 & US 2002/110836 A1 & US 2002/164600 A1	1-11,23-33
X	EP 670369 A2 (ONO PHARMACEUTICAL CO., LTD.), 06 September, 1995 (06.09.95), Particularly, abstract; Claims; page 5, line 52 to page 6, line 6 & EP 670369 A3 & US 5629204 A & US 5698520 A & JP 7-291996 A & JP 2003-230395 A & CA 2143491 A & CN 1113518 A & CN 1080311 B	1-11,23-33
X	BROWN, Julia A. et al., Blockade of PD-1 Ligands on dendritic cells enhances T cell activation and cytokine production., FASEB Journal, March 2002, Vol.16, No.4, pp.A710,517.4	1-11,23-33
P,X	WO 03/042402 A2 (DANA-FARBER CANCER INSTITUTE, INC.), 22 May, 2003 (22.05.03), Particularly, abstract; Claims; page 56 line 21 to page 59, line 2 (Family: none)	1-11,23-33
P,X	WO 03/011911 A1 (ONO PHARMACEUTICAL CO., LTD.), 13 February, 2003 (13.02.03), Particularly, abstract; Claims; page 14, lines 17 to 19 (Family: none)	1-11,23-33
P,X	WO 02/078731 A1 (WYETH), 10 October, 2002 (10.10.02), Particularly, abstract; Claims; page 74, line 27 to page 77, line 29 (Family: none)	1-11,23-33
P,X	BROWN, Julia A. et al., Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production., The Journal of Immunology., February 2003, Vol.170, No.3, 1257-66	1-11,23-33

Form PCT/ISA/210 (continuation of second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP03/08420

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. [X] Claims Nos.: 12-22

because they relate to subject matter not required to be searched by this Authority, namely:

Claims 12-22 pertain to methods for treatment of the human body by therapy and thus relates to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. [ ] Claims Nos.:

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

3. [ ] Claims Nos.:

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

Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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

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

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

国際調査報告

国際出願番号　ＰＣＴ／ＪＰ０３／０８４２０

Ａ．　発明の属する分野の分類（国際特許分類（ＩＰＣ））

Int.Cl⁷ A61K 45/00, 38/17, 39/395, 31/7088, A61P31/00, 35/00, 35/04, 37/04, C12N5/10, C12Q1/02, G01N33/50, A01K6//027

Ｂ．　調査を行った分野

調査を行った最小限資料（国際特許分類（ＩＰＣ））

Int.Cl⁷ A61K 45/00, 38/17, 39/395, 31/7088, A61P31/00, 35/00, 35/04, 37/04, C12N5/10, C12Q1/02, G01N33/50, A01K6//027

最小限資料以外の資料で調査を行った分野に含まれるもの

国際調査で使用した電子データベース（データベースの名称、調査に使用した用語）

CAPLUS(STN), MEDLINE(STN), EMBASE(STN), BIOSIS(STN), JICST(JOIS)

Ｃ．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
X	WO 01/14557 A1(DANA-FARBER CANCER INSTITUTE, INC.) 2001.03.01 特に、Abstract, Claims, 第82ﾍﾟｰｼﾞ第8行-第86ﾍﾟｰｼﾞ第10行 & EP 1210428 A1 & US 2002/102651 A1 & US 2002/160000 A1 & BR 2000013581 A & NO 2002000859 A	1-11, 23-33

☒ Ｃ欄の続きにも文献が列挙されている。　　☐ パテントファミリーに関する別紙を参照。

＊　引用文献のカテゴリー
「Ａ」特に関連のある文献ではなく、一般的技術水準を示すもの
「Ｅ」国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの
「Ｌ」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献（理由を付す）
「Ｏ」口頭による開示、使用、展示等に言及する文献
「Ｐ」国際出願日前で、かつ優先権の主張の基礎となる出願の日の後に公表された文献
「Ｔ」国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
「Ｘ」特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
「Ｙ」特に関連のある文献であって、当該文献と他の１以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
「＆」同一パテントファミリー文献

国際調査を完了した日	国際調査報告の発送日
０４．１０．０３	21.10.03

国際調査機関の名称及びあて先	特許庁審査官（権限のある職員）	４Ｃ	９２８４
日本国特許庁（ＩＳＡ／ＪＰ） 郵便番号１００－８９１５ 東京都千代田区霞が関三丁目４番３号	瀬下　浩一　印		
	電話番号　０３－３５８１－１１０１　内線　３４５２		

様式ＰＣＴ／ＩＳＡ／２１０（第２ページ）（１９９８年７月）

Ｃ（続き）．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
X	WO 02/00730 A2(GENETICS INSTITUTE, INC.)2002.01.03 特に、Abstract, Claims, 第104ﾍﾟｰｼﾞ第9行-第108ﾍﾟｰｼﾞ第13行 & WO 02/00730 A3 & WO 02/00730 C2 & EP 1297135 A2 & US 2002/110836 A1 & US 2002/164600 A1 & AU 2001071731 A5	1-11, 23-33
X	WO 02/00692 A2(GENETICS INSTITUTE, INC.)2002.01.03 特に、Abstract, Claims, 第104ﾍﾟｰｼﾞ第9行-第108ﾍﾟｰｼﾞ第13行 & WO 02/00692 A3 & WO 02/00692 C2 & EP 1320599 A2 & US 2002/110836 A1 & US 2002/164600 A1	1-11, 23-33
X	EP 670369 A2(ONO PHARMACEUTICAL CO., LTD.)1995.09.06 特に、Abstract, Claims, 第5ﾍﾟｰｼﾞ第52行-第6ﾍﾟｰｼﾞ第6行 & EP 670369 A3 & US 5629204 A & US 5698520 A & JP 7-291996 A & JP 2003-230395 A & CA 2143491 A & CN 1113518 A & CN 1080311 B	1-11, 23-33
X	BROWN, Julia A. et al, Blockade of PD-1 Ligands on dendritic cells enhances T cell activation and cytokine production., FASEB Journal, Mar. 2002, Vol.16, No.4, pp. A710, 517.4	1-11, 23-33
ＰＸ	WO 03/042402 A2(DANA-FARBER CANCER INSTITUTE,INC.) 2003.05.22 特に、Abstract, Claims, 第56ﾍﾟｰｼﾞ第21行-第59ﾍﾟｰｼﾞ第2行 （ファミリーなし）	1-11, 23-33
ＰＸ	WO 03/011911 A1(小野薬品工業株式会社)2003.02.13 特に、Abstract, 請求の範囲, 第14ﾍﾟｰｼﾞ第17-19行 （ファミリーなし）	1-11, 23-33
ＰＸ	WO 02/078731 A1(WYETH)2002.10.10 特に、Abstract, Claims, 第74ﾍﾟｰｼﾞ第27行-第77ﾍﾟｰｼﾞ第29行 （ファミリーなし）	1-11, 23-33
ＰＸ	BROWN, Julia A. et al, Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production., The Jounrnal of Immunology. Feb. 2003 , Vol.170, No.3, 1257-66	1-11, 23-33

国際調査報告

国際出願番号　ＰＣＴ／ＪＰ０３／０８４２０

第Ⅰ欄　請求の範囲の一部の調査ができないときの意見（第１ページの２の続き）

法第８条第３項（ＰＣＴ１７条(2)(a)）の規定により、この国際調査報告は次の理由により請求の範囲の一部について作成しなかった。

1．[X] 請求の範囲　１２－２２　は、この国際調査機関が調査をすることを要しない対象に係るものである。
つまり、
請求の範囲１２－２２は治療による人体の処置方法に関するものであって、ＰＣＴ１７条(2)(a)(i)及びＰＣＴ規則39.1(iv)の規定により、この国際調査機関が調査をすることを要しない対象に係るものである。

2．[ ] 請求の範囲　＿＿＿＿＿＿＿＿　は、有意義な国際調査をすることができる程度まで所定の要件を満たしていない国際出願の部分に係るものである。つまり、

3．[ ] 請求の範囲　＿＿＿＿＿＿＿＿　は、従属請求の範囲であってＰＣＴ規則6.4(a)の第２文及び第３文の規定に従って記載されていない。

第Ⅱ欄　発明の単一性が欠如しているときの意見（第１ページの３の続き）

次に述べるようにこの国際出願に二以上の発明があるとこの国際調査機関は認めた。

1．[ ] 出願人が必要な追加調査手数料をすべて期間内に納付したので、この国際調査報告は、すべての調査可能な請求の範囲について作成した。

2．[ ] 追加調査手数料を要求するまでもなく、すべての調査可能な請求の範囲について調査することができたので、追加調査手数料の納付を求めなかった。

3．[ ] 出願人が必要な追加調査手数料を一部のみしか期間内に納付しなかったので、この国際調査報告は、手数料の納付のあった次の請求の範囲のみについて作成した。

4．[ ] 出願人が必要な追加調査手数料を期間内に納付しなかったので、この国際調査報告は、請求の範囲の最初に記載されている発明に係る次の請求の範囲について作成した。

追加調査手数料の異議の申立てに関する注意
[ ] 追加調査手数料の納付と共に出願人から異議申立てがあった。
[ ] 追加調査手数料の納付と共に出願人から異議申立てがなかった。

様式ＰＣＴ／ＩＳＡ／２１０（第１ページの続葉（１））（１９９８年７月）

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 November 2013 (21.11.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/173223 A1

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C07K 16/28 (2006.01)

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13 May 2013 (13.05.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/647,442 15 May 2012 (15.05.2012) US
61/790,747 15 March 2013 (15.03.2013) US

(71) Applicant: **BRISTOL-MYERS SQUIBB COMPANY** [US/US]; Route 206 and Province Line Road, Princeton, New Jersey 08543-4000 (US).

(72) Inventors: **COGSWELL, John P.;** c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **GOLDBERG, Stacie M.;** c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **GUPTA, Ashok K.;** c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **JURE-KUNKEL, Maria;** c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **WANG, Xi Tao;** c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **WIGGINTON, Jon M.;** c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US).

(74) Agents: **DELAUNEY, Ashton J.** et al; Bristol-Myers Squibb Company, P.O. Box 4000, Princeton, New Jersey 08543-4000 (US).

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

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

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

[Continued on nextpage]

(54) Title: CANCER IMMUNOTHERAPY BY DISRUPTING PD-l/PD-Ll SIGNALING

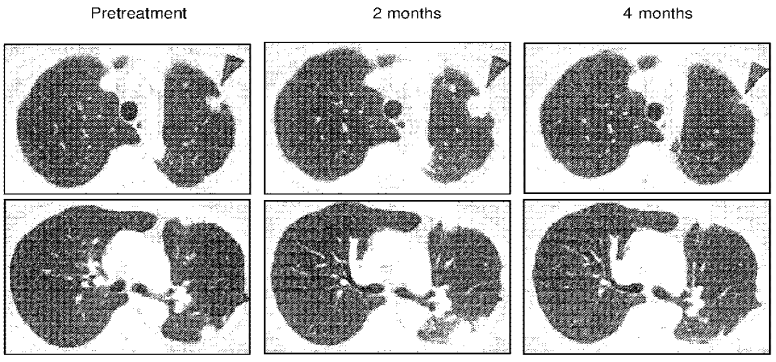

FIG. 7

(57) Abstract: The disclosure provides a method for immunotherapy of a cancer patient, comprises administering to the patient an Ab that inhibits signaling from the PD-l/PD-Ll signaling pathway, or a combination of such Ab and an anti-CTLA-4 Ab. This disclosure also provides a method for immunotherapy of a cancer patient comprising selecting a patient who is a suitable candidate for immunotherapy based on an assessment that the proportion of cells in a test tissue sample from the patient that express PD-Ll on the cell surface exceeds a predetermined threshold level, and administering an anti-PD- 1 Ab to the selected subject. The disclosure additionally provides rabbit mAbs that bind specifically to a cell surface-expressed PD-Ll antigen in a FFPE tissue sample, and an automated IHC method for assessing cell surface expression in FFPE tissues using the provided anti-PD-Ll Abs.

WO 2013/173223 A1

Published:

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

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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