

ANTI-PD1 ANTIBODIES AND METHODS OF USE

FIELD OF THE INVENTION

The present invention relates to anti-PD1 antibodies and methods of using the same.

BACKGROUND

5 PD-1

Co-stimulation or the provision of two distinct signals to T-cells is a widely accepted model of lymphocyte activation of resting T lymphocytes by antigen-presenting cells (APCs) (Lafferty et al., Aust. J. Exp. Biol. Med. Sci. 53: 27-42 (1975)).

10 This model further provides for the discrimination of self from non-self and immune tolerance (Bretscher et al., Science 169: 1042-1049 (1970); Bretscher, P.A., P.N.A.S. USA 96: 185-190 (1999); Jenkins et al., J. Exp. Med. 165: 302-319 (1987)). The primary signal, or antigen specific signal, is transduced through the T-cell receptor (TCR) following recognition of foreign antigen peptide presented in
15 the context of the major histocompatibility-complex (MHC). The second or co-stimulatory signal is delivered to T-cells by co-stimulatory molecules expressed on antigen-presenting cells (APCs), and induces T-cells to promote clonal expansion, cytokine secretion and effector function (Lenschow et al., Ann. Rev. Immunol. 14:233 (1996)). In the absence of co-stimulation, T-cells can become refractory to
20 antigen stimulation, do not mount an effective immune response, and further may result in exhaustion or tolerance to foreign antigens.

The simple two-signal model can be an oversimplification because the strength of the TCR signal actually has a quantitative influence on T-cell activation and differentiation (Viola et al., Science 273: 104-106 (1996); Sloan-Lancaster, Nature
25 363: 156-159 (1993)). Moreover, T-cell activation can occur even in the absence of co-stimulatory signals if the TCR signal strength is high. More importantly, T-cells receive both positive and negative secondary co-stimulatory signals. The regulation of such positive and negative signals is critical to maximize the host's protective immune responses, while maintaining immune tolerance and preventing
30 autoimmunity.

Negative secondary signals seem necessary for induction of T-cell tolerance, while positive signals promote T-cell activation. While the simple two-signal model still provides a valid explanation for naive lymphocytes, a host's immune response is a dynamic process, and co-stimulatory signals can also be provided to antigen-exposed T-cells.

The mechanism of co-stimulation is of therapeutic interest because the manipulation of co-stimulatory signals has shown to provide a means to either enhance or terminate cell-based immune response. Recently, it has been discovered that T cell dysfunction or anergy occurs concurrently with an induced and sustained expression of the inhibitory receptor, programmed death 1 polypeptide (PD-1). As a result, therapeutic targeting of PD-1 is an area of intense interest.

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) *Immunogenetics* 10:247-260). PD-1 was discovered through screening for differential expression in apoptotic 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) *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, MX. (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. Acad. Sci. USA 99: 12293-7; Brown et al. (2003) J. Immunol. 170:1257-66).

PD1 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 171:1-8). 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, PD1 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 11:510). In a murine B cell tumor line, the ITSM of PD1 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).

Various patent applications disclose production of anti-PD-1 antibodies and/or methods of enhancing immune responses with an agent (including an anti-PD-1 antibody) that interferes with PD-L1 binding and/or PD-1 signaling, including the following:

US2003/0039653, US2004/0213795, US2006/0110383, US2007/0065427, US2007/0122378, US2012/237522, WO2004/072286, WO2006/121168, WO2006/133396, WO2007/005874, WO2008/083174, WO2008/156712, WO2009/024531, WO2009/014708, WO2009/114335, WO2010/027828, WO2010/027423, WO2010/036959, WO2010/029435, WO2010/029434, WO2010/063011, WO2010/089411, WO2011/066342, WO2011/110604, WO2011/110621, and WO2012/145493.

SUMMARY

The disclosure provides anti-PD1 antibodies and methods of using the same.

The disclosure provides an anti-PD1 antibody, wherein the antibody:

- 5 i) competes for binding to PD-1 with an anti-PD1 antibody comprising the VH and VL of PD1-0103, and/or
- ii) binds to a human and cynomolgus PD-1; and/or
- iii) enhances the interferon-gamma (IFN-gamma) secretion by allogenic stimulated T cells by 85% or more at an antibody concentration of 10µg/ml ; and/ or
- 10 iv) enhances the tumor necrosis factor alpha (TNF alpha) secretion by allogenic stimulated T cells by 200% or more at an antibody concentration of 10µg/ml.

15 The disclosure also provides for an antibody that binds to human PD1, wherein the antibody enhances the interferon-gamma (IFN-gamma) secretion by allogenic stimulated T cells by 85% or more at an antibody concentration of 10µg/ml in a Mixed Lymphocyte Reaction (MLR) assay.

The disclosure also provides for an antibody that binds to human PD1, wherein the antibody enhances the tumor necrosis factor alpha (TNF alpha) secretion by allogenic stimulated T cells by 200% or more at an antibody concentration of 10µg/ml in a Mixed Lymphocyte Reaction (MLR) assay.

- 5 In one aspect, the disclosure provides for an isolated antibody that binds to human PD1, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:71; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:72; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:73; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:74; (e) 10 HVR-L2 comprising the amino acid sequence of SEQ ID NO:75; (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:76, and (g) FR-H3 comprising the amino acid sequence of SEQ ID NO: 77 (of RDN) at positions of 71, 72 and 73 according to Kabat numbering.

15 The disclosure provides an isolated antibody that binds to human PD1, wherein the antibody comprises

- A) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (e) HVR-L2 20 comprising the amino acid sequence of SEQ ID NO:5; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; or
- B) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:9; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:10; (c) HVR-

H3 comprising the amino acid sequence of SEQ ID NO:11; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:13; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:14; or

5 C) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:19; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:20; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (f) HVR-L3
10 comprising the amino acid sequence of SEQ ID NO:22; or

D) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:28; (e) HVR-L2
15 comprising the amino acid sequence of SEQ ID NO:29; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30; or

E) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:33; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:34; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:35; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:36; (e) HVR-L2
20 comprising the amino acid sequence of SEQ ID NO:37; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:38; or

F) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:41; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:42; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:43; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:44; (e) HVR-L2
25 comprising the amino acid sequence of SEQ ID NO:45; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:46; or

G) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:49; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:50; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:51; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:52; (e) HVR-L2
30 comprising the amino acid sequence of SEQ ID NO:53; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:54.

In another aspect, the disclosure provides an isolated antibody that binds to human PD1, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

The disclosure further provides an isolated antibody that binds to human PD1, wherein the antibody comprises

- 10 A) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:3; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; or
- 15 B) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:9, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:10, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:11; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:13 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:14; or
- 20 C) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:19; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:21 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22; or.
- 25 D) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26, and (iii) HVR-H3 comprising an amino acid
- 30

sequence selected from SEQ ID NO:27; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:28; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:29 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30; or

- 5 E) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:33, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:34, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:35; and (b) a VL domain comprising

(i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:36; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:37 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:38; or

- 5 F) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:41, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:42, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:43; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:44; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:45 and (iii) 10 HVR-L3 comprising the amino acid sequence of SEQ ID NO:46; or
- G) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:51; and (b) a VL domain comprising 15 (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:52; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:53 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:54.

20 In another aspect, the disclosure provides an isolated antibody that binds to human PD1, wherein the antibody comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:3; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (ii) HVR-L2

comprising the amino acid sequence of SEQ ID NO:5 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

The disclosure further provides an isolated antibody that binds to human PD1, wherein the antibody

5 A)

- i) comprises a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8;
- ii) or humanized variant of the VH and VL of the antibody under i);

or B)

- 10 i) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:58.
- ii) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:59.
- 15 iii) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:60.

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- iv) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:61.

or C)

- 5 i) comprises a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:16;
- ii) or humanized variant of the VH and VL of the antibody under i);

or D)

- i) comprises a VH sequence of SEQ ID NO:23 and a VL sequence of SEQ ID NO:24;
- 10 ii) or humanized variant of the VH and VL of the antibody under i);

or E)

- i) comprises a VH sequence of SEQ ID NO:31 and a VL sequence of SEQ ID NO:32;
- ii) or humanized variant of the VH and VL of the antibody under i);

15 or F)

- i) comprises a VH sequence of SEQ ID NO:39 and a VL sequence of SEQ ID NO:40;
- ii) or humanized variant of the VH and VL of the antibody under i);

or G)

- 20 i) comprises a VH sequence of SEQ ID NO:47 and a VL sequence of SEQ ID NO:48;
- ii) or humanized variant of the VH and VL of the antibody under i);

or H)

- 25 i) comprises a VH sequence of SEQ ID NO:55 and a VL sequence of SEQ ID NO:56;

ii) or humanized variant of the VH and VL of the antibody under i).

In another aspect, the disclosure provides an isolated antibody that binds to human PD1, wherein the antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:58.

5 In another aspect, the disclosure provides an isolated antibody that binds to human PD1, wherein the antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:59.

In another aspect, the disclosure provides an isolated antibody that binds to human PD1, wherein the antibody comprises a VH sequence of SEQ ID NO:57 and a VL
10 sequence of SEQ ID NO:60.

In another aspect, the disclosure provides an isolated antibody that binds to human PD1, wherein the antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:61.

In one embodiment the anti-PD1 antibody according to the disclosure is a
15 monoclonal antibody.

In one embodiment the anti-PD1 antibody according to the disclosure is a human, humanized, or chimeric antibody.

In one embodiment the anti-PD1 antibody according to the disclosure which is an antibody fragment that binds to PD1.

In one embodiment the anti-PD1 antibody according to the disclosure which is Fab fragment.

- 5 In another aspect, the disclosure provides an isolated nucleic acid encoding the antibody as described herein.

In another aspect, the disclosure provides a host cell comprising such nucleic acid.

- 10 In another aspect, the disclosure provides a method of producing an antibody comprising culturing the host cell as described herein so that the antibody is produced.

The disclosure provides such method of producing an antibody, further comprising recovering the antibody from the host cell.

In another aspect, the disclosure provides a pharmaceutical formulation comprising the antibody described herein and a pharmaceutically acceptable carrier.

- 15 In another aspect, the disclosure provides the antibody described herein for use as a medicament.

In another aspect, the disclosure provides the antibody described herein for use in treating cancer.

- 20 The disclosure provides the use of the antibody described herein in the manufacture of a medicament. In one embodiment the medicament is for treatment of cancer. In another aspect, the disclosure provides use of the antibody as described herein in the manufacture of a medicament for the treatment of cancer.

- 25 The disclosure provides a method of treating an individual having cancer comprising administering to the individual an effective amount of the antibody described herein.

BRIEF DESCRIPTION OF THE FIGURES

- Figure 1:** Blockade of PD1 with chimeric PD1-0103 strongly enhances IFN-gamma secretion by allogenic stimulated primary human T cells.
- 5 **Figure 2:** Blockade of PD1 with chimeric PD1-0103 strongly increases interferon-gamma (IFN-g) secretion by allogenic stimulated primary human T cells.
- Figure 3:** Blockade of PD1 with chimeric PD1-0103 strongly increases tumor necrosis factor alpha (TNF) secretion by allogenic stimulated primary human T cells.
- 10 **Figure 4:** 4A) frequency of CD4 T cells producing Granzyme B and 4B) Amount of IFN- γ detected by absorbance (Optical Density, O.D.) in the supernatant of the MLR in presence of increasing concentrations of different anti-PD-1 antibodies.
- 15 **Figure 5:** 5A) Impact of PD1/PD-L1 blockade on reactivation of suppressed T cell receptor signalig in presence of different anti-PD-1 antibodies 5B) Impact of PD1/PD-L1 blockade on reactivation of suppressed T cell receptor signalig in presence of different anti-PD-1 antibodies.
- Figure 6:** Structure of PD1-ECD in complex with Fab of PD1-0103.
- 20 **Figure 7:** Structure of PD1-ECD complex with Fab PD1-0103: *Glycosylation at ASN58 on PD1 is involved in the interaction.*
- Figure 8:** Structure of PD1-ECD complex Structure of PD1-ECD complex with Fab PD1-0103:*View on epitope/paratop.*
- Figure 9:** Contacts PD1 core sugar side chain at Asn58 – Fab PD1-0103 Heavy chain: contacts identified by distance cutoff of 5Å.
- 25 **Figure 10:** Residues of PD1-ECD that are interacting with the antibody-*Sequence view with detailed contact properties – PD-I.*
- Figure 11:** Residues of the antibody that are interacting with PD1-ECD -*Sequence view with detailed contact properties – heavy chain.*

Figure 12: Residues of the antibody that are interacting with PD1-ECD-*Sequence view with detailed contact properties – light chain.*

Figure 13A: Binding of different antibodies to PD1 aglycosylated at Asn58 (left) and to PD1 glycosylated at Asn58 (right) (Biacore sensorgramms).

5 **Figure 13B:** Binding of different antibodies to PD1 aglycosylated at Asn58 and to PD1 glycosylated at Asn58 - On-off-rate mab determined by Biacore.

Figure 14A: In vivo tumor growth inhibition of PD1-0103-0312 (aPD-1) compared to nivolumab in combination with a bispecific CEA-CD3 antibody - at high doses.

10 **Figure 14B:** In vivo tumor growth inhibition of PD1-0103-0312 (aPD-1) compared to nivolumab in combination with a bispecific CEA-CD3 antibody - at high doses.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

15 An “acceptor human framework” for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some
20 embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

25 When used herein, the term “PD1”, “human PD1”, “PD-1” or “human PD-1” refers to the human protein PD1 (SEQ ID NO: 68) (protein without signal sequence) / (SEQ ID NO: 70) (protein with signal sequence). As used herein, an antibody “binding to human PD1”, “specifically binding to human PD1”, “that binds to human PD1” or “anti-PD1 antibody” refers to an antibody specifically binding to
30 the human PD1 antigen or its Extracellular Domain (ECD) with a binding affinity of a K_D -value of 1.0×10^{-8} mol/l or lower, in one embodiment of a K_D -value of 1.0×10^{-9} mol/l or lower, in one embodiment of a K_D -value of 1.0×10^{-9} mol/l to

1.0×10^{-13} mol/l. The binding affinity is determined with a standard binding assay, such as surface plasmon resonance technique (BIAcore®, GE-Healthcare Uppsala, Sweden) e.g. using the PD1 extracellular domain.

Human PD1 has N-linked glycosylation sites at PD-1 residues 49, 58, 74 of SEQ ID NO. 70 (see e.g. D.Y. Lin et al, PNAS 105 (2008) 3011–3016). The core sugar chain (N-linked glycosylation) tree at position Asn58 of PD-1 has the following structure with respect to the monosaccharides. In one embodiment the core sugar chain at Asn58 of PD1 refers to the first 5 sugars (monosaccharides) which are attached to PD1 at Asn58.

Asn58-N-GlcNAc(FUC) – GlcNAc – BMA – MAN (see Figure 9) wherein the following abbreviations are used.

[GlcNAc] = NGA = N-acetyl-beta-D-galactosamine = 2-(acetylamino)-2-deoxy-beta-D-galactopyranose

[FUC] = alpha-L-fucose

[BMA] = beta-D-mannopyranose

[MAN] = alpha-D-mannopyranose

The first GlcNAc in the sugar chain is fucosylated which abbreviated as GlcNAc(FUC).

In one embodiment the core sugar chain at Asn58 of PD1 refers to the first 5 sugars (monosaccharides) GlcNAc, FUC, GlcNAc, BMA, MAN which are attached to PD1 at Asn58.

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody

molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

5 An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

10 The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

15 The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively.

20 The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents;
25 enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

30 An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region.

5 The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat, E.A. et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991), NIH Publication 91-3242.

10 "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

15 The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

20 The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as
25 screened or selected for in the originally transformed cell are included herein.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human
30 antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human

immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat, E.A. et al., Sequences of Proteins of Immunological Interest, 5th ed., Bethesda MD (1991), NIH Publication 91-3242, Vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra*. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.

A “humanized” antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term “hypervariable region” or “HVR” as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence (“complementarity determining regions” or “CDRs”) and/or form structurally defined loops (“hypervariable loops”) and/or contain the antigen-contacting residues (“antigen contacts”). Generally, antibodies comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:

- (a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));
- (b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991));
- (c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)); and

- (d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

5 Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

An “immunoconjugate” is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

10 An “individual” or “subject” is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

15 An “isolated” antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity see, e.g., Flatman, S. et al., J. Chromatogr. B 848 (2007) 79-87.

20 An “isolated” nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

25 “Isolated nucleic acid encoding an anti-PD1 antibody” refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

30 The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such

variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

A “naked antibody” refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation. (Include if Prior art has immunoconjugates)

“Native antibodies” refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

“Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference

polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

- 5 A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject., A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

- 10 As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, 15 preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

- 20 The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt, T.J. et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., N.Y. (2007), page 91) A single VH or VL domain may be 25 sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See e.g., Portolano, S. et al., J. Immunol. 150 (1993) 880-887; 30 Clackson, T. et al., Nature 352 (1991) 624-628).

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are

capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors".

I. COMPOSITIONS AND METHODS

In one aspect, the invention is based, in part, on the finding that the selected anti-PD1 antibodies of the invention bind to certain epitopes of PD1, and have ability to increase the activation of different immune cells (e.g. T-cells, B-cells, NK cells, dendritic cells (DC), monocytes and macrophages). E.g. they increase immunomodulating cytokines (e.g. interferon gamma and granzyme B) release (secretion). Other immunomodulating cytokines which are or can be increased are e.g. tumor necrosis factor alpha (TNF alpha) secretion and IL-12. As used herein the terms interferon-gamma (IFN-gamma) tumor necrosis factor alpha (TNF alpha) secretion, IL-12 etc refer to the human cytokines.

In certain embodiments, antibodies that bind to PD1 are provided. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of cancer.

A. Exemplary Anti-PD1 Antibodies

In one aspect, the invention provides isolated antibodies that bind to human PD1.

In certain embodiments, an anti-PD1 is provided wherein the antibody:

- i) competes for binding to PD-1 with an anti-PD1 antibody comprising the VH and VL of PD1-0103, and
- ii) binds to a human and cynomolgus PD-1; and
- iii) enhances the interferon-gamma (IFN-gamma) secretion by allogenic stimulated T cells by 85% or more (in one preferred embodiment by 90% or more, in one preferred embodiment by 95% or more) at an antibody concentration of 10 µg/ml (wherein the secretion without antibody is set as 0% (basal level of IFN gamma) and the secretion with 20 EU/ml recombinant human IL-2 is set as 100% (in a (allogenic) Mixed lymphocyte reaction (MLR) assay according to Example 3); and/ or
- iv) enhances the tumor necrosis factor alpha (TNF alpha) secretion by allogenic stimulated T cells by 200% or more (in one preferred

embodiment by 250% or more) at an antibody concentration of 10µg/ml (wherein the secretion without antibody is set as 0% (basal level of IFN gamma) and the secretion with 20 EU/ ml recombinant human IL-2 is set as 100% (in a (allogenic) Mixed lymphocyte reaction (MLR) assay according to Example 3).

In one aspect, the invention provides an anti-PD1 antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:3; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

In one embodiment such anti-PD1 antibody comprises

- i) a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8;
- ii) or humanized variant of the VH and VL of the antibody under i).

In one embodiment such anti-PD1 antibody comprises

- i) a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:58; or
- ii) a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:59; or
- iii) a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:60; or

- iv) a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:61.

In one embodiment such anti-PD1 antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:58.

- 5 In one embodiment such anti-PD1 antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:59.

In one embodiment such anti-PD1 antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:60.

- 10 In one embodiment such anti-PD1 antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:61.

- 15 In one aspect, the invention provides an anti-PD1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:9; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:10; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:11; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:13; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.

- 20 In one aspect, the invention provides an anti-PD1 antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:9; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:10; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:11; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:13; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.

- 25 In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:9, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:10, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:11; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:13 and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.
- 30

5 In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:9, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:10, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:11; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:13 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.

In one embodiment such anti-PD1 antibody comprises

- 10 i) comprises a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:16;
- ii) or humanized variant of the VH and VL of the antibody under i).

15 In one aspect, the invention provides an anti-PD1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:19; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:20; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22.

20 In one aspect, the invention provides an anti-PD1 antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:19; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:20; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22.

25

30 In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:19; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:20;

(ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:21 and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22.

5 In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:19; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:21 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22.

10 In one embodiment such anti-PD1 antibody comprises

- i) comprises a VH sequence of SEQ ID NO:23 and a VL sequence of SEQ ID NO:24;
- ii) or humanized variant of the VH and VL of the antibody under i).

15 In one aspect, the invention provides an anti-PD1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:28; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:29; and
20 (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30.

In one aspect, the invention provides an anti-PD1 antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:28; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:29; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30.

30 In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:27; and (b) a VL

domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:28; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:29 and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30.

- 5 In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:27; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of
10 SEQ ID NO:28; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:29 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30.

In one embodiment such anti-PD1 antibody comprises

- i) comprises a VH sequence of SEQ ID NO:31 and a VL sequence of SEQ ID NO:32;
- 15 ii) or humanized variant of the VH and VL of the antibody under i).

- In one aspect, the invention provides an anti-PD1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:33; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:34; (c) HVR-H3 comprising the amino acid sequence of
20 SEQ ID NO:35; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:36; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:37; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:38.

- In one aspect, the invention provides an anti-PD1 antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:33; (b) HVR-H2
25 comprising the amino acid sequence of SEQ ID NO:34; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:35; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:36; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:37; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:38.

- 30 In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:33, (ii) HVR-H2

comprising the amino acid sequence of SEQ ID NO:34, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:35; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:36;
5 (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:37 and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:38.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:33, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:34, and (iii)
10 HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:35; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:36; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:37 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:38.

In one embodiment such anti-PD1 antibody comprises

- 15 i) comprises a VH sequence of SEQ ID NO:39 and a VL sequence of SEQ ID NO:40;
- ii) or humanized variant of the VH and VL of the antibody under i).

In one aspect, the invention provides an anti-PD1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the
20 amino acid sequence of SEQ ID NO:41; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:42; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:43; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:44; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:45; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:46.

25 In one aspect, the invention provides an anti-PD1 antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:41; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:42; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:43; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:44; (e) HVR-L2 comprising the amino acid sequence
30 of SEQ ID NO:45; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:46.

5 In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:41, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:42, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:43; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:44; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:45 and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:46.

10 In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:41, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:42, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:43; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of
15 SEQ ID NO:44; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:45 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:46.

In one embodiment such anti-PD1 antibody comprises

- i) comprises a VH sequence of SEQ ID NO:47 and a VL sequence of SEQ ID NO:48;
- 20 ii) or humanized variant of the VH and VL of the antibody under i).

In one aspect, the invention provides an anti-PD1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:49; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:50; (c) HVR-H3 comprising the amino acid sequence of
25 SEQ ID NO:51; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:52; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:53; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:54.

In one aspect, the invention provides an anti-PD1 comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:49; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:50; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:51; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:52; (e) HVR-L2 comprising the amino acid sequence of SEQ ID
30 NO:53; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:54.

5 In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:51; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:52; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:53 and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:54.

10 In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:51; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of
15 SEQ ID NO:52; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:53 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:54.

In one embodiment such anti-PD1 antibody comprises

- i) comprises a VH sequence of SEQ ID NO:47 and a VL sequence of SEQ ID NO:48;
- 20 ii) or humanized variant of the VH and VL of the antibody under i).

In one preferred embodiment an antibody is provided that binds to the same epitope as an anti-PD1 antibody comprising a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8.

25 In one preferred embodiment an antibody is provided that competes for binding to human PD1 with anti- PD1 antibody comprising a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8 (as determined in a competition assay described in Example 2 (Epitope mapping ELISA/ Binding competition assay)).

In one aspect, the invention provides an anti-PD1 antibody (e.g. an antibody that binds to human PD1) comprising

- 30 A) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; (d) HVR-L1

comprising the amino acid sequence of SEQ ID NO:4; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; or

In another aspect the invention provides an anti-PD1 antibody (e.g. an antibody that binds to human PD1) comprising

- 5 (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:3; and (b) a VL domain comprising (i) HVR-L1
- 10 comprising the amino acid sequence of SEQ ID NO:4; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

In one aspect, the invention provides an antibody that binds to human PD1 that

A)

- 15 i) comprises a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8;
- ii) or humanized variant of the VH and VL of the antibody under i);

or B)

- 20 i) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:58.
- ii) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:59.
- iii) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:60.
- 25 iv) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:61.

or C)

- i) comprises a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:16;
- ii) or humanized variant of the VH and VL of the antibody under i);

5 or D)

- i) comprises a VH sequence of SEQ ID NO:23 and a VL sequence of SEQ ID NO:24;
- ii) or humanized variant of the VH and VL of the antibody under i);

or E)

- 10
- i) comprises a VH sequence of SEQ ID NO:31 and a VL sequence of SEQ ID NO:32;
 - ii) or humanized variant of the VH and VL of the antibody under i);

or F)

- 15
- i) comprises a VH sequence of SEQ ID NO:39 and a VL sequence of SEQ ID NO:40;
 - ii) or humanized variant of the VH and VL of the antibody under i);

or G)

- 20
- i) comprises a VH sequence of SEQ ID NO:47 and a VL sequence of SEQ ID NO:48;
 - ii) or humanized variant of the VH and VL of the antibody under i);

or H)

- i) comprises a VH sequence of SEQ ID NO:55 and a VL sequence of SEQ ID NO:56;
- ii) or humanized variant of the VH and VL of the antibody under i).

In one aspect, the invention provides an antibody that binds to human PD1 that

- i) comprises a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8;
- ii) or humanized variant of the VH and VL of the antibody under i);

5 In one aspect, the invention provides an antibody that binds to human PD1 that comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:58.

In one aspect, the invention provides an antibody that binds to human PD1 that comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:59.

10 In one aspect, the invention provides an antibody that binds to human PD1 that comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:60.

In one aspect, the invention provides an antibody that binds to human PD1 that comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:61.

In another aspect the invention provides an anti-PD1 antibody (e.g. an antibody that binds to human PD1) comprising

15 A) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and (f) HVR-L3
20 comprising the amino acid sequence of SEQ ID NO:6; or

B) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:9; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:10; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:11; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (e) HVR-L2
25 comprising the amino acid sequence of SEQ ID NO:13; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:14; or

C) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:19; (d) HVR-L1
30 comprising the amino acid sequence of SEQ ID NO:20; (e) HVR-L2

comprising the amino acid sequence of SEQ ID NO:21; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22; or

- 5 D) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:28; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:29; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30; or
- 10 E) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:33; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:34; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:35; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:36; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:37; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:38; or
- 15 F) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:41; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:42; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:43; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:44; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:45; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:46; or
- 20 G) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:49; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:50; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:51; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:52; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:53; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:54;
- 25

wherein the antibody is characterized independently by one or more of the following properties: the anti-PD-1 antibody

- 30 i) competes for binding to PD-1 with an anti-PD-1 antibody comprising the VH and VL of PD1-0103, and/ or
- ii) binds to a human and cynomolgus PD-1; and/ or

- 5 iii) enhances the interferon-gamma (IFN-gamma) secretion by allogenic stimulated T cells by 85% or more (in one preferred embodiment by 90% or more, in one preferred embodiment by 95% or more) at an antibody concentration of 10 μ g/ml (wherein the secretion without antibody is set as 0% (basal level of IFN gamma) and the secretion with 20 EU/ ml recombinant human IL-2 is set as 100% (in a (allogenic) Mixed lymphocyte reaction (MLR) assay according to Example 3) ; and/ or
- 10 iv) enhances the tumor necrosis factor alpha (TNF alpha) secretion by allogenic stimulated T cells by 200% or more (in one preferred embodiment by 250% or more) at an antibody concentration of 10 μ g/ml (wherein the secretion without antibody is set as 0% (basal level of IFN gamma) and the secretion with 20 EU/ ml recombinant human IL-2 is set as 100% (in a (allogenic) Mixed lymphocyte reaction (MLR) assay according to Example 3).
- 15

In another aspect the invention provides an anti-PD1 antibody (e.g. an antibody that binds to human PD1) comprising

- 20 A) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:3; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; or
- 25 B) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:9, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:10, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:11; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (ii)
- 30 HVR-L2 comprising the amino acid sequence of SEQ ID NO:13 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:14; or
- C) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18, and (iii) HVR-H3 comprising an amino acid

sequence selected from SEQ ID NO:19; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:21 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22; or.

5 D) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:27; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:28; (ii) 10 HVR-L2 comprising the amino acid sequence of SEQ ID NO:29 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30; or

 E) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:33, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:34, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:35; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:36; (ii) 15 HVR-L2 comprising the amino acid sequence of SEQ ID NO:37 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:38; or

 F) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:41, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:42, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:43; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:44; (ii) 20 HVR-L2 comprising the amino acid sequence of SEQ ID NO:45 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:46; or 25

 G) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:51; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:52; (ii) 30 HVR-L2 comprising the amino acid sequence of SEQ ID NO:53 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:54;

wherein the antibody is characterized independently by one or more of the following properties: the anti-PD-1 antibody

- i) competes for binding to PD-1 with an anti-PD-1 antibody comprising the VH and VL of PD1-0103, and/ or
- 5 ii) binds to a human and cynomolgus PD-1; and/ or
- iii) enhances the interferon-gamma (IFN-gamma) secretion by allogenic stimulated T cells by 85% or more (in one preferred embodiment by 90% or more, in one preferred embodiment by 95% or more) at an antibody concentration of 10µg/ml (wherein the secretion without
10 antibody is set as 0% (basal level of IFN gamma) and the secretion with 20 EU/ ml recombinant human IL-2 is set as 100% (in a (allogenic) Mixed lymphocyte reaction (MLR) assay according to Example 3) ; and/ or
- iv) enhances the tumor necrosis factor alpha (TNF alpha) secretion by
15 allogenic stimulated T cells by 200% or more (in one preferred embodiment by 250% or more) at an antibody concentration of 10µg/ml (wherein the secretion without antibody is set as 0% (basal level of IFN gamma) and the secretion with 20 EU/ ml recombinant human IL-2 is set as 100% (in a (allogenic) Mixed lymphocyte
20 reaction (MLR) assay according to Example 3).

In a further aspect of the invention, an anti-PD1 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-PD1 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')₂ fragment. In another
25 embodiment, the antibody is a full length antibody, e.g., an intact IgG1 or IgG4 antibody or other antibody class or isotype as defined herein.

In a further aspect, an anti-PD1 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

1. Antibody Affinity

In certain embodiments, an antibody provided herein has a dissociation constant KD of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M , e.g., from 10^{-9} M to 10^{-13} M).

5 In one preferred embodiment, KD is measured using surface plasmon resonance assays using a BIACORE[®] at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N*'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 $\mu\text{g/ml}$ (~0.2 μM) before injection at a flow rate of 5 $\mu\text{l/minute}$ to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in 10 PBS with 0.05% polysorbate 20 (TWEEN-20TM) surfactant (PBST) at 25°C at a flow rate of approximately 25 $\mu\text{l/min}$. Association rates (k_{on} or k_a) and dissociation rates (k_{off} or k_d) are calculated using a simple one-to-one Langmuir binding model (BIACORE[®] Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant KD is calculated as the ratio k_d/k_a ($k_{\text{off}}/k_{\text{on}}$). See, e.g., Chen, Y. et al., J. Mol. Biol. 293 (1999) 865-881. If the on-rate exceeds $10^6 \text{ M}^{-1} \text{ s}^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCOTM spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

30 In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')₂, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson, P.J. et al., Nat. Med. 9 (2003) 129-134. For a

review of scFv fragments, see, e.g., Plueckthun, A., In; The Pharmacology of Monoclonal Antibodies, Vol. 113, Rosenberg and Moore (eds.), Springer-Verlag, New York (1994), pp. 269-315; see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')₂ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 0 404 097; WO 1993/01161; Hudson, P.J. et al., Nat. Med. 9 (2003) 129-134; and Holliger, P. et al., Proc. Natl. Acad. Sci. USA 90 (1993) 6444-6448. Triabodies and tetrabodies are also described in Hudson, P.J. et al., Nat. Med. 9 (2003) 129-134).

Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516 B1).

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

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

In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which

5 HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

10 Humanized antibodies and methods of making them are reviewed, e.g., in Almagro, J.C. and Fransson, J., *Front. Biosci.* 13 (2008) 1619-1633, and are further described, e.g., in Riechmann, I. et al., *Nature* 332 (1988) 323-329; Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86 (1989) 10029-10033; US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri, S.V. et al., *Methods* 36 (2005) 25-34 (describing SDR (a-CDR) grafting); Padlan, E.A., *Mol. Immunol.* 28 (1991) 489-498 (describing "resurfacing"); Dall'Acqua, W.F. et al., *Methods* 36 (2005) 43-60 (describing "FR shuffling"); and Osbourn, J. et al., *Methods* 36 (2005) 61-68 and Klimka, A. et al., *Br. J. Cancer* 83 (2000) 252-260 (describing the "guided selection" approach to FR shuffling).

20 Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims, M.J. et al., *J. Immunol.* 151 (1993) 2296-2308; framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter, P. et al., *Proc. Natl. Acad. Sci. USA* 89 (1992) 4285-4289; and Presta, L.G. et al., *J. Immunol.* 151 (1993) 2623-2632); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro, J.C. and Fransson, J., *Front. Biosci.* 13 (2008) 1619-1633); and framework regions derived from screening FR libraries (see, e.g., Baca, M. et al., *J. Biol. Chem.* 272 (1997) 10678-10684 and Rosok, M.J. et al., *J. Biol. Chem.* 271 (1996) 22611-22618).

4. Human Antibodies

30 In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk, M.A. and van de Winkel, J.G., *Curr. Opin. Pharmacol.* 5 (2001) 368-374 and Lonberg, N., *Curr. Opin. Immunol.* 20 (2008) 450-459.

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

Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor, D., J. Immunol. 133 (1984) 3001-3005; Brodeur, B.R. et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York (1987), pp. 51-63; and Boerner, P. et al., J. Immunol. 147 (1991) 86-95) Human antibodies generated via human B-cell hybridoma technology are also described in Li, J. et al., Proc. Natl. Acad. Sci. USA 103 (2006) 3557-3562. Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, J., Xiandai Mianyixue 26 (2006) 265-268 (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers, H.P. and Brandlein, S., Histology and Histopathology 20 (2005) 927-937 and Vollmers, H.P. and Brandlein, S., Methods and Findings in Experimental and Clinical Pharmacology 27 (2005) 185-191.

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

5. Library-Derived Antibodies

Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom, H.R. et al., *Methods in Molecular Biology* 178 (2001) 1-37 and further described, e.g., in the McCafferty, J. et al., *Nature* 348 (1990) 552-554; Clackson, T. et al., *Nature* 352 (1991) 624-628; Marks, J.D. et al., *J. Mol. Biol.* 222 (1992) 581-597; Marks, J.D. and Bradbury, A., *Methods in Molecular Biology* 248 (2003) 161-175; Sidhu, S.S. et al., *J. Mol. Biol.* 338 (2004) 299-310; Lee, C.V. et al., *J. Mol. Biol.* 340 (2004) 1073-1093; Fellouse, F.A., *Proc. Natl. Acad. Sci. USA* 101 (2004) 12467-12472; and Lee, C.V. et al., *J. Immunol. Methods* 284 (2004) 119-132.

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter, G. et al., *Ann. Rev. Immunol.* 12 (1994) 433-455. Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths, A.D. et al., *EMBO J.* 12 (1993) 725-734. Finally, naive libraries can also be made synthetically by cloning non-rearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom, H.R. and Winter, G., *J. Mol. Biol.* 227 (1992) 381-388. Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

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

6. Multispecific Antibodies

In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for PD1 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of PD1. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express PD1. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein, C. and Cuello, A.C., *Nature* 305 (1983) 537-540, WO 93/08829, and Traunecker, A. et al., *EMBO J.* 10 (1991) 3655-3659), and “knob-in-hole” engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan, M. et al., *Science* 229 (1985) 81-83); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny, S.A. et al., *J. Immunol.* 148 (1992) 1547-1553; using “diabody” technology for making bispecific antibody fragments (see, e.g., Holliger, P. et al., *Proc. Natl. Acad. Sci. USA* 90 (1993) 6444-6448); and using single-chain Fv (sFv) dimers (see, e.g. Gruber, M et al., *J. Immunol.* 152 (1994) 5368-5374); and preparing trispecific antibodies as described, e.g., in Tutt, A. et al., *J. Immunol.* 147 (1991) 60-69).

Engineered antibodies with three or more functional antigen binding sites, including “Octopus antibodies,” are also included herein (see, e.g. US 2006/0025576).

The antibody or fragment herein also includes a “Dual Acting Fab” or “DAF” comprising an antigen binding site that binds to PD1 as well as another, different antigen (see, US 2008/0069820, for example).

The antibody or fragment herein also includes multispecific antibodies described in WO 2009/080251, WO 2009/080252, WO 2009/080253, WO 2009/080254, WO 2010/112193, WO 2010/115589, WO 2010/136172, WO 2010/145792, and

WO 2010/145793, WO2011/117330, WO2012/025525, WO2012/025530, WO2013/026835, WO2013/026831, WO2013/164325, or WO 2013/174873.

7. Antibody Variants

5 In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or
10 substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

a) Substitution, Insertion, and Deletion Variants

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

Table 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn

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

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

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- 5 (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

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

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications

(e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR “hotspots,” i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, P.S., *Methods Mol. Biol.* 207 (2008) 179-196), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom, H.R. et al. in *Methods in Molecular Biology* 178 (2002) 1-37. In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

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

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

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

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

b) Fc region variants

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

Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields, R.L. et al., J. Biol. Chem. 276 (2001) 6591-6604).

In one embodiment the invention such antibody is a IgG1 with mutations L234A and L235A or with mutations L234A, L235A and P329G. In another embodiment or IgG4 with mutations S228P and L235E or S228P, L235E or and P329G (numbering according to EU index of Kabat et al , Kabat et al., Sequences of
5 Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991).

Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer, R.L. et al., J. Immunol. 117 (1976) 587-593, and Kim, J.K. et al., J.
10 Immunol. 24 (1994) 2429-2434), are described in US 2005/0014934. Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434,
15 e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

See also Duncan, A.R. and Winter, G., Nature 322 (1988) 738-740; US 5,648,260; US 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

c) Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered
20 antibodies, e.g., “thioMAbs,” in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug
25 moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in
30 U.S. Patent No. 7,521,541.

d) Antibody Derivatives

In certain embodiments, an antibody provided herein may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily

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

In another embodiment, conjugates of an antibody and non-proteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the non-proteinaceous moiety is a carbon nanotube (Kam, N.W. et al., Proc. Natl. Acad. Sci. USA 102 (2005) 11600-11605). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the non-proteinaceous moiety to a temperature at which cells proximal to the antibody-non-proteinaceous moiety are killed.

B. Recombinant Methods and Compositions

Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-PD1 antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid

that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-PD1 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

For recombinant production of an anti-PD1 antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., US 5,648,237, US 5,789,199, and US 5,840,523. (See also Charlton, K.A., In: Methods in Molecular Biology, Vol. 248, Lo, B.K.C. (ed.), Humana Press, Totowa, NJ (2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, T.U., Nat. Biotech. 22 (2004) 1409-1414; and Li, H. et al., Nat. Biotech. 24 (2006) 210-215.

Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of

invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

5 Plant cell cultures can also be utilized as hosts. *See*, e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIESTM technology for producing antibodies in transgenic plants).

10 Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham, F.L. et al., J. Gen Virol. 36 (1977) 59-74); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, J.P., Biol. Reprod. 23 (1980) 243-252); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather, J.P. et al., Annals N.Y. Acad. Sci. 383 (1982) 44-68; MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR⁻ CHO cells (Urlaub, G. et al., Proc. Natl. Acad. Sci. USA 77 (1980) 4216-4220); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki, P. and Wu, A.M., Methods in Molecular Biology, Vol. 248, Lo, B.K.C. (ed.), Humana Press, Totowa, NJ (2004), pp. 255-268.

25 C. Assays

Anti-PD1 antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1. Binding assays and other assays

30 In one aspect, an antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc.

In another aspect, competition assays may be used to identify an antibody that competes with PD1-0103 (comprising a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8) for binding to PD1 (or alternatively with the humanized PD1-0103 variants antibodies PD1-0103-0312, PD1-0103-0313, PD1-0103-0314, PD1-0103-0315, with the identical 5 to 6 HVRs) . One embodiment of the invention is antibody which competes for binding to human PD1 with an anti-PD1 antibody comprising all 3 HVRs of VH sequence of SEQ ID NO:7 and all 3 HVRs of VL sequence of SEQ ID NO:8. One embodiment of the invention is antibody which competes for binding to human PD1 with an anti-PD1 antibody comprising all 3 HVRs of VH sequence of SEQ ID NO:57 and all 3 HVRs of VL sequence of SEQ ID NO:58. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by anti-PD1 antibody PD1-0103. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris, G.E. (ed.), Epitope Mapping Protocols, In: Methods in Molecular Biology, Vol. 66, Humana Press, Totowa, NJ (1996).

In an exemplary competition assay, immobilized PD1(-ECD) is incubated in a solution comprising a first labeled antibody that binds to PD1 (e.g., anti-PD1 antibody PD1-0103 or humanized antibody PD1-0103-0312) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to PD1. The second antibody may be present in a hybridoma supernatant. As a control, immobilized PD1 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to PD1, excess unbound antibody is removed, and the amount of label associated with immobilized PD1 is measured. If the amount of label associated with immobilized PD1 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to PD1. See Harlow, E. and Lane, D., Antibodies: A Laboratory Manual, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1988). For another exemplary competition assay see Example 2 (Epitope mapping ELISA/ Binding competition assay).

2. Activity assays

In one aspect, assays are provided for identifying anti-PD1 antibodies thereof having biological activity. Biological activity may include, e.g., the ability to

enhance the activation and/or proliferation of different immune cells especially T-cells. E.g. they enhance secretion of immunomodulating cytokines (e.g. interferon-gamma (IFN-gamma) and/or tumor necrosis factor alpha (TNF alpha)). Other immunomodulating cytokines which are or can be enhance are e.g. IL12, Granzyme B etc. Biological activity may also include, cynomolgous binding crossreactivity, as well as binding to different cell types. Antibodies having such biological activity in vivo and/or in vitro are also provided.

In certain embodiments, an antibody of the invention is tested for such biological activity as described e.g. in Examples below.

10 **D. Immunoconjugates (Cancer only or modify for target)**

The invention also provides immunoconjugates comprising an anti-PD1 antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see US 5,208,020, US 5,416,064 and EP 0 425 235 B1); an auristatin such as monomethyl auristatin drug moieties DE and DF (MMAE and MMAF) (see US 5,635,483, US 5,780,588, and US 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see US 5,712,374, US 5,714,586, US 5,739,116, US 5,767,285, US 5,770,701, US 5,770,710, US 5,773,001, and US 5,877,296; Hinman, L.M. et al., Cancer Res. 53 (1993) 3336-3342; and Lode, H.N. et al., Cancer Res. 58 (1998) 2925-2928); an anthracycline such as daunomycin or doxorubicin (see Kratz, F. et al., Curr. Med. Chem. 13 (2006) 477-523; Jeffrey, S.C. et al., Bioorg. Med. Chem. Lett. 16 (2006) 358-362; Torgov, M.Y. et al., Bioconjug. Chem. 16 (2005) 717-721; Nagy, A. et al., Proc. Natl. Acad. Sci. USA 97 (2000) 829-834; Dubowchik, G.M. et al., Bioorg. & Med. Chem. Letters 12 (2002) 1529-1532; King, H.D. et al., J. Med. Chem. 45 (2002) 4336-4343; and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including

but not limited to 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, Phytolacca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, saponaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example TC^{99m} or I¹²³, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 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, E.S. et al., Science 238 (1987) 1098-1104. Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triamine pentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO 94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari, R.V. et al., Cancer Res. 52 (1992) 127-131; U.S. Patent No. 5,208,020) may be used.

The immunoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to,

BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce
5 Biotechnology, Inc., Rockford, IL., U.S.A).

E. Methods and Compositions for Diagnostics and Detection

In certain embodiments, any of the anti-PD1 antibodies provided herein is useful for detecting the presence of PD1 in a biological sample. The term “detecting” as used herein encompasses quantitative or qualitative detection. In certain
10 embodiments, a biological sample comprises a cell or tissue, such as immune cell or T cell infiltrates.

In one embodiment, an anti-PD1 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of PD1 in a biological sample is provided. In certain embodiments, the method
15 comprises contacting the biological sample with an anti-PD1 antibody as described herein under conditions permissive for binding of the anti-PD1 antibody to PD1, and detecting whether a complex is formed between the anti-PD1 antibody and PD1. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-PD1 antibody is used to select subjects eligible for therapy with an anti-PD1
20 antibody, e.g. where PD1 is a biomarker for selection of patients.

In certain embodiments, labeled anti-PD1 antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly,
25 e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P , ^{14}C , ^{125}I , ^3H , and ^{131}I , fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin,
30 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β -galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP,

lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

F. Pharmaceutical Formulations

Pharmaceutical formulations of an anti-PD1 antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed.) (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyl dimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as poly(vinylpyrrolidone); amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rhuPH20 (HYLENEX[®], Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rhuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO 2006/044908, the latter formulations including a histidine-acetate buffer.

The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

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

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

G. Therapeutic Methods and Compositions

Any of the anti-PD1 antibodies (or antigen binding proteins) provided herein may be used in therapeutic methods.

In one aspect, an anti-PD1 antibody for use as a medicament is provided. In further aspects, an anti-PD1 antibody or use in treating cancer is provided. In certain embodiments, an anti-PD1 antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-PD1 antibody for use in a method of treating an individual having cancer comprising administering to the individual an effective amount of the anti-PD1 antibody.

In further embodiments, the invention provides an anti-PD1 antibody for use as immunostimulatory agent/ or stimulating interferon-gamma (IFN-gamma) secretion. In certain embodiments, the invention provides an anti-PD1 antibody for use in a method of immunostimulation/ or stimulating interferon-gamma (IFN-

gamma) secretion in an individual comprising administering to the individual an effective of the the anti-PD1 antibody for immunostimulation/ or stimulating interferon-gamma (IFN-gamma) secretion.

5 In further embodiments, the invention provides an anti-PD1 antibody for use as immunostimulatory agent/ or stimulating tumor necrosis factor alpha (TNF alpha) secretion. In certain embodiments, the invention provides an anti-PD1 antibody for use in a method of immunostimulation/ or stimulating tumor necrosis factor alpha (TNF alpha) secretion in an individual comprising administering to the individual an effective of the the anti-PD1 antibody for
10 immunostimulation/ or stimulating tumor necrosis factor alpha (TNF alpha) secretion.

An “individual” according to any of the above embodiments is preferably a human. In a further aspect, the invention provides for the use of an anti-PD1 antibody in the manufacture or preparation of a medicament. In one embodiment, the
15 medicament is for treatment of cancer. In a further embodiment, the medicament is for use in a method of treating cancer comprising administering to an individual having cancer an effective amount of the medicament. In a further embodiment, the medicament is for inducing cell mediated lysis of cancer cells In a further embodiment, the medicament is for use in a method of inducing cell mediated lysis
20 of cancer cells in an individual suffering from cancer comprising administering to the individual an amount effective of the medicament to induce apoptosis in a cancer cell/ or to inhibit cancer cell proliferation. An “individual” according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for treating cancer. In one
25 embodiment, the method comprises administering to an individual having cancer an effective amount of an anti-PD1. An “individual” according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for inducing cell mediated lysis of cancer cells in an individual suffering from cancer. In one embodiment, the
30 method comprises administering to the individual an effective amount of an anti-PD1 to induce cell mediated lysis of cancer cells in the individual suffering from cancer. In one embodiment, an “individual” is a human.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-PD1 antibodies provided herein, e.g., for use in any of the above

therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-PD1 antibodies provided herein and a pharmaceutically acceptable carrier.

5 An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending
10 in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this
15 context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to
20 prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is
25 empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is
30 administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.5mg/kg - 10 mg/kg) of antibody can be an initial
35 candidate dosage for administration to the patient, whether, for example, by one or

more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a
5 desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the
10 patient receives from about two to about twenty, or e.g. about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of the antibody. However, other dosage regimens may be useful.
15 The progress of this therapy is easily monitored by conventional techniques and assays.

It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-PD1 antibody.

20 It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-PD1 antibody.

II. Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials
25 useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds
30 a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package

insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-PD1 antibody.

Description of the amino acid sequences

SEQ ID NO: 1	heavy chain HVR-H1, PD1-0103
SEQ ID NO: 2	heavy chain HVR-H2, PD1-0103
20 SEQ ID NO: 3	heavy chain HVR-H3, PD1-0103
SEQ ID NO: 4	light chain HVR-L1, PD1-0103
SEQ ID NO: 5	light chain HVR-L2, PD1-0103
SEQ ID NO: 6	light chain HVR-L3, PD1-0103
SEQ ID NO: 7	heavy chain variable domain VH, PD1-0103
25 SEQ ID NO: 8	light chain variable domain VL, PD1-0103
SEQ ID NO: 9	heavy chain HVR-H1, PD1-0098
SEQ ID NO: 10	heavy chain HVR-H2, PD1-0098
SEQ ID NO: 11	heavy chain HVR-H3, PD1-0098
30 SEQ ID NO: 12	light chain HVR-L1, PD1-0098
SEQ ID NO: 13	light chain HVR-L2, PD1-0098
SEQ ID NO: 14	light chain HVR-L3, PD1-0098
SEQ ID NO: 15	heavy chain variable domain VH, PD1-0098
SEQ ID NO: 16	light chain variable domain VL, PD1-0098

	SEQ ID NO: 17	heavy chain HVR-H1, PD1-0050
	SEQ ID NO: 18	heavy chain HVR-H2, PD1-0050
	SEQ ID NO: 19	heavy chain HVR-H3, PD1-0050
	SEQ ID NO: 20	light chain HVR-L1, PD1-0050
5	SEQ ID NO: 21	light chain HVR-L2, PD1-0050
	SEQ ID NO: 22	light chain HVR-L3, PD1-0050
	SEQ ID NO: 23	heavy chain variable domain VH, PD1-0050
	SEQ ID NO: 24	light chain variable domain VL, PD1-0050
10	SEQ ID NO: 25	heavy chain HVR-H1, PD1-0069
	SEQ ID NO: 26	heavy chain HVR-H2, PD1-0069
	SEQ ID NO: 27	heavy chain HVR-H3, PD1-0069
	SEQ ID NO: 28	light chain HVR-L1, PD1-0069
	SEQ ID NO: 29	light chain HVR-L2, PD1-0069
15	SEQ ID NO: 30	light chain HVR-L3, PD1-0069
	SEQ ID NO: 31	heavy chain variable domain VH, PD1-0069
	SEQ ID NO: 32	light chain variable domain VL, PD1-0069
	SEQ ID NO: 33	heavy chain HVR-H1, PD1-0073
20	SEQ ID NO: 34	heavy chain HVR-H2, PD1-0073
	SEQ ID NO: 35	heavy chain HVR-H3, PD1-0073
	SEQ ID NO: 36	light chain HVR-L1, PD1-0073
	SEQ ID NO: 37	light chain HVR-L2, PD1-0073
	SEQ ID NO: 38	light chain HVR-L3, PD1-0073
25	SEQ ID NO: 39	heavy chain variable domain VH, PD1-0073
	SEQ ID NO: 40	light chain variable domain VL, PD1-0073
	SEQ ID NO: 41	heavy chain HVR-H1, PD1-0078
	SEQ ID NO: 42	heavy chain HVR-H2, PD1-0078
30	SEQ ID NO: 43	heavy chain HVR-H3, PD1-0078
	SEQ ID NO: 44	light chain HVR-L1, PD1-0078
	SEQ ID NO: 45	light chain HVR-L2, PD1-0078
	SEQ ID NO: 46	light chain HVR-L3, PD1-0078
	SEQ ID NO: 47	heavy chain variable domain VH, PD1-0078
35	SEQ ID NO: 48	light chain variable domain VL, PD1-0078

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	SEQ ID NO: 49	heavy chain HVR-H1, PD1-0102
	SEQ ID NO: 50	heavy chain HVR-H2, PD1-0102
	SEQ ID NO: 51	heavy chain HVR-H3, PD1-0102
	SEQ ID NO: 52	light chain HVR-L1, PD1-0102
5	SEQ ID NO: 53	light chain HVR-L2, PD1-0102
	SEQ ID NO: 54	light chain HVR-L3, PD1-0102
	SEQ ID NO: 55	heavy chain variable domain VH, PD1-0102
	SEQ ID NO: 56	light chain variable domain VL, PD1-0102
10	SEQ ID NO: 57	humanized variant -heavy chain variable domain VH of PD1-0103_01
	SEQ ID NO: 58	humanized variant -light chain variable domain VL of PD1-0103_01
15	SEQ ID NO: 59	humanized variant -light chain variable domain VL of PD1-0103_02
	SEQ ID NO: 60	humanized variant -light chain variable domain VL of PD1-0103_03
	SEQ ID NO: 61	humanized variant -light chain variable domain VL of PD1-0103_04
20	SEQ ID NO: 62	human kappa light chain constant region
	SEQ ID NO: 63	human lambda light chain constant region
	SEQ ID NO: 64	human heavy chain constant region derived from IgG1
	SEQ ID NO: 65	human heavy chain constant region derived from IgG1 with mutations L234A and L235A
25	SEQ ID NO: 66	human heavy chain constant region derived from IgG1 with mutations L234A, L235A and P329G
	SEQ ID NO: 67	human heavy chain constant region derived from IgG4
	SEQ ID NO: 68	exemplary human PD1 sequence (without signal sequence)
	SEQ ID NO: 69	human PD1 Extracellular Domain (ECD)
30	SEQ ID NO: 70	exemplary human PD1 sequence (including signal sequence)
	SEQ ID NO: 71:	Minimal HVR1 of PD1-0103 and PD1-0103 humanized variant PD1-0103-0312, PD1-0103-0313, PD1-0103-0314 , and PD1-0103-0315
35	SEQ ID NO: 72:	Minimal HVR2 of PD1-0103 and PD1-0103 humanized variant PD1-0103-0312, PD1-0103-0313, PD1-0103-0314 , and PD1-0103-0315

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- SEQ ID NO: 73: Minimal HVR3 of PD1-0103 and PD1-0103 humanized variant PD1-0103-0312, PD1-0103-0313, PD1-0103-0314 , and PD1-0103-0315
- 5 SEQ ID NO: 74: Minimal LVR1 of PD1-0103 and PD1-0103 humanized variant PD1-0103-0312, PD1-0103-0313, PD1-0103-0314 , and PD1-0103-0315
- SEQ ID NO: 75: Minimal LVR2 of PD1-0103 and PD1-0103 humanized variant PD1-0103-0312, PD1-0103-0313, PD1-0103-0314 , and PD1-0103-0315
- 10 SEQ ID NO: 76: Minimal LVR3 of PD1-0103 and PD1-0103 humanized variant PD1-0103-0312, PD1-0103-0313, PD1-0103-0314 , and PD1-0103-0315
- SEQ ID NO: 77: fragment of FR-H3 comprising the amino acid sequence RDN at positions of 71, 72, 73 according to Kabat numbering
- 15

In the following the amino acid sequences of the VH und VL domains including marked HVRs (HVRs in bold, underlined letters) of anti-PD1 antibodies PD1-0016 (and its humanized versions PD1-0103-0312, PD1-0103-0313, PD1-0103-0314 and PD1-0103-0315), PD1-0098, PD1-0050, PD1-0069, PD1-0073, PD1-0078 and PD1-0102 are listed:

20

anti-PD1 PD1-0103:

VH PD1-0103:

25 EVILVESGGGLVKPGGSLKLSCAAS**GFSFSSY**TMSWVRQTPEKRLDWVATISG**GGR**DIYYPDSVKGR
FTISRDNAKNTLYLEMSSLMSEDTALYYCVLL**TGRVYFALD**SWGQGTSTVTVSS

VL PD1-0103:

30 KIVLTQSPASLPVSLGQRATISCRAS**ESVDTS****NSF**IHWYQQRPGQSPKLLIY**RSS**TLESQVTPARFS
GSGSRTDFTLTIDPVEADDVATYYC**QNYDVPW**TFGGGTKLEIK

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Humanized anti-PD1 PD1-0103 versions PD1-0103-0312, PD1-0103-0313,
PD1-0103-0314 and PD1-0103-0315:

5 VH PD1-0103-0312= VH PD1-0103-0313= VH PD1-0103-0314= VH PD1-0103-
0315:

EVQLLESQGGGLVQPGGSLRLSCAASGFSFSSYTMSWVRQAPGKGLEWVATISGGGRDIYYPD^{SV}KGR
FTISRDN^{SK}NTLYLQMNSLRAEDTAVYYCVLLTGRVYFALDSWGQGT^{LV}TVSS

VL PD1-0103-0312:

10 DIVMTQSPDSLAVSLGERATINCKASESVDTSDNSFIHWYQQKPGQSPKLLIYRSSTLESGVPDRFS
GSGSGTDFTLT^{IS}SLQAEDVAVYYCQQNYDVPWTFGQGTKVEIK

VL PD1-0103-0313:

DVVM^{TQ}SP^{LS}LPVTLGQPASISCRASESVDTSDNSFIHWYQQRPQGSPRLLIYRSSTLESGVPDRFS
GSGSGTDFTLKISRVEAEDVGVYYCQQNYDVPWTFGQGTKVEIK

15 VL PD1-0103-0314:

EIVLTQSPATLSLSPGERATLSCRASESVDTSDNSFIHWYQQKPGQSPRLLIYRSSTLESGIPARFS
GSGSGTDFTLT^{IS}SLPEPDAVYYCQQNYDVPWTFGQGTKVEIK

VL PD1-0103-0315:

20 EIVLTQSPATLSLSPGERATLSCRASESVDTSDNSFIHWYQQKPGQSPRLLIYRSSTLESGIPARFS
GSGSGTDFTLT^{IS}SLPEPDAVYYCQQNYDVPWTFGQGTKVEIK

anti-PD1 PD1-0098:

VH PD1-0098:

25 DVQLQESGPGLVKPSQSLSLTCTVTGYSITSDYAWN^{WIR}QFP^{GD}KLEWLGYITYSGFTN^{YN}PSLKSR
ISISRDT^{SK}NQFFLQLNSVATEDTATYYCARWHGSAPWYFDYWGRGTT^{LV}TVSS

VL PD1-0098:

DVLMTQTPLSLPVSLGDQASISCRSQNIVHSDGNTYLEWYLQKPGQSPNLLIYKVSRRRFS^{GP}DRF
SGSGSGTDFTLKISRVEAEDLGVYYCFQGSHFPLTFGAGTKLELK

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VH: 0050

DVQLQESGPGLVKPSQSLSLTCTVTGYSITSDYAWNWIWQFPGNKLEWMGYITYYTGRTSYNPSLKSR
 ISITRDTSKNQFFLQLNSVTTEDTATYYCAREMDYYGSTLDYWGQGTTLTVSS

5 VL: 0050

KIVLTQSPASLAVSLRQRATISCRASSESVDRYGNSFIHWYQQKPGQPPKVLIIRASNLESGFPARFS
 GSGSRTDFTLTIDPVEADDAATYYCQQNNEDPYTFGSGTKLEIK

VH: 0069

10 QVQLQQSGPELVRPGVSVKISCKGSGYTFTDYAMHWVKQSHARTLEWIGVISTYSGDTNYNQKFQDK
 ATMTVDKSSSTAYLELARMTSEDSAIYYCARLGITTGFAYWGQGTTLTVSA

VL: 0069

DIVLTQSPASLAVSLGQRATISCRASSKGVSTSSYSFMHWYQQKPRQPPKLLIKYASYLESGVPARFS
 GSGSGTDFTLNHPVEEEDAATYYCHHSREFPWTFGGGKLEIK

VH: 0073

15 EVKLVESGGGLVKPGGSLKLSCAASGFTFSNYGMSWIRQTPEKGLEWVATISGGGRDTYYPDSVKGR
 FTISRDNVKNLNLQMSLRSEDATFYCASYYYGIDYWGQGTSTVTVSS

VL: 0073

DIVMTQPHKFMSTSVGDRVRITCKASQDVTTAVAWYQQKPGQSPKLLIIWASTRHTGVPDRFTGSGS
 GTEFTLTITSSVQAEDLALYYCQQHYSIPWTFGGGKLEIK

20 VH: 0078

QVQLQQPGAELVKPGASVKMSCKASGYTFTSTWMHWVKQRPQGQLEWIGAIDPSDSYTTYNQKFQKGK
 ATLTVDTSSSTAYMQLSSLTSEDSAVYYCTRSPFDYWGQGTTLTVSS

VL: 0078

25 DIVMTQSHKFMSTSVGDRVSITCKASQDVSTAVAWYQQKPGQSPKLLIISASYRYTGVPDRFTGSGS
 GTDFTFAISSVQAEDLAVYYCQQHYSHPFTFGSGTKLEIK

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VH: 0102

DVQLQESGPD LVKPSQSLSLTCTVTGYSITSGYSWHWIRQFPGNKLEWMGFIHSSGDTNYPNPSLKSR
ISFTRDTSKNQFFLQLSSLTDEDATYYCATYRNWFYDVWGAGTTVTVSS

VL: 0102

5 DIVMTQSPSSLTVTAGEKVTMRCKSSQSLNLSGTQKNYLTWYQQKPGQPPKLLIYWASTRESGVPNR
FTGSGSGTDFTLTITSSVQAEDLSVYYCQSDYTFPLTFGGGTKLELK

In the following specific embodiments of the invention are listed:

1. An isolated antibody that binds to human PD1, wherein the antibody binds to the (core) sugar chain at Asn58 of glycosylated human PD1 of SEQ ID NO: 70 which is glycosylated at Asn58.
2. The antibody according to claim 1 wherein the antibody binds additionally to one or more amino acids of positions 60 to 64, 68, 78 to 84, 126 to 134 of human PD1.
3. The antibody according to any one of claims 1 or 2, wherein the antibody binds with its heavy chain to the the sugar chain at Asn58.
4. The antibody according to any one of claims 2 to 3, wherein the antibody binds to one or more amino acids of positions 61, 62, 64, 83, 126, 128, 132, 134 of human PD1.
5. The antibody according to any one of claims 2 to 3, wherein the antibody binds to amino acids of positions 61, 62, 64, 83, 126, 128, 132, 134 of human PD1.
6. The antibody according to any one of claims 2 to 3, wherein the antibody binds to acids of positions 60, 61, 62, 63, 64, 68, 78, 82, 83, 84, 126, 127, 128, 130, 131, 132, 133, 134 of human PD1.
7. The antibody according to any one of claims 1 to 6, wherein the antibody binds to human PD1, wherein the antibody binds to the first and second GlNAc, FUC, BMA and MAN within the (core) sugar chain at Asn58 of glycosylated human PD1 of SEQ ID NO: 70, which is glycosylated at Asn58.

8. The antibody according to any one of claims 1 to 7, wherein the antibody shows reduced binding to human PD1 of SEQ ID NO: 70 which is not glycosylated at Asn58 compared to the binding to human PD1 which is glycosylated at Asn58.
- 5 9. An isolated antibody that binds to human PD1, wherein the antibody comprises
- (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:71; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:72; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:73; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:74; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:75; (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:76, and (g) FR-H3 comprising the amino acid sequence of SEQ ID NO: 77 (of RDN) at positions of 71, 72 and 73 according to Kabat numbering
- 10 10. The isolated antibody that binds to human PD1 according to claim 9, wherein the antibody
- 15 A)
- i) comprises a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8;
- 20 ii) or humanized variant of the VH and VL of the antibody under i);
- or B)
- i) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:58.
- 25 ii) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:59.
- iii) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:60.
- iv) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:61.

In the following specific embodiments of the invention are listed:

1. An isolated antibody that binds to human PD1, wherein the antibody comprises

- 5 A) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; or
- 10 B) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:9; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:10; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:11; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:13; and (f) HVR-L3
- 15 comprising the amino acid sequence of SEQ ID NO:14; or
- C) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:19; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:20; (e) HVR-L2
- 20 comprising the amino acid sequence of SEQ ID NO:21; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22; or
- D) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27; (d) HVR-L1
- 25 comprising the amino acid sequence of SEQ ID NO:28; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:29; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30; or
- E) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:33; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:34; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:35; (d) HVR-L1
- 30 comprising the amino acid sequence of SEQ ID NO:36; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:37; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:38; or

- 5 F) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:41; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:42; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:43; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:44; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:45; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:46; or
- 10 G) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:49; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:50; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:51; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:52; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:53; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:54.

2. An isolated antibody that binds to human PD1, wherein the antibody comprises

- 15 A) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:3; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; or
- 20 B) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:9, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:10, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:11; and (b) a VL domain comprising
- 25 (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:13 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:14; or
- 30 C) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:19; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:21 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22; or.

- 5 D) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:27; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:28; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:29 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30; or
- 10 E) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:33, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:34, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:35; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:36; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:37 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:38; or
- 15 F) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:41, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:42, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:43; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:44; (ii) 20 HVR-L2 comprising the amino acid sequence of SEQ ID NO:45 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:46; or
- 25 G) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:51; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:52; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:53 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:54.

3. An isolated antibody that binds to human PD1, wherein the antibody

- 30 A)
- i) comprises a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8;

ii) or humanized variant of the VH and VL of the antibody under i);

or B)

i) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:58.

5 ii) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:59.

iii) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:60.

10 iv) comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:61.

or C)

i) comprises a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:16;

ii) or humanized variant of the VH and VL of the antibody under i);

15 or D)

i) comprises a VH sequence of SEQ ID NO:23 and a VL sequence of SEQ ID NO:24;

ii) or humanized variant of the VH and VL of the antibody under i);

or E)

20 i) comprises a VH sequence of SEQ ID NO:31 and a VL sequence of SEQ ID NO:32;

ii) or humanized variant of the VH and VL of the antibody under i);

or F)

25 i) comprises a VH sequence of SEQ ID NO:39 and a VL sequence of SEQ ID NO:40;

ii) or humanized variant of the VH and VL of the antibody under i);

or G)

- i) comprises a VH sequence of SEQ ID NO:47 and a VL sequence of SEQ ID NO:48;
- ii) or humanized variant of the VH and VL of the antibody under i);

5 or H)

- i) comprises a VH sequence of SEQ ID NO:55 and a VL sequence of SEQ ID NO:56;
- ii) or humanized variant of the VH and VL of the antibody under i).

4. An isolated antibody that binds to human PD1, wherein the antibody

- 10 i) comprises a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8;
- ii) or humanized variant of the VH and VL of the antibody under i);

5. An isolated antibody that binds to human PD1, wherein the antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:58.

15 6. An isolated antibody that binds to human PD1, wherein the antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:59.

7. An isolated antibody that binds to human PD1, wherein the antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:60.

20 8. An isolated antibody that binds to human PD1, wherein the antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:61.

9. The anti-PD1 antibody according to any one of the preceding embodiments

wherein the antibody is characterized independently by one or more of the following properties: the anti-PD-1 antibody

- 25 i) competes for binding to PD-1 with an anti-PD-1 antibody comprising the VH with the amino acid sequence of SEQ ID NO:7 and VL with the amino acid sequence of SEQ ID NO:8, and/ or

- ii) binds to a human and cynomolgus PD-1; and/ or
 - iii) enhances the interferon-gamma (IFN-gamma) secretion by allogenic stimulated T cells by 85% or more at an antibody concentration of 10µg/ml ; and/ or
- 5 iv) enhances the tumor necrosis factor alpha (TNF alpha) secretion by allogenic stimulated T cells by 200% or more at an antibody concentration of 10µg/ml.
- 10. An isolated antibody that binds to PD1, wherein the antibody enhances the tumor necrosis factor alpha (TNF alpha) secretion by allogenic stimulated T
- 10 cells by 200% or more (in one preferred embodiment by 250% or more) at an antibody concentration of 10µg/ml in a Mixed lymphocyte reaction (MLR) assay.
- 11. An isolated antibody that binds to PD1, wherein the antibody enhances the interferon-gamma (IFN-gamma) secretion by allogenic stimulated T cells
- 15 by 85% or more (in one preferred embodiment by 90% or more, in one preferred embodiment by 95% or more) at an antibody concentration of 10µg/ml in a Mixed lymphocyte reaction (MLR) assay.
- 12. An isolated antibody that binds to human PD-1, wherein the antibody:
 - 20 i) competes for binding to PD-1 with an anti-PD1 antibody comprising the VH with the amino acid sequence of SEQ ID NO:7 and VL with the amino acid sequence of SEQ ID NO:8, and/ or
 - ii) binds to a human and cynomolgus PD-1; and
 - iii) enhances the interferon-gamma (IFN-gamma) secretion by allogenic stimulated T cells by 85% or more at an antibody concentration of 10µg/ml; and
 - 25 iv) enhances the tumor necrosis factor alpha (TNF alpha) secretion by allogenic stimulated T cells by 200% or more at an antibody concentration of 10µg/ml.
- 13. The antibody of any of the preceding embodiments, which is a monoclonal
- 30 antibody.

14. The antibody according to any of the preceding embodiments, which is a human, humanized, or chimeric antibody.
15. The antibody according to any of the preceding embodiments, which is an antibody fragment that binds to PD1.
- 5 16. The antibody according to any one of the preceding embodiments, which is a full length IgG1 antibody.
17. The antibody of according to any one of the preceding embodiments, which is a full length IgG1 antibody with mutations L234A, L235A and P329G (numbering according to the EU index of Kabat).
- 10 18. Isolated nucleic acid encoding the antibody according to any one of the preceding embodiments.
19. A host cell comprising the nucleic acid of embodiment 19.
20. A method of producing an antibody comprising culturing the host cell of embodiment 20 so that the antibody is produced.
- 15 21. The method of embodiment 21, further comprising recovering the antibody from the host cell.
22. A pharmaceutical formulation comprising the antibody according any one of embodiments 1 to 18 and a pharmaceutically acceptable carrier.
- 20 23. The antibody according any one of embodiments 1 to 18 for use as a medicament.
24. The antibody according any one of embodiments 1 to 18 for use in treating cancer.
25. Use of the antibody according any one of embodiments 1 to 18 in the manufacture of a medicament.
- 25 26. The use of embodiment 26, wherein the medicament is for treatment of cancer.
27. A method of treating an individual having cancer comprising administering to the individual an effective amount of the antibody of embodiment 1.

III. EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

5 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

10 **Example 1:**
Generation of anti-PD-1 antibodies

Immunization of mice

15 NMRI mice were immunized genetically, using a plasmid expression vector coding for full-length human PD-1 by intradermal application of 100 ug vector DNA (plasmid15300_hPD1-fl), followed by Electroporation (2 square pulses of 1000 V/cm, duration 0.1 ms, interval 0.125 s; followed by 4 square pulses of 287.5 V/cm, duration 10 ms, interval 0.125 s. Mice received either 6 consecutive immunizations at days 0, 14, 28, 42, 56, 70, and 84. Blood was taken at days 36, 78 and 92 and serum prepared, which was used for titer determination by ELISA (see
20 below). Animals with highest titers were selected for boosting at day 96, by intravenous injection of 50 ug of recombinant human PD1 human Fc chimera, and monoclonal antibodies were isolated by hybridoma technology, by fusion of splenocytes to myeloma cell line 3 days after boost. Determination of serum titers (ELISA).

25 Human recombinant PD1 human Fc chimera was immobilized on a 96-well NUNC Maxisorp plate at 0.3 ug/ml, 100 ul/well, in PBS, followed by: blocking of the plate with 2% Crotein C in PBS, 200 ul/well; application of serial dilutions of antisera, in duplicates, in 0.5% Crotein C in PBS, 100 ul/well; detection with HRP-conjugated goat anti-mouse antibody (Jackson Immunoresearch/Dianova 115-036-
30 071; 1/16 000). For all steps, plates were incubated for 1 h at 37⁰ C. Between all steps, plates were washed 3 times with 0.05% Tween 20 in PBS. Signal was developed by addition of BM Blue POD Substrate soluble (Roche), 100 ul/well; and stopped by addition of 1 M HCl, 100 ul/well. Absorbance was read out at

450 nm, against 690 nm as reference. Titer was defined as dilution of antisera resulting in half-maximal signal.

Example 2:

Characterization anti-PD1 antibodies

5 Binding of anti-PD1 antibodies to human PD1

ELISA for hu PD1

Nunc maxisorp streptavidin coated plates (MicroCoat #11974998001) were coated with 25 µl/well biotinylated PD1-ECD-AviHis and incubated at 4°C over night. After washing (3x90 µl/well with PBST-buffer) 25 µl anti PD1 samples or
 10 reference antibodies (human anti PD1; Roche/mouse anti PD1; Biolegend; cat.:329912) were added and incubated 1h at RT. After washing (3x90 µl/well with PBST-buffer) 25µl/well goat-anti-human H+L-POD (JIR, JIR109-036-088)/Sheep-anti-mouse-POD (GE Healthcare; NA9310) was added in 1:2000/1:1000 dilution and incubated at RT for 1 h on shaker. After washing (3x90 µl/well with
 15 PBST-buffer) 25 µl/well TMB substrate (Roche Catalogue No. 11835033001) was added and incubated until OD 2 – 3. Measurement took place at 370/492 nm.

ELISA results are listed as EC50-values [ng/ml] in summary Tables 2 and 3 below.

Cell ELISA for PD1

Adherent CHO-K1 cell line stably transfected with plasmid 15311_hPD1-fl_pUC_Neo coding for full-length human PD1 and selection with G418
 20 (Neomycin resistance marker on plasmid) were seeded at a concentration of 0.01x10E6 cells/well in 384-well flat bottom plates and grown over night.

The next day 25 µl/well PD1 sample or human anti PD1 (Roche)/mouse anti PD1(Biolegend; cat.:329912) reference antibody were added and incubated for 2h
 25 at 4°C (to avoid internalization). After washing carefully (1x90µl/well PBST) cells were fixed by adding 30µl/well 0,05% Glutaraldehyde (Sigma, Cat.No: G5882, 25%)diluted in 1xPBS-buffer and incubated for 10min at RT. After washing (3x90µl/well PBST) 25 µl/well secondary antibody was added for detection: goat-anti-human H+L-POD (JIR, JIR109-036-088)/Sheep-anti-mouse-POD (GE
 30 NA9310) followed by 1h incubation at RT on shaker. After washing (3x90µl/well PBST) 25 µl/well TMB substrate solution (Roche 11835033001) was added and incubated until OD 1.0 – 2.0. Plates were measured at 370/492 nm.

Cell ELISA results are listed as “EC50 CHO-PD1”-values [ng/ml] in summary table Table 3 below.

ELISA for cyno PD1

5 Nunc maxisorp streptavidin coated plates (MicroCoat #11974998001) were coated with 25 µl/well biotinylated cynoPD1-ECD-Biotin and incubated at 4°C over night. After washing (3x90 µl/well with PBST-buffer) 25 µl anti PD1 samples or reference antibodies (human anti PD1; Roche) were added and incubated 1h at RT on shaker. After washing (3x90 µl/well with PBST-buffer) 25µl/well goat-anti-human H+L-POD (JIR, JIR109-036-088) was added in 1:1000 dilution and
10 incubated at RT for 1 h on shaker. After washing (3x90 µl/well with PBST-buffer) 25 µl/well TMB substrate (Roche, 11835033001) was added and incubated until OD 2 – 3. Measurement took place at 370/492 nm.

ELISA results are listed as EC50-values [ng/ml] in summary Table 2 and 3 below.

PD Ligand 1 replacing assay

15 Nunc maxisorp streptavidin coated plates (MicroCoat #11974998001) were coated with 25 µl/well biotinylated PD1-ECD-AviHis and incubated at 4°C over night. After washing (3x90 µl/well with PBST-buffer) 25 µl anti PD1 samples or reference antibodies (mouse anti PD1; Biolegend; cat.:329912) were added and incubated 1h at RT on shaker. After washing (3x90 µl/well with PBST-buffer)
20 25µl/well PD-L1 (Recombinant human B7-H1/PD-L1 Fc Chimera; 156-B7, R&D) was added and incubated 1h at RT on shaker. After washing (3x90 µl/well with PBST-buffer) 25µl/well goat-anti-human H+L-POD (JIR, 109-036-088) was added in 1:1000 dilution and incubated at RT for 1 h on shaker. After washing (3x90 µl/well with PBST-buffer) 25 µl/well TMB substrate (Roche, 11835033001) was
25 added and incubated until OD 2 – 3. Measurement took place at 370/492 nm.

ELISA results are listed as IC50-values [ng/ml] in summary Table 2 below.

PD Ligand 2 replacing assay

30 Nunc maxisorp streptavidin coated plates (MicroCoat #11974998001) were coated with 25 µl/well biotinylated PD1-ECD-AviHis and incubated at 4°C over night. After washing (3x90 µl/well with PBST-buffer) 25 µl anti PD1 samples or reference antibodies (mouse anti huPD1; Roche) were added and incubated 1h at RT on shaker. After washing (3x90 µl/well with PBST-buffer) 25µl/well PD-L2

(Recombinant human B7-DC/PD-L2 Fc Chimera; 1224-PL-100, R&D) was added and incubated 1h at RT on shaker. After washing (3x90 µl/well with PBST-buffer) 25µl/well goat-anti-human H+L-POD (JIR, 109-036-088) was added in 1:2000 dilution and incubated at RT for 1 h on shaker. After washing (3x90 µl/well with PBST-buffer) 25 µl/well TMB substrate (Roche, 11835033001) was added and incubated until OD 2 – 3. Measurement took place at 370/492 nm.

ELISA results are listed as IC50-values [ng/ml] in summary Table 2 below.

Epitope mapping ELISA/ Binding competition assay

Nunc maxisorp plates (Nunc #464718) were coated with 25µl/well capture antibody (goat anti mouse IgG; JIR; 115-006-071) and incubated for 1h at RT on shaker. After washing (3x90µl/well with PBST-buffer) plates were blocked for 1h with 2% BSA containing PBS buffer at RT on shaker. After washing (3x90µl/well with PBST-buffer) 25µl mouse anti PD1 samples were added and incubated 1h at RT on shaker. After washing (3x90µl/well with PBST-buffer) capture antibody was blocked by 30µl/well mouse IgG (JIR; 015-000-003) for 1h at RT on shaker. At the same time biotinylated PD1-ECD-AviHis was preincubated with second sample antibody for 1h at RT on shaker. After washing assay plate (3x90 µl/well with PBST-buffer) the PD1 antibody mix was transferred to assay plate and incubated at RT for 1h on shaker. After washing (3x90 µl/well with PBST-buffer) 25µl/well streptavidin POD (Roche, #11089153001) was added in 1:4000 dilution and incubated at RT for 1 h on shaker. After washing (3x90 µl/well with PBST-buffer) 25 µl/well TMB substrate (Roche, #11089153001) was added and incubated until OD 1.5 – 2.5. Measurement took place at 370/492 nm. Epitope groups were defined by hierarchical clustering against reference antibodies.

Table 2: Binding, PD-L1 inhibition and epitope region groups of exemplary antibodies (ELISA)

Antibody	ELISA huPD1 EC50 [ng/ml]	ELISA cyPD1 EC50 [ng/ml]	ELISA PD-L1 inhibition IC50 [ng/ml]	ELISA PD- L2 inhibition IC50 [ng/ml]	Epitope region group By competition assay)
PD1- 0050	17.9	9.8	128	34	1
PD1- 0069	45.7	22.7	225	89	6
PD1- 0073	15.1	8.3	124	65	5
PD1- 0078	26.3	22.4	x	86	2
PD1- 0098	50.8	54.6	174	45	5
PD1- 0102	34.2	52.7	>35.5 µg/ml	140	4
PD1-0103	33.7	36.9	182	51	5

Table 3: Biochemical- and Cell-binding of humanized PD1 antibodies derived from parental mouse antibody PD1-0103 (ELISA).

5

Humanize d antibody	ELISA huPD1 EC50 [ng/ml]	ELISA cyPD1 EC50 [ng/ml]	ELISA CHO- PD1 EC50 [ng/ml]
PD1-0103- 0312	11	8.3	10.1
PD1-0103- 0313	15	11	10.8
PD1-0103- 0314	11	8.3	7.7
PD1-0103- 0315	10	7.9	7.3

Biacore characterization of the humanized anti-PD-1 antibodies

A surface plasmon resonance (SPR) based assay has been used to determine the kinetic parameters of the binding between several murine PD1 binders as well as commercial human PD1 binding references. Therefore, an anti-human IgG was immobilized by amine coupling to the surface of a (Biacore) CM5 sensor chip. The samples were then captured and hu PD1-ECD was bound to them. The sensor chip surface was regenerated after each analysis cycle. The equilibrium constant and kinetic rate constants were finally gained by fitting the data to a 1:1 langmuir interaction model.

About 2000 response units (RU) of 20 µg/ml anti-human IgG (GE Healthcare #BR-1008-39) were coupled onto the flow cells 1 and 2 (alternatively: 3 and 4) of a CM5 sensor chip in a Biacore T200 at pH 5.0 by using an amine coupling kit supplied by GE Healthcare.

The sample and running buffer was HBS-EP+ (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.05 % v/v Surfactant P20, pH 7.4). Flow cell temperature was set to 25 °C and sample compartment temperature to 12 °C. The system was primed with running buffer.

The samples were injected for 20 seconds with a concentration of 10 nM and bound to the second flow cell. Then a complete set of human PD1-ECD concentrations (144 nM, 48 nM, 16 nM, 5.33 nM, 1.78 nM, 0.59 nM, 0.20 nM and 0 nM) was injected over each sample for 120s followed by a dissociation time of 30/300s and two 20s regeneration steps with 3 M MgCl₂, of which the last one contained an “extra wash after injection” with running buffer.

Finally the double referenced data was fitted to a 1:1 langmuir interaction model with the Biacore T200 Evaluation Software. Resulting K_D , k_a and k_d values are shown in Table 4.

Table 4: Kinetic rate constants and equilibrium constant for chimeric PD1-0103 and humanized PD1-Abs determined by Biacore (see next page).

Ligand	$k_a [M^{-1} s^{-1}]$	$k_d [s^{-1}]$	$K_D [nM]$
chimeric PD1-0103	3.86E+05	3.07E-04	0.8
PD1-0103-0312	1.95E+05	3.45E-04	1.8
PD1-0103-0313	1.60E+05	3.67E-04	2.3
PD1-0103-0314	1.87E+05	2.79E-04	1.5
PD1-0103-0315	1.89E+05	2.91E-04	1.5

As shown in Table 4, all the humanized versions of chimeric PD1-0103 (generation see Example 6) display kinetic properties similar to the parental antibody (chimeric PD1-0103).

Kinetics

5 A CM5 sensor series S was mounted into the Biacore 4000 System and the detection spots were hydrodynamically addressed according to the manufacturer's instructions.

10 The polyclonal rabbit IgG antibody <IgGFC γ M>R (Jackson ImmunoResearch Laboratories Inc.) was immobilized at 10 000 Ru on the detection spots 1 and 5 in the flow cells 1,2,3 and 4. Coupling was done via EDC/NHS chemistry according to the manufacturer's instructions. The remaining spots in the flow cells served as a reference. The sample buffer was the system buffer supplemented with 1 mg/ml carboxymethyl dextrane.

15 In one embodiment the assay was driven at 25 °C. In another embodiment the assay was driven at 37 °C. 50 nM of each murine monoclonal antibody was captured on the sensor surface by a 1 min injection at 10 μ l/min. Subsequently the respective antigens were injected in a concentration series of 100 nM, 2x 33 nM, 11 nM, 4 nM, 1 nM and system buffer 0 nM at 30 μ l/min for 4 min association phase time. The dissociation was monitored for another 4 min. The capture system was
20 regenerated using a 3 min injection of 10 mM glycine pH 1.5 at 30 μ l/min. Relevant kinetic data was calculated using the Biacore evaluation software according to the manufacturer's instructions.

Epitope Mapping

25 A Biacore 4000 instrument was mounted with a Biacore CAP sensor and was prepared like recommended by the manufacturer. The instrument buffer was HBS-ET (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% w/v Tween 20). The instrument was running at 25 °C.

All samples were diluted in system buffer. A 35kDa biotinylated antigen PD1-ECD-AviHis was captured at 200 RU on the CAP sensor surface by a 1 min injection at 30µl/min in the flow cells 1, 2, 3 and 4 in the spots 1 and 5. Spots 2, 3 and 4 served as a reference. In another embodiment, a 35 kDa biotinylated antigen PD1-ECD-AviHis was captured at 200 RU on the CAP sensor in the same manner.

Subsequently a primary antibody was injected at 100 nM for 3 min at 30 µl/min followed by the injection of a secondary antibody at 100 nM for 3 min at 30 µl/min. The primary antibody was injected until full saturation of the surface presented antigen. At the end of the primary and secondary antibody injection phases report points "Binding Late" (BL) were set to monitor the binding response of the respective antibodies. The Molar Ratio, a quotient between the secondary antibody binding response "BL2" and the primary antibody response "BL1" was calculated. The Molar Ratio was used as an indicator of the antigen accessibility of the secondary antibody, when the antigen was already complexed by the primary antibody.

The complexes were completely removed from the sensor surface by an injection for 2 min at 30µl/min 2M guanidine-HCL 250 mM NaOH regeneration buffer as recommended by the manufacturer, followed by a 1 min injection at 30µl /min of system buffer.

Example 3: Effect of different anti-PD-1 Antibodies on Cytokine Production in a Mixed Lymphocyte Reaction (MLR)

3A) The Mixed Lymphocyte Reaction (MLR) is a immune cell assay which measures the activation of lymphocytes from one individual (donor X) to lymphocytes from another individual (donor Y). A mixed lymphocyte reaction was used to demonstrate the effect of blocking the PD1 pathway to lymphocyte effector cells. T cells in the assay were tested for activation and their IFN-gamma secretion in the presence or absence of an anti-PD1 mAbs.

To perform an allogeneic MLR, peripheral blood mononuclear cells (PBMCs) from at least four healthy donors of unknown HLA type were isolated by density gradient centrifugation using Leukosep (Greiner Bio One, 227 288). Briefly, heparinized blood samples were diluted with the three fold volume of PBS and 25 ml aliquots of the diluted blood were layered in 50 ml Leukosep tubes. After centrifugation at 800 x g for 15 min at room temperature (w/o break) the

lymphocyte containing fractions were harvested, washed in PBS and used directly in functional assay or resuspended in freezing medium (10% DMSO, 90 %FCS) at 1.0E+07 cells/ml and stored in liquid nitrogen. Individual 2-way MLR reactions were set up by mixing PBMCs from two different donors at a 1:1 stimulator/responder cell ratio and co-cultures were done at least in duplicate in flat-bottomed 96-well plates for 6 days at 37°C, 5% CO₂, in the presence or w/o of a different concentration range of purified anti-PD1 monoclonal antibodies PD1-0050, PD1-0069, PD1-0073, PD1-0078, PD1-0098, PD1-0102, PD1-0103. As reference anti-PD1 antibodies, antibodies comprising the VH and VL domains of either nivolumab (also known as MDX-5C4 or MDX-1106) or pembrolizumab (also known as MK-3475 or Org 1.09A) were synthesized and cloned with backbones of human IgG1 (with mutations L234A, L235A and P329G (EU index of Kabat)). Either no antibody or an isotype control antibody was used as a negative control and rec hu IL-2 (20 EU/ml) was used as positive control. After day 6 100 µl of medium was taken from each culture for cytokine measurement. The levels of IFN-gamma were measured using OptEIA ELISA kit (BD Biosciences).

The results are shown in Table 5 (IFN-g secretion/release). The anti-PD1 monoclonal antibodies promoted T cell activation and IFN-gamma secretion in concentration dependent manner. The value of % increase of IFN-g secretion was calculated in relation to IFN-g production of MLR w/o adding of any blocking mAbs (basal allogeneic stimulation induced IFN-g value as E-c) and MLR with adding of 20 EU/ml rec hu IL-2 (positive control = 100% IFN-g value as E+c) and was calculated according to formula: Rel.Stimulation [%] = ((Esampel - E-c)/(E+c - E-c))*100

Table 5: Percentage of of IFN gamma secretion after allogenic stimulation and treatment with anti-PD-1 antibody in comparison to effect of recombinant human IL-2 treatment (20 EU/ml) (= 100% increase) as positive control

	Concentration (µg/ml)	1:12	1:120	1:1200	Effect in MLR
PD1-0050	44	136	96	33	+++
PD1-0069	60	76	71	55	+++
PD1-0073	43	103	63	38	++
PD1-0078	64	99	72	21	++

Several PD1 blocking antibodies PD1-0050, PD1-0069, PD1-0073, PD1-0078, PD1-0098, PD1-0102, PD1-0103 demonstrated strong immune modulating activity

by enhancing secretion of interferon gamma (IFN-g) (data not shown for all antibodies).

5 **3B)** In a further experiment chimeric PD1-0103 (human IgG1 isotype with mutations L234A, L235A and P329G (EU index of Kabat)) was evaluated. Blockade of PD1 with chimeric PD1-0103 strongly enhances IFN-gamma secretion by allogenic stimulated primary human T cells. Chimeric PD1-0103 is more potent than reference anti-PD1 antibodies (see Figure 1).

10 For comparison the reference anti-PD1 antibodies comprising the VH and VL domains of either nivolumab (also known as MDX5C4 or MDX-1106) and pembrolizumab (also known as MK-3475 or Org 1.09A) were synthesized and cloned with backbones of human IgG1 (with mutations L234A, L235A and P329G (EU index of Kabat)) were used.

15 **3C)** In additional experiments the immune modulating activity of the humanized variants of anti-PD-1 antibody PD1-0103 (humanized antibodies PD1-0103-0312, , PD1-0103-0314, in figures 2 and 3, see also Example 6 below) the a) IFNrelease (secretion) b) TNF-alpha release (secretion) was evaluated in MLR as described above. The effect of the chimeric PD1-0103 antibody and its humanized versions were compared to the reference anti-PD1 antibodies comprising the VH and VL domains of either nivolumab (also known as MDX5C4 or MDX-1106) and
20 pembrolizumab (also known as MK-3475 or Org 1.09A) with backbones of human IgG1 (with mutations L234A, L235A and P329G (EU index of Kabat)). After 6 days of MLR culture 50 µl of supernatant was taken and multiple cytokines were measured in a single culture using Bio-Plex Pro™ Human Cytokine Th1/Th2 Assay (Bio-Rad Laboratories Inc.). (data not shown for all cytokines).

25 The chimeric PD1-0103 antibody and its humanized versions (PD1-0103_0312 and PD1-0103_0314) were more potent compared to the reference anti-PD1 antibodies in enhancing the T cell activation and IFN-gamma secretion (see Figure 2).

30 Further the chimeric PD1-0103 antibody and its humanization variants increase tumor necrosis factor alpha (TNF alpha) (see Figure 3) and IL-12 (data not shown) secretion by antigen presenting cells and enhance capacity of monocytes /macrophages or antigen presenting cells to stimulate a T cell.

Example 4:**Effect of anti-PD-1 blockade on cytotoxic Granzyme B release and IFN- γ secretion by human CD4 T cells cocultured with allogeneic mature dendritic cells**

5 To further investigate the effect of anti-PD-1 treatment in an allogeneic setting we developed an assay in which freshly purified CD4 T cells are cocultured for 5 days in presence of monocyte-derived allogeneic mature dendritic cells (mDCs). Monocytes were isolated from fresh PBMCs one week before through plastic adherence followed by the removal of the non-adherent cells. We then generated
10 immature DCs from the monocytes by culturing them for 5 days in media containing GM-CSF (50 ng/ml) and IL-4 (100 ng/ml). To induce iDCs maturation, we added TNF-alpha, IL-1beta and IL-6 (50 ng/ml each) to the culturing media for 2 additional days. We then assessed DCs maturation by measuring their surface expression of Major Histocompatibility Complex Class II (MHCII), CD80, CD83
15 and CD86 thorough flow cytometry (LSRFortessa, BD Biosciences).

On the day of the minimal mixed lymphocyte reaction (mMLR), CD4 T cells were enriched via a microbead kit (Miltenyi Biotec) from 108 PBMCs obtained from an unrelated donor. Prior culture, CD4 T cells were labeled with 5 μ M of Carboxy-Fluorescein-Succinimidyl Esther (CFSE). 105 CD4 T cells were then plated in a 96
20 well plate together with mature allo-DCs (5:1) in presence or absence of blocking anti-PD1 antibody (either PD1-0103, chimeric PD1-0103, or humanized antibodies PD1-0103-0312, PD1-0103-0313, PD1-0103-0314, PD1-0103-0315, abbreviated as 0312, 0313, 0314, 0315 in figures 4A and 4 B), at the concentration of 10 μ g/ml if not differently indicated in the figures.

25 Five days later we collected the cell-culture supernatants, used later to measure the IFN-gamma levels by ELISA (R&D systems), and left the cells at 37 C degrees for additional 5 hours in presence of Golgi Plug (Brefeldin A) and Golgi Stop (Monensin). The cells were then washed, stained on the surface with anti-human CD4 antibody and the Live/Dead fixable dye Aqua (Invitrogen) before being
30 fixed/permeabilized with Fix/Perm Buffer (BD Bioscience). We performed intracellular staining for Granzyme B (BD Bioscience), IFN-gamma and IL-2 (both from eBioscience). Results are shown in Figures 4A and 4 B.

We also tested different concentrations of the humanized variants PD1-0103 (humanized antibodies PD1-0103-0312, PD1-0103-0313, PD1-0103-0314, PD1-

0103-0315, abbreviated as 0312, 0313, 0314, 0315 in the figures, see also Example 6 below) and found them to be equally good in enhancing granzyme B and interferon gamma. DP47 is a non binding human IgG with a LALA mutation in the Fc portion to avoid recognition by FcgammaR and was used as negative control.

5 **Example 5:**

Chimeric antibodies derivatives

Chimeric PD1 antibodies were generated by amplifying the variable heavy and light chain regions of the anti-PD1 mouse antibodies PD1-0098, PD1-0103 via PCR and cloning them into heavy chain expression vectors as fusion proteins with human IgG1 backbones / human CH1-Hinge-CH2-CH3 with mutations L234A, L235A and P329G (EU index of Kabat)) (Leucine 234 to Alanine, Leucine 235 to Alanine, Proline 329 to Glycine) abrogating effector functions and light chain expression vectors as fusion proteins to human C-kappa. LC and HC Plasmids were then cotransfected into HEK293 and purified after 7 days from supernatants by standard methods for antibody purification. The chimeric PD1-antibodies were renamed chimeric chiPD1-0098 (chiPD1-0098) and chimeric PD1-0103 (chiPD1-0103). For comparison the reference anti-PD1 antibodies comprising the VH and VL domains of either nivolumab (also known as MDX-5C4 or MDX-1106) and pembrolizumab (also known as MK-3475 or Org 1.09A) were synthesized and cloned with backbones of human IgG1 (with mutations L234A, L235A and P329G (EU index of Kabat)) were used.

Example 6:

Generation, Expression and Purification of humanized variants of anti-PD1 antibody PD-0103 (huMab PD-0103) and characterization

25 Humanization of the VH and VL domains of murine anti-PD1 antibody 0103

Based upon the amino acid sequence of the murine VH and VL domains of murine anti-PD1 antibody 0103 (SEQ ID NO: 7 and 8), humanized anti- anti-PD1 antibody variants were generated.

30 The humanized VH-variant is based on the human germline IMGT_hVH_3_23 in combination with the human J-element germline IGHJ5-01 with several mutations. (resulting in SEQ ID NO: 57).

The humanized variants of VL are based on the human germlines IMGT_hVK_4_1, IMGT_hVK_2_30, IMGT_hVK_3_11 and IMGT_hVK_1_39 in combination with the human J-element germline IGKJ1-01. Different mutations resulted in humanized variants of SEQ ID NO: 58 to SEQ ID NO: 61.

- 5 The humanized amino acid sequences for heavy and light chain variable regions of PD1-0103 were backtranslated in to DNA and the resulting cDNA were synthesized (GenArt) and then cloned into heavy chain expression vectors as fusion proteins with human IgG1 backbones /human CH1-Hinge-CH2-CH3 with LALA and PG mutations (Leucine 234 to Alanine, Leucine 235 to Alanine, Proline 329 to
- 10 Glycine) abrogating effector functions or into light chain expression vectors as fusion proteins to human C-kappa. LC and HC Plasmids were then cotransfected into HEK293 and purified after 7 days from supernatants by standard methods for antibody purification. The resulting humanized PD1-antibodies named as follows:

Table 6: VH and VL sequences of humanized variant antibodies of PD1-0103

Humanized antibodies of PD1-0103	humanized variant of VH/SEQ ID NO:	humanized variant of VL/SEQ ID NO:
PD1-0103-0312	SEQ ID NO: 57	SEQ ID NO: 58
PD1-0103-0313	SEQ ID NO: 57	SEQ ID NO: 59
PD1-0103-0314	SEQ ID NO: 57	SEQ ID NO: 60
PD1-0103-0315	SEQ ID NO: 57	SEQ ID NO: 61

15 **Table 7: HVR sequences of humanized variant antibodies of PD1-0103**

Humanized antibodies of PD1-0103	HVR-H1, HVR-H2, and HVR-H3 of humanized variant/SEQ ID NO:	HVR-L1, HVR-L2, and HVR-L3 of humanized variant/SEQ ID NO:
PD-0103-0312	SEQ ID NOs: 1 , 2 and 3	SEQ ID NOs: 4 , 5 and 6
PD-0103-0313	SEQ ID NOs: 1 , 2 and 3	SEQ ID NOs: 4 , 5 and 6
PD-0103-0314	SEQ ID NOs: 1 , 2 and 3	SEQ ID NOs: 4 , 5 and 6
PD-0103-0315	SEQ ID NOs: 1 , 2 and 3	SEQ ID NOs: 4 , 5 and 6

Humanized PD1-0103 antibody variants and parental chimeric PD1-0103 were characterized as described above. Results are shown in Table 8.

Table 8: Summary of results for humanized PD1-0103 antibody variants and parental chimeric PD1-0103

Assay	chimeric PD1-0103	PD-0103- 0312	PD-0103- 0313	PD-0103- 0314	PD-0103- 0315
Affinity K_D 37°C [nM] *)	2.0 / 0.8	1.5 / 1.8	1.9 / 2.3	1.6 / 1.5	1.7 / 1.5
ELISA EC50 [nM]	0,2	0,1	0,07	0,07	0,06
CHO-PD1 EC50	+	+	+	+	+
IC50 PD-L1, 2 [nM]	1,35	tbd	tbd	tbd	tbd
Mixed Lymphocyte Reaction assay	+++	+++	+++	++++	++
cynomolgus crossreactivity (EC50 [nm])	+	0,08	0,06	0,05	0,04

5

**Example 7:
Neutralizing potency PD-1 antibodies**

To test the neutralizing potency of inhouse generated PD-1 antibodies in mimicking a restoration of a suppressed T cell response in vitro a commercially available PD1/PD-L1 reporter assay (Promega) was used. This system consists of PD1+ NFAT Jurkat cells and a PD-L1+ CHO counterpart, which also gives the

10

activation signal. In principle, the reporter system is based on three steps: (1) TCR-mediated NFAT activation, (2) inhibition of NFAT signal upon activation by the PD-1/PD-L1 axis and (3) recovery of the NFAT signal by PD-1 blocking antibodies.

5 Material and Methods

- PD-L1 Medium: PAN Biotech (#P04-03609); FBS (10%) and L-Gln (4mM)
- Assay Medium: RPMI 1640 (#31870; Invitrogen), 25mM HEPES, 2mM L-Gln, FBS (2%)
- 10 • Cells used for this assay (both cell types purchased by Promega):

PD-L1+ CHO cells (batch no. #139147): 2-3x10⁴ cells/96well

PD-1+ NFAT Jurkat cells (batch no. #133024): 3.5x10⁴ cells/well

On day 1, PD-L1+ cells were thawed, seeded at the indicated cell concentration in the above mentioned medium and cultured over night at 37°C and 5% CO₂. On
15 the next day, medium was removed and PD-L1+ cells were incubated with the prepared antibodies at indicated concentrations (in Assay Medium). In parallel, PD-1+ NFAT Jurkat cells were thawed and above mentioned cell numbers were transferred to and co-cultured with the PD-L1+ cells. After an incubation of 6 hrs at 37°C and 5% CO₂, Bio-Glo substrate was warmed to room temperature (1-2 hrs
20 prior addition). The cell culture plate was removed from the incubator and adjusted to room temperature (10min) before 80µl Bio-Glo solution was added per well, incubated for 5-10 min before the luminescence was measured at a Tecan Infinite reader according to the kit's manufacturer's recommendation. Results can be seen in the Figures 5A and 5 B where the restoration of a PD-1/PD-L1 mediated
25 suppression of the NFAT signal by different PD-1 antibodies upon TCR stimulation is shown: Figure 5 A: Chimeric PD1_0103 showed a reproducibly superior effect when compared to a reference antibody. As reference an anti-PD1 antibody comprising the VH and VL domains nivolumab (also known as MDX-5C4 or MDX-1106) was synthesized and cloned with backbones of human IgG1 (with mutations L234A, L235A and P329G (EU index of Kabat)). Figure 5B: The
30 four humanized variants of PD1_0103 demonstrated a similar in vitro potency to the lead antibody and were also slightly superior to the reference antibody.

Example 8:**Crystallization of Fab PD1-0103 with PD-1 ectodomain:**

For complex formation Fab PD1-0103 was mixed in a 1.1 molar excess with the PD-1 ectodomain. After incubation on ice for 1 hour the complex was
5 deglycosylated by a PNGase step to remove glycans which are not involved in complex formation. Crystallization screening for complex crystals of Fab fragment PD1-0103 (with human CH1 and CL) with the PD-1 ECD was performed at a concentration of 15mg/ml. Crystallization droplets were set up at 21 °C by mixing
10 0.1 µl of protein solution with 0.1 µl reservoir solution in vapor diffusion sitting drop experiments. Crystals appeared out of various conditions containing PEG as precipitating agent. Crystals used to determine the structure appeared within 4 days out of 30% PEG1500 and grew to final size of 0.03x0.06x0.02 µm within 7 days.

Crystals were transferred into reservoir solution supplemented with 20% Glycerol as cryoprotectant and then flash-cooled in liquid N₂. Diffraction images were
15 collected with a Pilatus 6M detector at a temperature of 100K at the beam line X10SA of the Swiss Light Source and processed with the XDS package [*Kabsch, W. Automatic processing of rotation diffraction data from crystals of initially unknown symmetry and cell constants. J. Appl. Cryst. 26, 795-800 (1993)*]. Data from one crystal were merged to yield a 1.9 Å resolution data set in space group P1
20 with two complex molecules per crystallographic asymmetric unit (see Table 1).

The structure was determined by molecular replacement using the coordinates of a Fab fragment from PDB-ID 3UTZ as search model. As search coordinates for the PD-1 ECD the PDB-ID 3RRQ was used. The Fab was split into constant and variable domains and with both separate searches in the CCP4 program PHASER
25 CCP4 were performed [*CCP4 (Collaborative Computational Project, N. The CCP4 suite: programs for protein crystallography. Acta Crystallogr. D, 760-763 (1994)*] in order to account for possible changes in the elbow angle. The model was rebuilt in COOT (*Emsley, P., Lohkamp, B., Scott, WG. & Cowtan, K. Features and development of COOT. Acta Crystallogr. D Biol. Crystallogr. 60, 486-501 (2010)*)
30 and refined with the CCP4 program REFMAC. The final refinement steps were performed with the program BUSTER (*Bricogne G., Blanc E., Brandl M., Flensburg C., Keller P., Paciorek W., Roversi P, Sharff A., Smart O.S., Vonrhein C., Womack T.O. (2016). BUSTER version 2.11.6. Cambridge, United Kingdom: Global Phasing Ltd.*).

Table 9: Data collection and structure refinement statistics for Fab PD1-0103-PD-1 ECD crystal

Data Collection	
Wavelength (Å)	1.0
Resolution ¹ (Å)	48.27 - 1.90 (1.99 - 1.90)
Space group	P1
Unit cell (Å, °)	66.37 69.82 86.09 99.17 98.01 119.40
Total reflections	170515 (20750)
Unique reflections	97997 (12250)
Multiplicity	1.72 (1.66)
Completeness (%)	0.97 (0.96)
Mean I/σ(I)	8.02 (0.86)
Wilson B-factor	30.30
R-meas	0.093 (0.610)
CC1/2	0.999 (0.290)
Refinement	
Reflections used in refinement	97986 (6792)
Reflections used for R-free	4754 (355)
R-work ³	0.1899 (0.2290)
R-free ⁴	0.2291 (0.2628)
Number of non-hydrogen atoms	9235
macromolecules	8199
Carbohydrate	162
Protein residues	1068
RMS bonds (Å)	0.013
RMS angles (°)	1.81
Ramachandran favored (%)	97
Ramachandran allowed (%)	2.9
Ramachandran outliers (%)	0.38
Rotamer outliers (%)	2.1
Clashscore	2.60

Average B-factor ($\text{\AA}^2$)	36.98
macromolecules	36.01
Carbohydrate	49.62
solvent	38.12

¹ Values in parentheses refer to the highest resolution bins.

² $R_{\text{merge}} = \sum |I - \langle I \rangle| / \sum I$ where I is intensity.

³ $R_{\text{work}} = \sum |F_o - \langle F_c \rangle| / \sum F_o$ where F_o is the observed and F_c is the calculated structure factor amplitude.

5 ⁴ R_{free} was calculated based on 5% of the total data omitted during refinement.

Structure determination of Fab PD1-0103 in complex with the PD-1 ectodomain

10 In order to characterize the epitope and paratope in detail we determined the crystal structure of the PD-1 ectodomain in complex with Fab PD1-0103 to a resolution of 1.9 $\text{\AA}$. The structure reveals Fab PD1-0103 to recognize an epitope formed by the BC and FG loop regions and by residues of β -strands CC'FG of the front β -sheet of the PD-1 V-type Ig domain. In addition the epitope includes the N-linked glycosylation tree at the position Asn58 which is part of the BC loop of PD-1. All CDRs except CDR2 of the light chain of Fab PD1-0103 contribute to the paratope.

15 A surface area of 1063 $\text{\AA}^2$ of PD-1 is covered by Fab PD1-0103 with 743 $\text{\AA}^2$ contributed by the heavy chain and 320 $\text{\AA}^2$ by the light chain. Analysis of the binding interface with the program PISA reveals an interaction pattern of Fab PD1-0103 with the PD-1 ECD via 6 hydrogen bonds and Van der Waals forces. Side chain hydrogen bonds are formed between residues of heavy chain CDR1 (Thr33) and CDR2 (Ser52, Arg56, Asp57) with Glu61 and Ser62 of the BC loop of PD-1. Van der Waals contacts are mainly driven by CDR3 of the light and heavy chain, in particular Phe105 of HCDR3, and by Tyr32 of HCDR1 which are in close distance to residues Val64 of the BC loop, Pro83 and to Ile126 and Leu128 of the FG loop. Further Van der Waals contacts are observed between FG loop residues Pro130, Ala132, Ile134 with the CDR2 of heavy chain and CDR3 of the light chain of Fab PD1-0103. The light chain of Fab PD1-0103 exclusively contacts the FG

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loop of PD-1. No contacts are provided by the CDR2 of the light chain for formation of the complex.

The N-linked glycosylation tree at position Asn58 of PD-1 is part of the epitope and interacts solely with residues of the heavy chain of Fab PD1-0103.

- 5 The core sugar chain (N-linked glycosylation) tree at position Asn58 of PD-1 has the following structure with respect to the monoscharides

Asn58-N-GlcNAc(FUC) – GlcNAc – BMA – MAN (see Figure 9) wherein the following abbreviations are used.

- 10 [GlcNAc]= NGA = N-acetyl-beta-D-galactosamine = 2-(acetylamino)-2-deoxy-beta-D-galactopyranose

[FUC] = alpha-L-fucose

[BMA] = beta-D-mannopyranose

[MAN] = alpha-D-mannopyranose

- 15 The first GlcNAc in the sugar chain is fucosylated which abbreviated as GlcNAc(FUC).

- 20 In the structure the core glycans are well defined in the electron density except one mannose unit. The fucose moiety points into a hydrophilic pocket formed by PD-1 with CDR1 and CDR2. Binding of the fucose is coordinated by a hydrogen bonding network with Ser30 and Ser31 of CDR1 together with Glu61 and Gln99 of PD-1. Further contacts are provided by hydrogen bonding of the first GlcNac to Arg56 and framework residues Arg72, Asp73, Asn74 to Man.

Table 10: List of contacts PD1 – Fab PD1-0103 Heavy chain**Contacts identified by distance cutoff of 5Å**

PD1	HC of PD-103
Ser60	Asp57, Tyr59
Glu61	Thr33, Ser52, Gly53, Gly54, Arg56, Asp57
Ser62	Thr33, Ser52, Asp57, Phe105
Phe63	Phe105
Val64	Gly101, Arg102, Phe105
Tyr68	Tyr104
Lys78	Arg102
Phe82	Ser31
Pro83	Ser31, Tyr32
Glu84	Tyr32
Ile126	Gly101, Tyr104, Phe105
Ser127	Phe105
Leu128	Tyr59, Leu99, Phe105
Pro130	Tyr59
Ile134	Tyr104

Table 11: List of contacts PD1 – Fab PD1-0103 light chain**Contacts identified by distance cutoff of 5Å**

	of PD-103
Ile126	Phe36
Leu128	Asn95, Trp100
Pro130	Asn95, Tyr96, Asp97, Val98
Lys131	Tyr96, Asp97
Ala132	Asn95, Tyr96, Asp97, Thr31, Phe36
Gln133	Thr31
Ile134	Thr31, Ser32, Asn34, Phe36

Table 12: List of contacts PD1 of core sugar chain at Asn58– Fab PD1-0103 Heavy chain**Contacts identified by distance cutoff of 5Å**

PD1 – N-Glycosylation at Asn58 (core sugar chain)	HC of PD-103
First GlcNAc	Arg56, Asp57
FUC	Ser30, Ser31, Tyr32, Gly53, Gly54,
Second GlcNAc	Gly54, Gly55, Arg56
BMA	Gly54, Asn74

PD1 – N-Glycosylation at Asn58 (core sugar chain)	HC of PD-103
MAN	Gly53, Gly54, Gly55, Arg72, Asp73, Asn74

Summary

- Epitope on PD1 resembles flat surface

-> Binding mainly by front b-sheet and CDR3 of PD1

- Interactions involve polar and van der Waal contacts
- Large interaction surface area of PD1 with heavy chain of Fab
- Glycosylation at position Asn 58 participates in binding of PD1 to Fab fragment
- Fucose unit occupies pocket formed by PD1 and heavy chain of Fab PD1-0103

Example 9:

Reduced antibody binding to human PD1 which is not glycosylated at Asn58 compared to the binding to human PD1 which is glycosylated at Asn58 (Biacore characterization of anti-PD-1 antibodies to glycosylated and non-glycosylated recombinant PD1)

A surface plasmon resonance (SPR) based assay has been used to determine the kinetic parameters of the binding between glycosylated PD1 and non-glycosylated recombinant human PD1. Therefore, an anti-human IgG was immobilized by amine coupling to the surface of a (Biacore) CM5 sensor chip. The samples were then captured and hu PD1-ECD was bound to them. The sensor chip surface was regenerated after each analysis cycle. The equilibrium constant and kinetic rate constants were finally gained by fitting the data to a 1:1 langmuir interaction model.

About 2000 response units (RU) of 20 µg/ml anti-human IgG (GE Healthcare #BR-1008-39) were coupled onto the flow cells 1 and 2 (alternatively: 3 and 4) of a

CM5 sensor chip in a Biacore T200 at pH 5.0 by using an amine coupling kit supplied by GE Healthcare.

The sample and running buffer was HBS-EP+ (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.05 % v/v Surfactant P20, pH 7.4). Flow cell temperature was set to 25 °C and sample compartment temperature to 12 °C. The system was primed with running buffer.

The samples were injected for 20 seconds with a concentration of 10 nM and bound to the second flow cell. Then a complete set of human PD1-ECD glycosylated or non-glycosylated) concentrations (200 nM, 66.6 nM, 22.2 nM, 7.4 nM, 2.46 nM and 0 nM) was injected over each sample for 200s followed by a dissociation time of 0/2000s(66.6 nM & 22.2nM) and two 20s regeneration steps with 3 M MgCl₂, of which the last one contained an “extra wash after injection” with running buffer.

Finally the double referenced data was fitted to a 1:1 langmuir interaction model with the Biacore T200 Evaluation Software. Resulting K_D , k_a and k_d values are shown in Table 13.

Table 13: Kinetic rate constants and equilibrium constant determined by Biacore.

Ligand	Sample	k_a (1/Ms)	k_d (1/s)	K_D (M)
PD1-0103-0312	PD1 aglycosylated at Asn58	3.36E+05	2.70E-02	8.02E-08
PD1-0103-0312	PD1 glycosylated at Asn58	7.77E+05	7.46E-05	9.61E-11
pembrolizumab	PD1 aglycosylated at Asn58	1.51E+06	2.46E-03	1.63E-09
pembrolizumab	PD1 glycosylated at Asn58	1.87E+06	4.50E-03	2.41E-09
nivolumab	PD1 aglycosylated at Asn58	5.49E+05	3.66E-03	6.66E-09

Ligand	Sample	ka (1/Ms)	kd (1/s)	KD (M)
nivolumab	PD1 glycosylated at Asn58	4.44E+05	1.63E-03	3.68E-09

There is a clear differentiation between the binding of PD-103-0312 to aglycosylated and glycosylated PD-1 in contrast to pembrolizumab and nivolumab (see also Figures 13A and 13 B).

5 **Example 10:**
in vivo anti-tumor efficacy of PD1 antibodies in combination with a T cell
bispecific antibody against CEA

Humanized animal were produced by conditioning NOG mice with subsequent adoptively transfer of human hematopoietic stem cells. The resulting mice display a
10 chimeric ratio between human and mouse leukocytes ranging from 20 to 85% of human derived cells. In such model , T cells are functional and can be activated to kill tumor cells by the bispecific antibody which binds to CEA and CD3 (which is described in WO2014/131712). Such humanized animals were then injected with one million CEA positive tumor cells, MKN45 gastric carcinoma, subcutaneously
15 in the lateral location. Tumor growth could be assessed by measuring the 3 dimensional axis of the tumor by a operator directed caliper, 3 times a week (Figure 14A and B). At day 9 after tumor injection, the mice were randomized based of tumor size to have homogenous animal groups and the therapeutic treatment started. With the exception of the vehicle groups (figure 14A and 14B, circles), all the mouse groups were administered intravenously with CEACD3TCB at a dose of 2.5mg/Kg twice a week. In addition each mouse group was also treated with one combination partner: anti-PD1 (PD1-0103-0312) at either 0.15mg/Kg weekly (Figure 14A, squares) or 1.5mg/Kg (Figure 14B, squares) weekly intraperitoneally; Nivolumab at either 0.15mg/Kg weekly (Figure 14A, diamonds)
20 or 1.5mg/Kg (Figure 14B, diamonds) weekly intraperitoneally. The mean of tumor size within one treatment group is displayed over time. The group were composed of 9-10 mice each and the measurement continue until there were at least 3 mice per group. The standardised Area under the curve (sAUC) has been calculated and the one way ANOVA analysis was use to calculate statistical significance.

Patent Claims

1. An isolated antibody that binds to human PD1, wherein the antibody comprises
 - (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:71; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:72; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:73; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:74; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:75; (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:76, and (g) FR-H3 comprising the amino acid sequence of SEQ ID NO: 77 (of RDN) at positions of 71, 72 and 73 according to Kabat numbering.

2. The isolated antibody that binds to human PD1 according to claim 1, wherein the antibody comprises
 - A)
 - i) a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8; or
 - ii) humanized variant of the VH and VL of the antibody under i);
 - or B)
 - i) a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:58;
 - ii) a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:59;
 - iii) a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:60; or
 - iv) a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:61.

3. An isolated antibody that binds to human PD1, wherein the antibody comprises
 - (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

4. An isolated antibody that binds to human PD1, wherein the antibody comprises

- (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:3; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:5 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.
5. An isolated antibody that binds to human PD1, wherein the antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:58.
 6. An isolated antibody that binds to human PD1, wherein the antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:59.
 7. An isolated antibody that binds to human PD1, wherein the antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:60.
 8. An isolated antibody that binds to human PD1, wherein the antibody comprises a VH sequence of SEQ ID NO:57 and a VL sequence of SEQ ID NO:61.
 9. The antibody according to any one of the preceding claims, which is a full length IgG1 antibody with mutations L234A, L235A and P329G (numbering according to the EU index of Kabat).
 10. Isolated nucleic acid encoding the antibody according to any one of the preceding claims.
 11. A host cell comprising the nucleic acid of claim 10.
 12. A method of producing an antibody comprising culturing the host cell of claim 11 so that the antibody is produced.
 13. The method of claim 12, further comprising recovering the antibody from the host cell.
 14. A pharmaceutical formulation comprising the antibody according to any one of claims 1 to 8 and a pharmaceutically acceptable carrier.
 15. The antibody according to any one of claims 1 to 8 for use as a medicament.

16. The antibody according to any one of claims 1 to 8 for use in treating cancer.
17. Use of the antibody according to any one of claims 1 to 8 in the manufacture of a medicament for the treatment of cancer.