ANTI-PD-1 ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to International Application No. PCT/CN2014/082721, filed July 22, 2014, which is incorporated herein by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to antibodies and antigen-binding fragments thereof that bind to PD-1, and to methods of using such antibodies and antigen-binding fragments.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0003] The content of the text file submitted electronically herewith is incorporated herein by reference in its entirety: A computer readable format copy of the Sequence Listing (filename: CRBI_006_01WO_SeqList_ST25); date recorded: July 14, 2015; file size 147KB).

BACKGROUND

[0004] Programmed death receptor 1 (PD-1) is primarily expressed on lymphocytes and has two ligands, PD-L1 and PD-L2. PD-1 is a 55 kDa protein encoded by a gene Pdcd1 and was shown to down-regulate antigen receptor signaling driven by its ligand's engagement (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). PD-1 belongs to the immunoglobulin superfamily which includes members such as CD28, CTLA-4, ICOS and BTLA. PD-1 is type I transmembrane glycoprotein containing an Ig variable-type (V-type) domain for ligand binding and a cytoplasmic tail for the binding of signaling molecules. PD-1 contains two cytoplasmic tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T cell stimulation, PD-1 recruits the tyrosine phosphatase SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 Zeta, PKC theta and ZAP70 that are involved in the CD3 T cell signaling cascade. In contrast, PD-1's ligands (PD-L1 and PD-L2) have two short cytoplasmic regions with no known functions. The ligands have an extracellular region containing IgV- and IgC-like domains and are constitutively expressed or can be induced

in a variety of cell types, including non-hematopoietic tissues as well as various tumor types. PD-L1 is not only expressed on B, T, myeloid and dendritic cells (DCs), but also on peripheral cells, like microvascular endothelial cells and non-lymphoid organs like heart, lung etc. In contrast, PD-L2 is only found on macrophages and DCs. The expression pattern of PD-1 ligands is suggestive of a role for PD-1 in maintaining peripheral tolerance and may serve regulate self-reactive T- and B-cell responses in the periphery. To date, numerous studies have shown that interaction of PD-1 with its ligands leads to the inhibition of lymphocyte proliferation in vitro and in vivo. Disruption of the PD-1/PDL1 interaction has been shown to increase T cell proliferation and promote cytokine production.

[0005] Thus, there is an important role for the PD-1/PD-L1 pathway in controlling immune responses. Dysfunction of PD-1/PD-L1 signaling appears to be correlated with initiation and development of diseases such as cancer and viral infection. Analysis of knockout animals has led to the understanding that PD-1 functions mainly in inducing and regulating peripheral tolerance. Thus, therapeutic blockade of the PD-1 pathway would be helpful in overcoming immune tolerance and in the treatment of cancer or infection as well as in boosting immunity during vaccination (either prophylactic or therapeutic). There is a need in the art for improved methods for blocking the PD-1 pathway.

SUMMARY OF THE INVENTION

[0006] In one aspect, the present invention provides antibodies and antigen-binding fragments thereof that bind to programmed death receptor 1 (PD-1). In some embodiments, the antibodies and antigen-binding fragments thereof bind to human PD-1. In some embodiments, the antibodies and antigen-binding fragments thereof bind to PD-1 and block binding of PD-L1 and/or PD-L2 to PD-1. In further embodiments, the anti-PD-1 antibodies and fragments thereof bind to PD-1 and disrupt the PD-1/PD-L1 or PD1/PD-L2 pathway. In one embodiment, the antibody or fragment thereof is a murine antibody, a chimeric antibody, a human antibody or a humanized antibody. In one embodiment, the anti-PD-1 antibody or fragment thereof is a monoclonal antibody, scFv, Fab fragment, Fab' fragment, F(ab)' fragment, bispecific antibody, immunoconjugate, or a combination thereof.

[0007] In one embodiment, the present invention provides an isolated antibody or fragment thereof comprising one or more CDRs selected from the group consisting of SEQ ID NOs: 19-

21, 24-26, 29-31, 34-36, 40-42, 45-47, 50-52, 55-57, 60-62, 65-67, 70-72, 75-77, 80-82, 85-87, 90-92, 95-97, 100-102, 105-107, 110-112, and 115-117.

[0008] In one embodiment, the antibody or fragment thereof comprises a light chain CDR1 sequence having at least 80% homology, at least 81% homology, at least 82% homology, at least 83% homology, at least 84% homology, at least 85% homology, at least 86% homology, at least 87% homology, at least 88% homology, at least 89% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 34, 45, 55, 65, 75, 85, 95, 105, and 115 .

[0009] In one embodiment, the antibody or fragment thereof comprises a light chain CDR2 sequence having at least 80% homology, at least 81% homology, at least 82% homology, at least 83% homology, at least 84% homology, at least 85% homology, at least 86% homology, at least 87% homology, at least 88% homology, at least 89% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 25, 35, 46, 56, 66, 76, 86, 96, 106, and 116.

[0010] In one embodiment, the antibody or fragment thereof comprises a light chain CDR3 sequence having at least 80% homology, at least 81% homology, at least 82% homology, at least 83% homology, at least 84% homology, at least 85% homology, at least 86% homology, at least 87% homology, at least 88% homology, at least 89% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 26, 36, 47, 57, 67, 77, 87, 97, 107, and 117.

[0011] In one embodiment, the antibody or fragment thereof comprises a heavy chain CDR1 sequence having at least 80% homology, at least 81% homology, at least 82% homology, at least 83% homology, at least 84% homology, at least 85% homology, at least 86% homology, at least 87% homology, at least 88% homology, at least 89% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least

95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 29, 40, 50, 60, 70, 80, 90, 100, and 110.

[0012] In one embodiment, the antibody or fragment thereof comprises a heavy chain CDR2 sequence having at least 80% homology, at least 81% homology, at least 82% homology, at least 83% homology, at least 84% homology, at least 85% homology, at least 86% homology, at least 87% homology, at least 88% homology, at least 89% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 30, 41, 51, 61, 71, 81, 91, 101, and 111.

[0013] In one embodiment, the antibody or fragment thereof comprises a heavy chain CDR3 sequence having at least 80% homology, at least 81% homology, at least 82% homology, at least 83% homology, at least 84% homology, at least 85% homology, at least 86% homology, at least 87% homology, at least 88% homology, at least 89% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 21, 31, 42, 52, 62, 72, 82, 92, 102, and 112.In one embodiment, the antibody or fragment thereof comprises a light chain CDR1 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 34, 45, 55, 65, 75, 85, 95, 105, and 115; a light chain CDR2 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 25, 35, 46, 56, 66, 76, 86, 96, 106, and 116; a light chain CDR3 consisting of an amino acid sequences selected from the group consisting of SEQ ID NOs: 26, 36, 47, 57, 67, 77, 87, 97, 107, and 117; a heavy chain CDR1 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 29, 40, 50, 60, 70, 80, 90, 100, and 110; a heavy chain CDR2 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 30, 41, 51, 61, 71, 81, 91, 101, and 111 and a heavy chain CDR3 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 21, 31, 42, 52, 62, 72, 82, 92, 102, and 112.

[0014] In one embodiment, the antibody or fragment thereof binds PD-1 and comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 24, 25, and 26, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 19, 20, and 21, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 24, 25, and 26, respectively, and a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 19, 20, and 21, respectively.

[0015] In one embodiment, the antibody or fragment thereof binds PD-1 and comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 34, 35, and 36, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 29, 30, and 31, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 34, 35, and 36, respectively, and a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 29, 30, and 31, respectively.

[0016] . In one embodiment, the antibody or fragment thereof binds PD-1 and comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92%

homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 45, 46, and 47, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 40, 41, and 42, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 45, 46, and 47, respectively, and a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 40, 41, and 42, respectively.

[0017] In one embodiment, the antibody or fragment thereof binds PD-1 and comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 55, 56, and 57, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 50, 51, and 52, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 55, 56, and 57, respectively, and a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 50, 51, and 52, respectively.

[0018] . In one embodiment, the antibody or fragment thereof binds PD-1 and comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 65, 66, and 67, respectively; and a heavy chain

CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 60, 61, and 62, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 65, 66, and 67, respectively, and a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 60, 61, and 62, respectively.

[0019] In one embodiment, the antibody or fragment thereof binds PD-1 and comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 75, 76, and 77, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 70, 71, and 72, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 75, 76, and 77, respectively, and a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 70, 71, and 72, respectively.

[0020] In one embodiment, the antibody or fragment thereof binds PD-1 and comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 85, 86, and 87, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at

least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 80, 81, and 82, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 85, 86, and 87, respectively, and a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 80, 81, and 82, respectively.

[0021] In one embodiment, the antibody or fragment thereof binds PD-1 and comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 95, 96, and 97, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 90, 91, and 92, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 95, 96, and 97, respectively, and a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 90, 91, and 92, respectively.

[0022] In one embodiment, the antibody or fragment thereof binds PD-1 and comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 105, 106, and 107, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 100, 101, and 102, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a light chain CDR1, CDR2,

and CDR3 according to SEQ ID NOs: 105, 106, and 107, respectively, and a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 100, 101, and 102, respectively.

[0023] In one embodiment, the antibody or fragment thereof binds PD-1 and comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 115, 116, and 117, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 110, 111, and 112, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 115, 116, 117, respectively, and a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 110, 111, and 112, respectively.

[0024] In one embodiment, the antibody or fragment thereof binds PD-1 and comprises a light chain variable region comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 23, 33, 44, 54, 64, 74, 84, 94, 104, 114, 133, 143, and 152; and a heavy chain variable region comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 28, 39, 49, 59, 69, 79, 89, 99, 109, 131, and 141. In a further embodiment, the isolated antibody or fragment thereof binds PD-1 and comprises a light chain variable region comprising, consisting essentially of, or consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 23, 33, 44, 54, 64, 74, 84, 94, 104, 114, 133, 143, and 152; and a heavy chain variable region

comprising, consisting essentially of, or consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 28, 39, 49, 59, 69, 79, 89, 99, 109, 131, and 141.

[0025] In one embodiment, the invention provides anti-PD-1 antibodies that comprise a variable light chain of an antibody selected from the group consisting of 10D1, 4C10, 7D3, 13F1, 15H5, 14A6, 22A5, 6E1, 5A8, 7A4, and 7A4D and a variable heavy chain of an antibody selected from the group consisting of 10D1, 4C10, 7D3, 13F1, 15H5, 14A6, 22A5, 6E1, 5A8, and 7A4. Thus, in one embodiment, the invention provides an antibody or fragment thereof comprising a light chain variable region comprising SEQ ID NO: 23 and a heavy chain variable region comprising SEQ ID NO: 18; a light chain variable region comprising SEQ ID NO: 33 and a heavy chain variable region comprising SEQ ID NO: 28; a light chain variable region comprising SEQ ID NO: 44 and a heavy chain variable region comprising SEQ ID NO: 39; a light chain variable region comprising SEQ ID NO: 54 and a heavy chain variable region comprising SEQ ID NO: 49; a light chain variable region comprising SEQ ID NO: 64 and a heavy chain variable region comprising SEQ ID NO: 59; a light chain variable region comprising SEQ ID NO: 74 and a heavy chain variable region comprising SEQ ID NO: 69; a light chain variable region comprising SEQ ID NO: 84 and a heavy chain variable region comprising SEQ ID NO: 79; a light chain variable region comprising SEQ ID NO: 94 and a heavy chain variable region comprising SEQ ID NO: 89; a light chain variable region comprising SEQ ID NO: 104 and a heavy chain variable region comprising SEQ ID NO: 99; a light chain variable region comprising SEQ ID NO: 114 and a heavy chain variable region comprising SEQ ID NO: 109; a light chain variable region comprising SEQ ID NO: 133 and a heavy chain variable region comprising SEQ ID NO: 131; a light chain variable region comprising SEQ ID NO: 143 and a heavy chain variable region comprising SEQ ID NO: 141; or a light chain variable region comprising SEQ ID NO: 152 and a heavy chain variable region comprising SEQ ID NO: 131.

[0026] In one embodiment, the present invention provides a chimeric anti-PD-1 antibody, wherein the antibody comprises a heavy chain having an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs; 119, 121, 125, and 127; and a light chain having an amino acid sequence having at least 80% homology, at least 85%

homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 123 and 129.

[0027] In one embodiment, the present invention provides a humanized anti-PD-1 antibody, wherein the antibody comprises a heavy chain variable region having an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 131 and 141. In another embodiment, the present invention provides a humanized anti-PD-1 antibody, wherein the antibody comprises a light chain variable region having an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 133, 143 and 152.

[0028] In another embodiment, the present invention provides a humanized anti-PD-1 antibody, wherein the antibody comprises a heavy chain variable region having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to SEQ ID NO: 131 and a light chain variable region having least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to SEQ ID NO: 133 or 152. In another embodiment, the present invention provides a humanized anti-PD-1 antibody, wherein the antibody comprises a heavy chain variable region having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to SEQ ID NO: 141 and a light chain variable region

having least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to SEQ ID NO: 143.

[0029] In one embodiment, the present invention provides a humanized anti-PD-1 antibody, wherein the antibody comprises a full heavy chain having an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 135, 137, 145, and 147. In another embodiment, the present invention provides a humanized anti-PD-1 antibody, wherein the antibody comprises a full light chain having an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 139, 149, and 153.

[0030] In one embodiment, the present invention provides a humanized anti-PD-1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 135 and a light chain according to SEQ ID NO: 139. In another embodiment, the present invention provides a humanized anti-PD-1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 137 and a light chain according to SEQ ID NO: 139. In another embodiment, the present invention provides a humanized anti-PD-1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 135 and a light chain according to SEQ ID NO: 153. In another embodiment, the present invention provides a humanized anti-PD-1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 137 and a light chain according to SEQ ID NO: 153. In another embodiment, the present invention provides a humanized anti-PD-1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 145 and a light chain according to SEQ ID NO: 149. In another embodiment, the present invention provides a humanized anti-PD-1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 147 and a light chain according to SEQ ID NO: 149. In one embodiment, the present invention provides anti-PD-1 antibodies or fragments thereof that bind

to the same epitope on PD-1 as any of the exemplary antibodies provided herein. In one embodiment, the antibodies or fragments thereof compete with any of the exemplary antibodies provided herein for binding to PD-1. Binding to PD-1 may be measured by ELISA, flow cytometry, surface plasmon resonance (SPR) assay, or any other method known in the art.

[0031] In one embodiment, the present invention provides anti-PD-1 antibodies and fragments thereof that bind to PD-1 with an affinity of about 1nM to about 0.01 nM. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of from about 0.5nM to about 0.1nM. In another embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 1 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.75 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.5 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.25 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.2 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.15 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.1 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.075 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.05 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.025 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.02 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.015 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.01 nM or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.0075 or less. In a further embodiment, the anti-PD-1 antibodies and fragments thereof provided herein bind to PD-1 with an affinity of about 0.005 or less.

[0032] In one embodiment, the anti PD-1 antibodies and fragments thereof provided herein have a binding EC50 for PD-1 of about 1 ng/mL to about 2000 ng/mL. In a further embodiment, the anti PD-1 antibodies and fragments thereof provided herein have a binding EC50 for PD-1 of about 1 ng/mL to about 1500 ng/mL. In a further embodiment, the anti PD-1 antibodies and fragments thereof provided herein have a binding EC50 for PD-1 of about 1 ng/mL to about 1000 ng/mL. In a further embodiment, the anti PD-1 antibodies and fragments thereof provided herein have a binding EC50 for PD-1 of about 2 ng/mL to about 500 ng/mL. In a further embodiment, the anti PD-1 antibodies and fragments thereof provided herein have a binding EC50 for PD-1 of about 2 ng/mL to about 200 ng/mL. In a further embodiment, the anti PD-1 antibodies and fragments thereof provided herein have a binding EC50 for PD-1 of about 5 ng/mL to about 100 ng/mL. In a further embodiment, the anti PD-1 antibodies and fragments thereof provided herein have a binding EC50 for PD-1 of about 5 ng/mL to about 50 ng/mL. In one embodiment, the anti PD-1 antibodies and fragments thereof provided herein have a binding EC50 for PD-1 of about 500 ng/mL or less, about 400 ng/mL or less, about 300 ng/mL or less, about 250 ng/mL or less, about 200 ng/mL or less, about 150 ng/mL or less, about 100 ng/mL or less, about 75 ng/mL or less, about 60 ng/mL or less, about 50 ng/mL or less, about 40 ng/mL or less, or about 30 ng/mL or less.

[0033] In one embodiment, the anti PD-1 antibodies and fragments thereof provided herein inhibit PD-L1 binding with an IC50 of about of about 1 ng/mL to about 1000 ng/mL. In a further embodiment, the anti PD-1 antibodies and fragments thereof provided herein inhibit PD-L1 binding with an IC50 of about 2 ng/mL to about 800 ng/mL. In a further embodiment, the anti PD-1 antibodies and fragments thereof provided herein inhibit PD-L1 binding with an IC50 of about 5 ng/mL to about 500 ng/mL. In a further embodiment, the anti PD-1 antibodies and fragments thereof provided herein inhibit PD-L1 binding with an IC50 of about 5 ng/mL to about 100 ng/mL. In a further embodiment, the anti PD-1 antibodies and fragments thereof provided herein inhibit PD-L1 binding with an IC50 of about 10 ng/mL to about 50 ng/mL. In one embodiment, the anti PD-1 antibodies and fragments thereof provided herein inhibit PD-L1 binding with an IC50 of about 800 ng/mL or less, about 400 ng/mL or less, about 300 ng/mL or less, about 250 ng/mL or less, about 200 ng/mL or less, about 150 ng/mL or less, about 100 ng/mL or less, about 75 ng/mL or less, about 60 ng/mL or less, about 50 ng/mL or less, about 40 ng/mL or less, or about 30 ng/mL or less.

[0034] In one embodiment, the anti-PD-1 antibody provided herein is a humanized antibody having a light chain variable region amino acid sequence according to SEQ ID NO: 133 and a heavy chain variable region amino acid according to SEQ ID NO: 131; or having a light chain variable region amino acid sequence according to SEQ ID NO: 143 and a heavy chain variable region amino acid sequence according to SEQ ID NO: 141; or having a light chain variable region amino acid sequence according to SEQ ID NO: 152 and a heavy chain variable region amino acid sequence according to SEQ ID NO: 131; wherein the anti-PD-1 antibody has a PD-1 binding EC50 of about 200ng/ml or less or about 150 ng/mL or less or about 100 ng/mL or less or about 80 ng/ml or less or about 60 ng/mL or less, as measured by ELISA or FACS. In another embodiment, the anti-PD-1 antibody provided herein is a humanized antibody having a light chain variable region amino acid sequence according to SEQ ID NO: 133 and a heavy chain variable region amino acid according to SEQ ID NO: 131; or having a light chain variable region amino acid sequence according to SEQ ID NO: 143 and a heavy chain variable region amino acid sequence according to SEQ ID NO: 141; or having a light chain variable region amino acid sequence according to SEQ ID NO: 152 and a heavy chain variable region amino acid sequence according to SEQ ID NO: 131; wherein the anti-PD-1 antibody has a PD-L1 blockage IC50 of about 1000 ng/mL or less, or about 800 ng/mL or less, or about 600 ng/mL or less, or about 500 ng/mL or less, or about 400 ng/mL or less, or about 300 ng/mL or less, or about 200 ng/mL or less, or about 100 ng/mL or less, or about 60 ng/mL or less, or about 30 ng/mL or less, or about 25 ng/mL or less, or about 20 ng/mL or less, or about 10 ng/mL or less, as measured by ELISA or FACS. In another embodiment, the anti-PD-1 antibody provided herein is a humanized antibody having a light chain variable region amino acid sequence according to SEQ ID NO: 133 and a heavy chain variable region amino acid according to SEQ ID NO: 131; or having a light chain variable region amino acid sequence according to SEQ ID NO: 143 and a heavy chain variable region amino acid sequence according to SEQ ID NO: 141; or having a light chain variable region amino acid sequence according to SEQ ID NO: 152 and a heavy chain variable region amino acid sequence according to SEQ ID NO: 131; wherein the anti-PD-1 antibody has an affinity for PD-1 of about 1 nM or less, or about 0.5 nM or less, or about 0.1 nM or less, or about 0.05nM or less. In a particular embodiment, the humanized anti-PD-1 antibody has an affinity for PD-1 of about 0.1 nM.

[0035] In one embodiment, the anti-PD-1 antibodies and fragments thereof provided bind to PD-1 on T cells, disrupting the PD-1/PD-L1 interaction and resulting in an increase in T cell activation. In a further embodiment, the antibodies and fragments thereof bind PD-1 and result in an increase in T cell proliferation and/or cytokine production. In a yet further embodiment, the antibodies and fragments thereof bind PD-1 and result in an increase of one or more cytokines selected from the group consisting of IL-2, IFNγ, TNF, IL-1, IL-4, IL-5, IL-6, IL-12, IL-13, IL-17, and GM-CSF. Thus, in one aspect, the present invention provides methods for modulating an immune response comprising contacting T cells with the anti-PD-1 antibody or fragment thereof. In one embodiment, the modulation of an immune response by the anti-PD-1 antibodies and fragments provided herein may be measured in a mixed lymphocyte (MLR) reaction. In one embodiment, the anti-PD-1 antibodies provided herein increase the level of cytokine production from lymphocytes in an MLR. In a further embodiment, the anti-PD-1 antibodies increase the level of IL-2 production and/or IFNγ production in an MLR. In a yet further embodiment, the anti-PD-1 antibodies increase the level of IL-2 production and IFNγ production in an MLR. In one embodiment, the anti-PD-1 antibodies enhance memory T cell responses. In a further embodiment, the anti-PD-1 antibodies enhance memory T cell responses as measured by an increase in IFNγ production from memory T cells.

[0036] In one embodiment, the anti-PD-1 antibodies and fragments thereof provided herein inhibit regulatory T cell function. In a further embodiment, the anti-PD-1 antibodies and fragments thereof inhibit the suppression of effector T cells by regulatory T cells. In another embodiment, the anti-PD-1 antibodies and fragments thereof restore the effector functions of T cells in the presence of regulatory T cells. In a further embodiment, the anti-PD-1 antibodies and fragments thereof restore the ability of effector T cells to proliferate and/or produce cytokines in the presence of regulatory T cells. Thus, in one embodiment, the present invention provides a method for inhibiting the suppressive effects of regulatory T cells in vitro or in a subject in need thereof.

[0037] In one aspect, an isolated antibody or fragment thereof that binds to PD-1 is provided, wherein the antibody is produced by a hybridoma selected from the group consisting of the hybridomas herein termed 10D1, 4C10, 7D3, 13F1, 15H5, 14A6, 22A5, 6E1, 5A8, 7A4, and 7A4D. Thus, the present invention also encompasses the hybridomas 10D1, 4C10, 7D3, 13F1, 15H5, 14A6, 22A5, 6E1, 5A8, 7A4, and 7A4D, as well as any hybridoma producing an antibody

disclosed herein. The present invention also provides isolated polynucleotides encoding the antibodies and fragments thereof provided herein. Expression vectors comprising the isolated polynucleotides, and host cells comprising such expression vectors, are also encompassed in the invention.

[0038] In one embodiment, the present invention provides anti-PD-1 antibody immunoconjugates. Thus, the present invention provides an antibody or fragment thereof that binds to PD-1 and that is linked or conjugated to a therapeutic agent. Therapeutic agents that may be linked or conjugated to the anti-PD-1 antibody may include, but are not limited to, cytotoxic drugs, radioactive isotopes, immunomodulators, or antibodies.

[0039] In one aspect, the present invention provides compositions comprising one or more anti-PD-1 antibody or fragment thereof provided herein, and a pharmaceutically acceptable carrier.

[0040] In one aspect, the present invention provides methods for modulating an immune response in a subject, the method comprising administering to the subject a therapeutically effective amount of an anti-PD-1 antibody or fragment thereof provided herein. In one embodiment, the present invention provides methods for treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an anti-PD-1 antibody or fragment thereof provided herein.

[0041] In one embodiment, the present invention provides a method for enhancing anti-tumor responses in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an anti-PD-1 antibody or fragment of the invention. In another embodiment, the present invention provides a method for reducing tumors or inhibiting the growth of tumor cells in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an anti-PD-1 antibody or fragment of the invention. In another embodiment, the present invention provides a method for treating cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an anti-PD-1 antibody or fragment of the invention. In a further embodiment, the cancer is selected from the group consisting of lymphoma, leukemia, melanoma, glioma, breast cancer, lung cancer, colon cancer, bone cancer, ovarian cancer, bladder cancer, kidney cancer, liver cancer, stomach cancer, rectal cancer, testicular cancer, salivary cancer, thyroid cancer, thymic cancer, epithelial cancer, head or neck cancer, gastric cancer, pancreatic cancer, or a combination thereof.

[0042] In one embodiment, the present invention provides a method for treating an infectious disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an anti-PD-1 antibody or fragment of the invention. In a further embodiment, the infectious disease is selected from the group consisting of candidiasis, candidemia, aspergillosis, streptococcal pneumonia, streptococcal skin and oropharyngeal conditions, gram negative sepsis, tuberculosis, mononucleosis, influenza, respiratory illness caused by Respiratory Syncytial Virus, malaria, schistosomiasis, and trypanosomiasis.

BRIEF DESCIRPTION OF THE FIGURES

[0043] Figures 1A and 1B are a graphs showing the blockage of PD-1 ligand PD-L1 and PD-L2 binding to PD-1 by murine anti-PD-1 antibodies as measured by FACS. **Figure 1A** shows the blockage of PD-L1's binding by murine anti-PD-1 antibodies and **Figure 1B** shows the blockage of PD-L2's binding by murine anti-PD-1 antibodies. The top panels of **Figure 1A and Figure 1B** show the MFI over a range of antibody concentrations. The blockage IC50 for the anti-PD-1 antibodies are shown in the bottom panels of **Figure 1A and Figure 1B**.

[0044] Figure 2 is a graph showing IL-2 (pg/mL) production in an MLR in response to different concentrations of murine anti-PD-1 antibodies. The anti-PD-1 antibodies tested were, from left to right, control mIgG1, 22A5-mIgG1, 6E1-mIgG1, 10D1-mIgG1, 4C10-mIgG1, 7D3-mIgG1, 13F1-mIgG1, 14A6-mIgG1, 15H5-mIgG1, 5A8-mIgG1, and 7A4-mIgG1. As shown on the x-axis, each antibody was tested at 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0045] Figure 3 is a graph showing IFN-γ (pg/mL) production in an MLR in response to different concentrations of murine anti-PD-1 antibodies. The anti-PD-1 antibodies tested were, from left to right, control mIgG1, 22A5-mIgG1, 6E1-mIgG1, 10D1-mIgG1, 4C10-mIgG1, 7D3-mIgG1, 13F1-mIgG1, 14A6-mIgG1, 15H5-mIgG1, 5A8-mIgG1, and 7A4-mIgG1. As shown on the x-axis, each antibody was tested at 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0046] Figure 4 is a graph showing IL-2 (pg/mL) production in an MLR in response to different concentrations of chimeric anti-PD-1 antibodies. The chimeric anti-PD-1 antibodies tested were, from left to right, control hIgG4, chimeric 4C10-hIgG4, chimeric 6E1-hIgG4, chimeric 7A4-hIgG4, chimeric 13F1-hIgG4, chimeric 15H5-hIgG4, chimeric 22A5-hIgG4, and chimeric 7D3-

hIgG4. As shown on the x-axis, each antibody was tested at 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0047] Figure 5 is a graph showing IFN-γ (pg/mL) production in an MLR in response to different concentrations of chimeric anti-PD-1 antibodies. The chimeric anti-PD-1 antibodies tested were, from left to right, control hIgG4, chimeric 4C10-hIgG4, chimeric 6E1-hIgG4, chimeric 7A4-hIgG4, chimeric 13F1-hIgG4, chimeric 15H5-hIgG4, chimeric 22A5-hIgG4, and chimeric 7D3-hIgG4. As shown on the x-axis, each antibody was tested at 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0048] Figure 6 shows the binding EC50 of humanized 13F1 (**Figure 6A**) and humanized 7A4 (**Figure 6B**) anti-PD-1 antibodies as measured by ELISA. The top panel of **Figure 6A** shows the absorbance over a range of concentrations of chimeric 13F1, humanized 13F1-hIgG1 (h13F1-IgG1), humanized 13F1-hIgG4 (h13F1-IgG4), or control hIgG4. The bottom panel of **Figure 6A** shows the calculated EC50 of each of the test antibodies. The top panel of **Figure 6B** shows the absorbance over a range of concentrations of chimeric 7A4- hIgG1, chimeric 7A4-hIgG4, humanized 784 -hIgG1 (h7A4hIgG1), humanized 7A4-hIgG4 (h7A4hIgG4), or control hIgG4. The bottom panel of **Figure 6B** shows the calculated EC50 of each of the test antibodies.

[0049] Figure 7 shows the binding EC50 of humanized 13F1 (**Figure 7A**) and humanized 7A4 (**Figure 7B**) anti-PD-1 antibodies as measured by FACS. The top panel of **Figure 7A** shows the mean fluorescence intensity (MFI) over a range of concentrations of control hIgG4, chimeric 13F1-hIgG4, humanized 13F1-hIgG1 (h13F1-hIgG1), or humanized 13F1-hIgG4 (h13F1-hIgG4). The bottom panel of **Figure 7A** shows the calculated EC50 of each of the test antibodies. The top panel of **Figure 7B** shows the MFI over a range of concentrations of control hIgG4, chimeric 7A4- hIgG4, chimeric 7A4-chimeric -IgG1, humanized 7A4-IgG4 (h7A4-hIgG4), or humanized 7A4- IgG1 (h7A4-hIgG1). The bottom panel of **Figure 7B** shows the calculated EC50 of each of the test antibodies.

[0050] Figure 8 shows the blockage of PD-L1 binding by humanized 13F1 (**Figure 8A**) and humanized 7A4 (**Figure 8B**) anti-PD-1 antibodies as measured by ELISA. **Figure 8A** shows the absorbance over a range of concentrations of control hIgG4, chimeric 13F1, humanized 13F1-hIgG1, or humanized 13F1-hIgG4. **Figure 8B** shows the absorbance over a range of concentrations of control hIgG4, chimeric 7A4 -hIgG1, chimeric 7A4- hIgG4, humanized 784 -

hIgG1 or humanized 7A4-hIgG4. **Figure 8C** shows the calculated PD-L1 blockage IC50 of the chimeric and humanized 13F1 and 7A4 antibodies.

[0051] Figure 9 shows the blockage of PD-L1 binding by humanized 13F1 and 7A4 antibodies as measured by FACS. The top panel of **Figure 9** shows the MFI over a range of antibody concentrations. The blockage IC50 for the humanized antibodies are shown in the bottom panel of **Figure 9**.

[0052] Figure 10 shows the binding data for PD-1 humanized monoclonal antibodies h13F1(top left panel) and h7A4 (top right panel), as measured by Biacore assay. The bottom panel provides the quantified binding data as measured by Biacore assay.

[0053] Figure 11 is a graph showing IL-2 production (pg/mL) in an MLR reaction in the presence of control hIgG4, murine 13F1-mIgG1 (13F1-mIgG1), humanized 13F1-hIgG1, humanized 13F1-hIgG4, or chimeric 7A4-hIgG4 at the following concentrations: 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0054] Figure 12 is a graph showing IFN-γ production (pg/mL) in an MLR reaction in the presence of control hIgG4, murine 13F1-mIgG1(13F1-mIgG1), humanized 13F1-hIgG1, humanized 13F1-hIgG4, or chimeric 7A4-hIgG4 at the following concentrations: 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0055] Figure 13 is a graph showing IL-2 production (pg/mL) in an MLR reaction in the presence of control hIgG4, chimeric 7A4-hIgG1, chimeric 7A4-hIgG4, humanized 7A4-hIgG1, or humanized 7A4-hIgG4 at the following concentrations: 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0056] Figure 14 is a graph showing IFN-γ production (pg/mL) in an MLR reaction in the presence of control hIgG4, chimeric 7A4-hIgG1, chimeric 7A4-hIgG4, humanized 7A4-hIgG1, or humanized 7A4-hIgG4 at the following concentrations: 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0057] Figure 15 shows the effect of humanized anti-PD-1 antibodies on memory T cell responses recalled by tetanus toxin, as measured by IFN-γ production (pg/mL). Negative control hIgG4, humanized 13F1-hIgG1, humanized 13F1-hIgG4, humanized 7A4-hIgG1, and humanized 7A4-hIgG4 antibodies were tested at the following concentrations: 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0058] Figure 16 shows IFN-γ production (pg/mL) from T cells in response to costimulation with autologous DCs and anti-CD3 antibody, in the presence of 10 μg/ml of humanized anti-PD-1 antibodies (h13F1-hIgG1, h13F1-hIgG4, h7A4-hIgG1, or h7A4-hIgG4), isotype control (hIgG4) antibody, or no antibody.

[0059] Figures 17A and **17B** show the data of Biacore based binding (Figure 17A) and FACS based blockage (Figure 17B) for PD-1 humanized monoclonal antibodies h7A4 and h7A4D. For Figure 17A, top left indicates h7A4 and top right indicates 7A4D, and the bottom panel of Figure 17A provides the quantified binding data as measured by Biacore analysis. Figure 17B indicates the blockage IC50 of PD-L1's binding to 293T-PD1 cells by 7A4D-hIgG4 antibody.

[0060] Figure 18 is a graph showing IL-2 production (pg/mL) in an MLR reaction in the presence of control hIgG4, humanized 7A4-hIgG4, or humanized 7A4D-hIgG4 at the following concentrations: 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0061] Figure 19 is a graph showing IFN-γ production (pg/mL) in an MLR reaction in the presence of control hIgG4, humanized 7A4-hIgG4, or humanized 7A4D-hIgG4 at the following concentrations: 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

DETAILED DESCRIPTION

[0062] Programmed death receptor 1 (PD-1) is a checkpoint receptor of immune system. It is primarily expressed on activated T and B cells, but also occurs on monocytes and CD4-CD8-double negative T cells and NK-T cells under thymic development (Agata et al., supra; Okazaki et al. (2002) Curr. Opin. Immunol. 14: 391779-82; Bennett et al. (2003) J Immunol 170:711-8). PD-1 has two ligands, PD-L1 and PD-L2. The interaction of PD-1 with either of the two ligands has been shown to attenuate T-cell responses in vitro and in vivo, which can, however, be reversed by inhibiting the local interaction of PD-1 with PD-L1, and the effect is additive when the interaction of PD-1 with PD-L2 is blocked as well (Iwai et al. (2002) Proc. Nat'l. Acad Sci. USA 99: 12293-7; Brown et al. (2003) J. Immunol. 170:1257-66).

[0063] PD-1 has been found to have a correlation with cancer growth and development due to its role in protecting tumor cells from efficient immune destruction. Its ligand, PD-L1, has been revealed to have significant expression on a number of mouse and human tumors, which is postulated to mediate immune evasion (Iwai, Y. et al., Proc. Natl. Acad. Sci. USA.99: 12293-

12297 (2002); Strome S. E. et al., Cancer Res., 63:6501-6505 (2003); Dong et al. (2002) Nat. Med. 8:787-9). In humans, expression of PD-1 (on tumor infiltrating lymphocytes) and/or PD-L1 (on tumor cells) has been found in a number of primary tumor biopsies as assessed by immunohistochemistry. Such tissues include cancers of the lung, liver, ovary, cervix, skin, colon, glioma, bladder, breast, kidney, esophagus, stomach, oral squamous cell, urothelial cell, and pancreas as well as tumors of the head and neck (Brown J. A.et al., J. Immunol. 170: 1257-1266 (2003); Dong H. et al., Nat.Med. 8: 793-800 (2002); Wintterle et al., Cancer Res. 63:7462-7467 (2003); Strome S. E. et al., Cancer Res., 63: 6501 -6505 (2003); Thompson R. H. et al., Cancer Res. 66: 3381-5(2006); Thompson et al., Clin. Cancer Res. 13: 1757-61(2007); Nomi T. et al., Clin. Cancer Res. 13: 2151-7. (2007)). More strikingly, PD-l ligand expression on tumor cells has been correlated to poor prognosis of cancer patients across multiple tumor types (reviewed in OkaZaki and Honjo, Int. Immunol. 19: 813-824 (2007)).

[0064] While 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: 3 07-3 14; Konishi et al. (2004) Clin. Cancer Res. 10:5094-100), blockade of the PD-1/PD-L1 interaction was accordingly shown to enhance tumor-specific T-cell immunity and be helpful in clearance of tumor cells by the immune system. In a murine model of aggressive pancreatic cancer, for example, Nomi T., et al. (Clin. Cancer Res. 13: 2151-2157, 2007) demonstrated the therapeutic efficacy of PD-1/PD-L1 blockade. Administration of either PD-1 or PD-L1 directed antibody significantly inhibited tumor growth. Antibody blockade effectively promoted tumor reactive CD8+ T cell infiltration into the tumor resulting in the up-regulation of anti-tumor effectors including IFN-γ, granzyme B and perforin. Additionally, the authors showed that PD-1 blockade can be effectively combined with chemotherapy to yield a synergistic effect. In another study, using a model of squamous cell carcinoma in mice, antibody blockade of PD-l or PD-Ll significantly inhibited tumor growth (Tsushima F. et al., Oral Oncol. 42:268-274 (2006)).

[0065] Furthermore, transfection of a murine mastocytoma line with PD-Ll led to decreased lysis of the tumor cells when co-cultured with a tumor-specific CTL clone. Lysis was restored when anti-PD-Ll mAb was added (Iwai Y. et al., Proc. Natl. Acad. Sci. USA. 99: 12293-12297 (2002)). In vivo, blocking the PDl/PD-Ll interaction was shown to increase the efficacy of

adoptive T cell transfer therapy in a mouse tumor model (Strome S. E. et al., Cancer Res. 63:6501-6505 (2003)). Further evidence for the role of PD-1 in cancer treatment comes from experiments performed with PD-l knockout mice. PD-Ll expressing myeloma cells grew only in Wild-type animals (resulting in tumor growth and associated animal death), but not in PD-1 deficient mice (Iwai Y., et al., Proc. Natl. Acad. Sci. USA. 99: 12293-12297(2002)).In human studies, R. M. Wong et al. (Int. Immunol. 19:1223-1234 (2007)) showed that PD-l blockade using a fully human anti-PD-l antibody augmented the absolute numbers of tumor-specific CD8+ T cells (CTLs) in ex vivo stimulation assays using vaccine antigens and cells from vaccinated individuals. In a similar study, antibody blockade of PD-Ll resulted in enhanced cytolytic activity of tumor-associated antigen-specific cytotoxic T cells and increased cytokine production by tumor specific TH cells (Blank C. et al., Int. J. Cancer 119: 317-327 (2006)). The same authors showed that PD-Ll blockade augments tumor-specific T cell responses in vitro when used in combination with anti-CTLA-4 blockade. Overall, the PD-l/PD-Ll pathway is a target for the development of antibody therapeutics for cancer treatment. Anti-PD-l antibodies may also be useful in chronic viral infection. Memory CD8+ T cells generated after an acute viral infection are highly functional and constitute an important component of protective immunity. In contrast, chronic infections are often characterized by varying degrees of functional impairment (exhaustion) of virus-specific T-cell responses, and this defect is a principal reason for the inability of the host to eliminate the persisting pathogen. Although functional effector T cells are initially generated during the early stages of infection, they gradually lose function during the course of a chronic infection. Barber et al. (Barber et al., Nature 439: 682-687 (2006)) showed that mice infected with a laboratory strain of LCMV developed chronic infection resulting in high levels of virus in the blood and other tissues. These mice initially developed a robust T cell response, but eventually succumbed to the infection upon T cell exhaustion. The authors found that the decline in number and function of the effector T cells in chronically infected mice could be reversed by injecting an antibody that blocked the interaction between PD-l and PD-Ll.

[0066] In one aspect, the present invention provides antibodies or antigen binding fragments thereof that bind to programmed cell death 1 (PD-1). PD-1. In one embodiment, the antibodies or fragments thereof bind to human PD-1. In another embodiment, the antibodies or fragments thereof bind to human and to cynomolgous PD-1. In another embodiment, the antibodies or fragments thereof block the interaction of PD-1 on T cells with its ligand PD-L1. In one

aspect, the present invention provides methods of making and using the anti-PD-1 antibodies or fragments thereof, and compositions comprising anti-PD-1 antibodies or fragments thereof, including pharmaceutical compositions.

[0067] As used herein, the term "antibody" refers to a binding protein having at least one antigen binding domain. The antibodies and fragments thereof of the present invention may be whole antibodies or any fragment thereof. Thus, the antibodies and fragments of the invention include monoclonal antibodies or fragments thereof and antibody variants or fragments thereof, as well as immunoconjugates. Examples of antibody fragments include Fab fragments, Fab' fragments, F(ab)' fragments, Fv fragments, isolated CDR regions, single chain Fv molecules (scFv), and other antibody fragments known in the art. Antibodies and fragments thereof may also include recombinant polypeptides, fusion proteins, and bi-specific antibodies. The anti-PD-1 antibodies and fragments thereof disclosed herein may be of an IgG1, IgG2, IgG3, or IgG4 isotype. The term "isotype" refers to the antibody class encoded by the heavy chain constant region genes. In one embodiment, the anti-PD-1 antibodies and fragments thereof disclosed herein are of an IgG1 or an IgG4 isotype. The PD-1 antibodies and fragments thereof of the present invention may be derived from any species including, but not limited to, mouse, rat, rabbit, primate, llama, and human. The PD-1 antibodies and fragments thereof may be chimeric, humanized, or fully human antibodies. In one embodiment, the anti-PD-1 antibodies are murine antibodies. In another embodiment, the anti-PD1 antibodies are chimeric antibodies. In a further embodiment, the chimeric antibodies are mouse-human chimeric antibodies. In another embodiment, the antibodies are derived from mice and are humanized.

[0068] A "chimeric antibody" is an antibody having at least a portion of the heavy chain variable region and at least a portion of the light chain variable region derived from one species; and at least a portion of a constant region derived from another species. For example, in one embodiment, a chimeric antibody may comprise murine variable regions and a human constant region.

[0069] A "humanized antibody" is an antibody containing complementarity determining regions (CDRs) that are derived from a non-human antibody; and framework regions as well as constant regions that are derived from a human antibody. For example, the anti-PD-1 antibodies provided herein may comprise CDRs derived from one or more murine antibodies and human framework and constant regions. Thus, in one embodiment, the humanized antibody provided herein binds

to the same epitope on PD-1 as the murine antibody from which the antibody's CDRs are derived. Exemplary humanized antibodies are provided herein. Additional anti-PD-1 antibodies comprising the heavy and light chain CDRs provided herein, or variants thereof, may be generated using any human framework sequence, and are also encompassed in the present invention. In one embodiment, framework sequences suitable for use in the present invention include those framework sequences that are structurally similar to the framework sequences provided herein. Further modifications in the framework regions may be made to improve the properties of the antibodies provided herein. Such further framework modifications may include chemical modifications; point mutations to reduce immunogenicity or remove T cell epitopes; or back mutation to the residue in the original germline sequence.

[0070] In some embodiments, such framework modifications include those corresponding to the mutations exemplified herein, including backmutations to the germline sequence. For example, in one embodiment, one or more amino acids in the human framework regions of the VH and/or VL of the humanized antibodies provided herein are back mutated to the corresponding amino acid in the parent murine antibody. As an example, as for VH and VL of 7A4 and 13F1, several sites of framework amino acid of the aforementioned template human antibody were back mutated to the corresponding amino acid sequences in mouse 7A4 and 13F1 antibody. In one embodiment, the amino acid at positions 40 and/or 45 and/or 70 and/or 72 of the light chain variable region is back mutated to the corresponding amino acid found at that position in the mouse 7A4 or 13F1 light chain variable region. In another embodiment, the amino acid at positions 2 and/or 26 and/or 46 and/or 48 and/or 49 and/or 67 and/or 70 and/or 71 of the heavy chain variable region is back mutated to the corresponding amino acid found at that position in the mouse 7A4 or 13F1 heavy chain variable region. In one embodiment, the humanized 7A4 antibody comprises a light chain variable region wherein the amino acid at position 40 is mutated from Tyr (Y) to Phe (F) and the amino acid at position 72 is mutated from Gly (G) to Arg (R); and a heavy chain variable region wherein the amino acid at position 2 is mutated from Val (V) to Ile (I), the amino acid at position 46 is mutated from Glu (E) to Lys (K), and the amino acid at position 70 is mutated from Phe (F) to Ile (I). In one embodiment, the humanized 13F1 antibody comprises a light chain variable region wherein the amino acid at position 45 is mutated from Leu (L) to Pro (P) and the amino acid at position 70 is mutated from Phe (F) to Tyr (Y); and a heavy chain variable region wherein the amino acid at position 26 is mutated from Gly (G) to

Tyr (Y), the amino acid at position 48 is mutated from Ile (I) to Met (M), the amino acid at position 49 is mutated from Gly (G) to Ala (A), the amino acid at position 67 is mutated from Val (V) to Ile (I), and the amino acid at position 71 is mutated from Val (V) to Arg (R). Additional or alternate back mutations may be made in the framework regions of the humanized antibodies provided herein in order to improve the properties of the antibodies.

[0071] The present invention also encompasses humanized antibodies that bind to PD-1 and comprise framework modifications corresponding to the exemplary modifications described herein with respect to any suitable framework sequence, as well as other framework modifications that otherwise improve the properties of the antibodies. For example, in some embodiments, the antibodies provided herein comprise one or more mutations to remove one or more deamidation sites or one or more oxidation sites. For example, in one embodiment, the antibodies provided herein comprise a mutation of one or more asparagine residues to remove one or more deamidation sites; and/or mutation of one or more methionine residues to remove one or more oxidation sites.

[0072] In other embodiments, the antibodies provided herein comprise one or more mutations to improve stability, improve solubility, alter glycosylation, and/or reduce immunogenicity, such as, for example, by targeted amino acid changes that reduce deamidation or oxidation, reduce isomerization, optimize the hydrophobic core and/or charge cluster residues, remove hydrophobic surface residues, optimize residues involved in the interface between the variable heavy and variable light chains, and/or modify the isoelectric point.

[0073] As used herein, the term “derived” when used to refer to a molecule or polypeptide relative to a reference antibody or other binding protein, means a molecule or polypeptide that is capable of binding with specificity to the same epitope as the reference antibody or other binding protein.

[0074] The antibodies and antigen-binding fragments thereof disclosed herein are specific for PD-1. In one embodiment, the antibodies and fragments thereof are specific for human PD-1. In one embodiment, the antibodies and fragments provided herein bind to human or primate PD-1 but not to PD-1 from any other mammal. In a further embodiment, the antibodies and fragments thereof do not bind to mouse PD-1. The terms “human PD-1,” “hPD-1”, and “huPD-1” and the like are used interchangeably herein and refer to human PD-1 and variants or isoforms of human PD-1. By “specific for” is meant that the antibodies and fragments thereof bind PD-1 receptor

with greater affinity than any other target. In one embodiment, the PD-1 antibodies and fragments provided herein are specific for PD-1 and do not cross react with CTLA4, ICOS, or CD28. As used herein, the term "EC50" refers to the effective concentration, 50% maximal response of the antibody. As used herein, the term "IC50" refers to the inhibitory concentration, 50% maximal response of the antibody. Both EC50 and IC50 may be measured by ELISA or FACS analysis, or any other method known in the art.

[0075] In one embodiment, the anti-PD1 antibodies and fragments or variants thereof have an affinity (KD) for PD-1 in the range of about 0.001 nM to about 100 nM, about 0.002 nM to about 50 nM, about 0.005 nM to about 5 nM, about 0.01 nM to about 1 nM, or about 0.05 nM to about 0.1 nM. In one embodiment, the antibodies and fragments thereof have an affinity (KD) for PD-1 of about 50 nM or less, about 25 nM or less, about 20 nM or less, about 15 nM or less, about 10 nM or less, about 8 nM or less, about 6 nM or less, about 4 nM or less, about 2 nM or less, about 1 nM or less, about 0.9 nM or less, about 0.8 nM or less, about 0.7 nM or less, about 0.6 nM or less, about 0.5 nM or less, about 0.4 nM or less, about 0.3 nM or less, about 0.2 nM or less, about 0.1 nM or less, about 0.09 nM or less, about 0.08 nM or less, about 0.07 nM or less, about 0.06 nM or less, about 0.05 nM or less, about 0.04 nM or less, about 0.03 nM or less, about 0.02 nM or less, about 0.01 nM or less, about 0.009 nM or less, about 0.008 nM or less, about 0.007 nM or less, about 0.006 nM or less, about 0.005 nM or less, about 0.004 nM or less, about 0.003 nM or less, about 0.002 nM or less, or about 0.001 nM or less. In one embodiment, the antibodies and fragments thereof have an affinity (KD) for PD-1 of about 10 nM, about 9 nM, about 8 nM, about 7 nM, about 6 nM, about 5 nM, about 4 nM, about 3 nM, about 2 nM, about 1 nM, about 0.9 nM, about 0.8 nM, about 0.7 nM, about 0.6 nM, about 0.5 nM, about 0.4 nM, about 0.3 nM, about 0.2 nM, about 0.1 nM, about 0.09 nM, about 0.08 nM, about 0.07 nM, about 0.06 nM, about 0.05 nM, about 0.04 nM, about 0.03 nM, about 0.02 nM, about 0.01 nM, about 0.009 nM, about 0.008 nM, about 0.007 nM, about 0.006 nM, about 0.005 nM, about 0.004 nM, about 0.003 nM, about 0.002 nM, or about 0.001.

[0076] In one embodiment, the antibodies and fragments provided herein comprise a light chain and a heavy chain, each of which comprises three CDR regions. Exemplary light chain CDR sequences (LCDR1, LCDR2, and LCDR3) for PD-1 antibodies of the invention are provided below in **Table 1**. Exemplary heavy chain CDR sequences (HCDR1, HCDR2, and HCDR3) for

PD-1 antibodies of the invention are provided below in **Table 2**. Exemplary variable regions and full antibody sequences for PD-1 antibodies of the invention are provided below in **Table 3**.

Table 1. Light Chain CDR sequences

Name	LCDR	SEQ ID NO	Sequence
10D1	1	24	RASQSISNNLH
	2	25	YASQSIS
	3	26	QQSNSWPLT
4C10	1	34	KASQSVSDDVA
	2	35	YAFNRYT
	3	36	QQDYRSPWT
7D3	1	45	RASQSISNDLH
	2	46	YVSQSIS
	3	47	QQSDSWPLT
13F1	1	55	RANSSVSSMH
	2	56	AISNLAF
	3	57	QQWSSRPPT
15H5	1	65	HASQSINVWLS
	2	66	ASNLHT
	3	67	QQGQSYPWT
14A6	1	75	RANSSVSSMH
	2	76	AISNLAF
	3	77	QQWNSRPPT
22A5	1	85	KASQDVDNAVA
	2	86	WASTRHH
	3	87	QQYSTFPYT

6E1	1	95	RASQSLSNNLH
	2	96	YASQSIS
	3	97	QQSNSWPLT
5A8	1	105	KASQSVSNDVA
	2	106	YAFTRYI
	3	107	QQDYSSPYT
7A4	1	115	RASESVDNYGYSFMN
	2	116	RASNLES
	3	117	QQSNADPT

Table 2. Heavy chain CDR sequences

Name	HCDR	SEQ ID NO	Sequence
10D1	1	19	SYGMS
	2	20	TMSGGGRDIYYPDSMKG
	3	21	QYYDDWFAY
4C10	1	29	TYGVH
	2	30	VIWSGGSTDYNAAFIS
	3	31	EKSVYGNYVGAMDY
7D3	1	40	SYGMS
	2	41	TISGGGRDIYYPDSVKG
	3	42	QYYDDWFAY
13F1	1	50	SDYAWN
	2	51	YISYSGYTSYNPSLKS
	3	52	SLDYDYGTMDY

15H5	1	60	SYDMS
	2	61	TISGGGSYTYYQDSVKG
	3	62	PYGPYFDY
14A6	1	70	SDYAWN
	2	71	YISYSGYTSYNPSLKS
	3	72	SLDYDYGTMDY
22A5	1	80	YYDMS
	2	81	TISGGGRNTYFIDSVKG
	3	82	PYEGAVDF
6E1	1	90	SYGMS
	2	91	TISGGGRDTYYLDSVKG
	3	92	QYYDDWFAY
5A8	1	100	NNWIG
	2	101	DFYPGGGYTNYNEKFKG
	3	102	GYGTNYWYFDV
7A4	1	110	NFGMN
	2	111	WISGYTREPTYAADFKG
	3	112	DVFDY

Table 3. Light chain and heavy chain variable region sequences and full antibody sequences

Name	Region[1]	SEQ ID NO	Sequence
10D1 murine	Light chain variable	23	D I V L T Q T P A T L S V T P G D S V S L S C R A S Q S I S N N L H W Y Q Q K S H E S P R L L I K Y A S Q S I S G I P S R F S G S G S G T D F T L N I N S V E T E D F G M Y F C Q Q S N S W P L T F G A G T K L E L K R

10D1 murine	Heavy chain variable	18	EVKLVESGGGLVKPGGSLKLSCAASGFTFSSYG MSWLRQTPEKRLEWVATMSGGGRDIYYPDSMK GRFTISRDNAKNNLYLQMSSLRSEDTALYYCAR QYYDDWFAYWGQGTLVTVSA
4C10 murine	Light chain variable	33	SIVMTQTPKFLLVSAGDRVTITCKASQSVSDDVA WYQQKPGQSPKLLIYYAFNRYTGVPDRFTGSGY GTDFTFTISTVQSEDLAVYFCQQDYRSPWTFGGG TKLEIKR
4C10 murine	Heavy chain variable	28	QVQLKQSGPGLVQPSQNLSVTCTVSGFSLTTYG VHWVRQSPGKGLEWLGVIWSGGSTDYNAAFISR LTISKDNARSQVFFKMNSLQVNDTAMYYCAREK SVYGNYVGAMDYWGQGTSVTVSS
7D3 murine	Light chain variable	44	DIVLTQSPATLSVTPGDSVSLSCRASQSISNDLH WYQQKSHESPRLLIKYVSQSISGIPSRFSGSGSGT DFTLSINSVETEDFGMYFCQQSDSWPLTFGAGTK LELKR
7D3 murine	Heavy chain variable	39	EVKLVESGGGLVKPGGSLKLSCGASGFTFSSYG MSWVRQTPEKRLEWVATISGGGRDIYYPDSVKG RLTISRDNAKNNLYLQMSSLRSEDTALYYCVRQ YYDDWFAYWGQGTLVTVSA
13F1 murine	Light chain variable	54	QIVLSQSPAILSASPGEKVTMTCRANSSVSSMHW YQQKPGSSPEPWIYAISNLAFGVPTRFSGSGSGT SYSLTISRVEAEDAATYFCQQWSSRPPTFGGGTK LEIKR
13F1 murine	Heavy chain variable	49	DVQLQESGPGLVKPSQSLSLTCTVTGYSITSDYA WNWIRQFPGNQLEWMAYISYSGYTSYNPSLKSR ISITRDTSKNQFFLQLNSVTTEDTATYYCARSLD YDYGTMDYWGQGTSVTVSS
15H5 murine	Light chain variable	64	DIQMNQSPSSLSASLGDTITITCHASQSINVWLS WYQQKPGNIPKLLIYRASNLHTGVPSRFSGSGSG TGFTLTISSLQPDDIATYYCQQGQSYPWTFGGGT KLEIKR
15H5 murine	Heavy chain variable	59	EVKLVESGGGLVKPGGSLKLSCAASGFAFRSYD MSWVRQTPEKILEWVATISGGGSYTYYQDSVKG RFTISRDNARNTLYLQMSSLRSEDTALYYCASPY GPYFDYWGQGTTLTVSS
14A6 murine	Light chain variable	74	QIVLSQSPAILSASPGEKVTMTCRANSSVSSMHW YQQKPGSSPEPWIYAISNLAFGVPARFSGSGSGT SYSLTISRVEAEDAATYFCQQWNSRPPTFGGGTK LEIKR
14A6 murine	Heavy chain variable	69	DVQLQESGPGLVKPSQSLSLTCTVTGYSITSDYA WNWIRQFPGNQLEWMAYISYSGYTSYNPSLKSR ISITRDTSRNQFFLQLNSVTTEDTATYYCARSLD YDYGTMDYWGQGTSVTVSS
22A5	Light chain	84	DIVMTQSHKVMSTSVGDRVSITCKASQDVDNAV AWYQQNPGQSPKLLIKWASTRHHGVPDRFTGSG

murine	variable		SGTDFTLTISTVQSEDLADFFCQQYSTFPYTFGG GTKLEIKR
22A5 murine	Heavy chain variable	79	EVKLVESGGGLVKPGGSLKLSCSASGFSFSYYD MSWVRQTPEKGLEWVATISGGGRNTYFIDSVKG RFTISRDNVKNNLYLLMSSLRSEDTALYYCASPY EGAVDFWGQGTSVTVSS
6E1 murine	Light chain variable	94	DIVLTQTPATLSVTPGDSVSLSCRASQSLSNNLH WYQQKSHESPRLLIKYASQSISGIPSRFSGSGSGT DFTLSINSVETEDFGMYFCQQSNSWPLTFGAGTK LEMKR
6E1 murine	Heavy chain variable	89	EVKLVESGGGLVKPGGSLKLSCAASGFTFSSYG MSWVRQTPEKRLEWVATISGGGRDTYYLDSVKG RFTISRDNAKNNLYLQMSSLRSEDTALYYCVRQ YYDDWFAYWGQGTLVSNSA
5A8 murine	Light chain variable	104	NIVMTQTPKILFISAGDRVTITCKASQSVSNDVA WYQQKPGQSPKLLIYYAFTRYIGVPDRFTGSGY GTDFTFTISTVQAEDLAVYFCQQDYSSPYTFGGG TKLEIKR
5A8 murine	Heavy chain variable	99	QVQLQQSGDELVRPGTSVKMSCKAAGYTFTNN WIGWVKQRPGHGLEWIGDFYPGGGYTNYNEKF KGKATLTADTSSSTAYMQLSSLTSEDSAIYYCAR GYGTNYWYFDVWGAGTTVTVSS
7A4 murine	Light chain variable	114	DIVLTQSPASLAVSLGQRATISCRASESVDNYGY SFMNWFQQKPGQPPKLLIYRASNLESGIPARFSG SGSRTNFTLTINPVEADDVATYFCQQSNADPTFG GGTNLEIKRA
7A4 murine	Heavy chain variable	109	QIHLVQSGPELKKPGETVKISCKASGYTFTNFGM NWVKQAPGKGLKWMGWISGYTREPTYAADFKG RFAISLETSASTAYLQINDLKNEDMATYFCARDV FDYWGQGTTLTVSS
7A4 chimeric	Full length heavy chain IgG1	119	QIHLVQSGPELKKPGETVKISCKASGYTFTNFGMNWVKQAPGKGLKWMGW ISGYTREPTYAADFKGRFAISLETSASTAYLQINDLKNEDMATYFCARDV FDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
7A4 chimeric	Full length heavy chain IgG4	121	QIHLVQSGPELKKPGETVKISCKASGYTFTNFGMNWVKQAPGKGLKWMGW ISGYTREPTYAADFKGRFAISLETSASTAYLQINDLKNEDMATYFCARDV FDYWGQGTTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
7A4	Full length	123	DIVLTQSPASLAVSLGQRATISCRASESVDNYGYSFMNWFQQKPGQPPKL LIYRASNLESGIPARFSGSGSRTNFTLTINPVEADDVATYFCQQSNADPT

chimeric	light chain		FGGGTNLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
13F1 chimeric	Full length heavy chain IgG1	125	DVQLQESGPGLVKPSQSLSLTCTVTGYSITSDYAWNWIRQFPGNQLEWMA YISYSGYTSYNPSLKSRISITRDTSKNQFFLQLNSVTTEDTATYYCARSL DYDYGTMDYWGQGTSVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
13F1 chimeric	Full length heavy chain IgG4	127	DVQLQESGPGLVKPSQSLSLTCTVTGYSITSDYAWNWIRQFPGNQLEWMA YISYSGYTSYNPSLKSRISITRDTSKNQFFLQLNSVTTEDTATYYCARSL DYDYGTMDYWGQGTSVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLSLG
13F1 chimeric	Full length light chain	129	QIVLSQSPAILSASPGEKVTMTCRANSSVSSMHWYQQKPGSSPEPWIYAI SNLAFGVPTRFSGSGSGTSYSLTISRVEAEDAATYFCQQWSSRPPTFGGG TKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
7A4 humanized	Heavy chain variable	131	QIQLVQSGSELKKPGASVKVSCKASGYTFTNFGMNWVRQAPGQGLKWMGW ISGYTREPTYAADFKGRFVISLDTSVSTAYLQISSLKAEDTAVYYCARDV FDYWGQGTLVTVSS
7A4 humanized	Light chain variable	133	DIVLTQSPASLAVSPGQRATITCRASESVDNYGYSFMNWFQQKPGQPPKL LIYRASNLESGVPARFSGSGSRTDFTLTINPVEANDTANYYCQQSNADPT FGQGTKLEIK
13F1 humanized	Heavy chain variable	141	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDYAWNWIRQPPGKGLEWMA YISYSGYTSYNPSLKSRITISRDTSKNQFSLKLSSVTAADTAVYYCARSL DYDYGTMDYWGQGTLVTVSS
13F1 humanized	Light chain variable	143	EIVLTQSPATLSLSPGERATLSCRANSSVSSMHWYQQKPGQSPEPWIYAI SNLAFGVPARFSGSGSGTDYTLTISSLEPEDFAVYYCQQWSSRPPTFGQG TKLEIK
7A4 humanized -IgG1 (D265A)	Full heavy chain	135	QIQLVQSGSELKKPGASVKVSCKASGYTFTNFGMNWVRQAPGQGLKWMGW ISGYTREPTYAADFKGRFVISLDTSVSTAYLQISSLKAEDTAVYYCARDV FDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

7A4 humanized – IgG4 (F234A/ L235A)	Full heavy chain	137	QIQLVQSGSELKKPGASVKVSCKASGYTFTNFGMNWVRQAPGQGLKWMGW ISGYTREPTYAADFKGRFVISLDTSVSTAYLQISSLKAEDTAVYYCARDV FDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
7A4 humanized	Full light chain	139	DIVLTQSPASLAVSPGQRATITCRASESVDNYGYSFMNWFQQKPGQPPKL LIYRASNLESGVPARFSGSGSRTDFTLTINPVEANDTANYYCQQSNADPT FGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
13F1 humanized -IgG1 (D265A)	Full heavy chain	145	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDYAWNWIRQPPGKGLEWMA YISYSGYTSYNPSLKSRITISRDTSKNQFSLKLSSVTAADTAVYYCARSL DYDYGTMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
13F1 humanized – IgG4 (F234A/ L235A)	Full heavy chain	147	QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDYAWNWIRQPPGKGLEWMA YISYSGYTSYNPSLKSRITISRDTSKNQFSLKLSSVTAADTAVYYCARSL DYDYGTMDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
13F1 humanized	Full light chain	149	EIVLTQSPATLSLSPGERATLSCRANSSVSSMHWYQQKPGQSPEPWIYAI SNLAFGVPARFSGSGSGTDYTLTISSLEPEDFAVYYCQQWSSRPPTFGQG TKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
Human IgG1 constant region	D265A mutation	150	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Human IgG4 constant region	F234A and L235A double mutation	151	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVES KYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLSLG

7A4D humanized	Light chain variable	152	DIVLTQSPASLAVSPGQRATITCRASESVDNYGYSFMNWFQQKPGQPPKL LIYRASNLESGVPARFSGSGSRTDFTLTINPVEADDTANYYCQQSNADPT FGQGTKLEIK
7A4D humanized	Full light chain	153	DIVLTQSPASLAVSPGQRATITCRASESVDNYGYSFMNWFQQKPGQPPKL LIYRASNLESGVPARFSGSGSRTDFTLTINPVEADDTANYYCQQSNADPT FGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC

[0077] In one embodiment, the invention provides anti-PD-1 antibodies that comprise the light chain CDRs and heavy chain CDRs of antibodies 10D1, 4C10, 7D3, 13F1, 15H5, 14A6, 22A5, 6E1, 5A8, and/or 7A4. The person of skill in the art will understand that the heavy and light chain CDRs of the antibodies provided herein may be independently selected, or mixed and matched, to form an antibody or binding fragment thereof comprising any light chain CDR1, CDR2, and CDR3; and any heavy chain CDR1, CDR2, and CDR3 from the antibodies provided herein. Thus, the invention provides anti-PD-1 antibodies that comprise a light chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 34, 45, 55, 65, 75, 85, 95, 105, and 115; a light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 25, 35, 46, 56, 66, 76, 86, 96, 106, and 116; a light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26, 36, 47, 57, 67, 77, 87, 97, 107, and 117; a heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 29, 40, 50, 60, 70, 80, 90, 100, and 110; a heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 30, 41, 51, 61, 71, 81, 91, 101, and 111; and a heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 21, 31, 42, 52, 62, 72, 82, 92, 102, and 112. In one embodiment, the present invention pvovides anti-PD-1 antibodies comprising heavy and light chain CDR regions comprising amino acid sequences having at least 75%, at least 80%, at least at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homology to the corresponding light or heavy chain CDR1, CDR2, or CDR3 provided herein. In one embodiment, the present invention pvovides anti-PD-1 antibodies comprising heavy and light chain CDR regions comprising amino acid sequences having 1, 2, 3, 4, 5, or 6 amino acid substitutions, deletions, or insertions relative to the corresponding light or heavy chain CDR1, CDR2, or CDR3 provided herein.

[0078] In one embodiment, the invention provides anti-PD-1 antibodies that comprise a variable light chain of an antibody selected from the group consisting of 10D1, 4C10, 7D3, 13F1, 15H5, 14A6, 22A5, 6E1, 5A8, 7A4 and 7A4D and a variable heavy chain of an antibody selected from the group consisting of 10D1, 4C10, 7D3, 13F1, 15H5, 14A6, 22A5, 6E1, 5A8, and 7A4. In one embodiment, the antibodies and fragments provided herein comprise a light chain variable region comprising an amino acid sequence that is at least 75%, at least 80%, at least at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homology to a light chain variable region according to SEQ ID NOs: 23, 33, 44, 54, 64, 74, 84, 94, 104, 114, 133, 143 and 152. In one embodiment, the antibodies and fragments provided herein comprise a light chain variable region comprising an amino acid sequence according to SEQ ID NO: 23, 33, 44, 54, 64, 74, 84, 94, 104, 114, 133, 143, 152, or a variant thereof, wherein the variant comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions or deletions, or a combination thereof. In a further embodiment, the amino acid substitutions are conservative substitutions. In another embodiment, the amino acid substitutions improve the properties of the antibodies as provided herein, for example, by removing a deamidation site. For example, in one embodiment, an asparagine (Asn; N) residue is mutated. In a further embodiment, the Asn is mutated to aspartic acid (Asp; D). In a yet further embodiment, the Asn at position 85 in framework region 3 of the light chain variable region is mutated to Asp. In one embodiment, the present disclosure provides humanized antibody 7A4D, which comprises the same amino acid sequence as humanized antibody 7A4 except with a mutation in framework 3 (position 85) of the light chain to remove the deamidation site.

[0079] In one embodiment, the antibodies and fragments provided herein comprise a heavy chain variable region comprising an amino acid sequence that is at least 75%, at least 80%, at least at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homology to a light chain variable region according to SEQ ID NOs: 18, 28, 39, 49, 59, 69, 79, 89, 99, 109, 131, and 141, or 84. In one embodiment, the antibodies and fragments provided herein comprise a heavy chain variable region comprising an amino acid sequence according to SEQ ID NO: 18, 28, 39, 49, 59, 69, 79, 89, 99, 109, 131, 141, or a variant thereof, wherein the variant comprises 1, 2, 3,

4, 5, 6, 7, 8, 9, 10, or more amino acid substitutions, insertions, or deletions, or a combination thereof. In a further embodiment, the amino acid substitutions are conservative substitutions. In another embodiment, the amino acid substitutions improve the properties of the antibodies as provided herein, for example, by removing a deamidation site. For example, in one embodiment, an asparagine (Asn; N) residue is mutated. In a further embodiment, the Asn is mutated to aspartic acid (Asp; D).

[0080] The anti-PD-1 antibodies disclosed herein having one or more amino acid substitution, insertion, deletion, or combination thereof in the CDR or variable light or heavy chain region retain the biological activity of the corresponding anti-PD-1 antibody that does not have an amino acid substitution, insertion, or deletion. Thus, the variant anti-PD-1 antibodies provided herein retain binding to PD-1. Percent homology, as used herein, refers to the number of identical amino acid sequences shared by two reference sequences, divided by the total number of amino acid positions, multiplied by 100.

[0081] In some embodiments, the anti-PD-1 antibodies provided herein comprise conservative amino acid substitutions. The person of skill in the art will recognize that a conservative amino acid substitution is a substitution of one amino acid with another amino acid that has a similar structural or chemical properties, such as, for example, a similar side chain. Exemplary conservative substitutions are described in the art, for example, in Watson *et al.*, *Molecular Biology of the Gene*, The Bengamin/Cummings Publication Company, 4th Ed. (1987).

[0082] The skilled person will understand that the variable light and variable heavy chains may be independently selected, or mixed and matched, from the antibodies provided herein. Thus, the present invention provides anti-PD-1 antibodies comprising a light chain variable region having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 23, 33, 44, 54, 64, 74, 84, 94, 104, 114, 133, 143, and 152; and a heavy chain variable region having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 28, 39, 49, 59, 69, 79, 89, 99, 109, 131, and 141.

[0083] In one embodiment, the present invention provides antibodies that bind to the same epitope as any one of the exemplary antibodies disclosed herein. Thus, in one embodiment, the present invention provides antibodies that compete for binding to PD-1 with the exemplary antibodies provided herein.

[0084] The anti-PD-1 antibodies and fragments thereof provided herein may further comprise Fc region modifications to alter effector functions. Fc modifications may be amino acid insertions, deletions, or substitutions, or may be chemical modifications. For example, Fc region modifications may be made to increase or decrease complement binding, to increase or decrease antibody-dependent cellular cytoxicity, or to increase or decrease the half life of the antibody. Some Fc modifications increase or decrease the affinity of the antibody for an Fcγ receptor such as FcγRI, FcγRII, FcγRIII, or FcRn. Various Fc modifications have been described in the art, for example, in Shields et al., *J Biol. Chem* 276; 6591 (2001); Tai et al. *Blood* 119; 2074 (2012); Spiekermann et al. *J Exp. Med* 196; 303 (2002); Moore et al. *mAbs* 2:2; 181 (2010); Medzihradsky *Methods in Molecular Biology* 446; 293 (2008); Mannan et al. *Drug Metabolism and Disposition* 35; 86 (2007); and Idusogie et al. *J Immunol* 164; 4178 (2000). In some embodiments, Fc region glycosylation patters are altered. In other embodiments, the Fc region is modified by pegylation (e.g., by reacting the antibody or fragment thereof with polyethylene glycol (PEG).

[0085] In one embodiment, the antibodies or fragments thereof provided herein are immunoconjugates comprising an anti-PD-1 antibody or fragment thereof and further comprising an agent selected from the group including an additional therapeutic agent, a cytotoxic agent, an immunoadhesion molecule, and an imaging agent. In some embodiments, the imaging agent is selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin. In some embodiments, the imaging agent is a radiolabel selected from the group consisting of: ^{3}H, ^{14}C, ^{35}S, ^{62}, ^{64}Cu, ^{89}Zr, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm. In some embodiments, the therapeutic agent or cytotoxic agent is selected from the group including a chemotherapeutic agent, an immunosuppressive agent, an immuno-stimulatory agent, an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, a toxin, and an apoptotic agent. In some embodiments, the binding protein is conjugated directly to the agent. In other embodiments, the binding protein is conjugated to the agent via a linker. Suitable linkers include, but are not limited to, amino acid and polypeptide linkers disclosed herein. Linkers may be cleavable or non-cleavable.

[0086] In one embodiment, the present invention provides bispecific or multispecific antibodies specific for PD-1 and at least one other antigen or epitope. The anti-PD-1 antibodies and

fragments thereof provided herein may be tested for binding to PD-1 using the binding assays provided herein, or any other binding assay known in the art.

[0087] Unless otherwise stated, the practice of the present invention employs conventional molecular biology, cell biology, biochemistry, and immunology techniques that are well known in the art and described, for example, in Methods in Molecular Biology, Humana Press; Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989), Current Protocols in Immunology (J. E. Coliganet al., eds., 1991); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Phage display: a laboratory manual (C. Barbas III et al, Cold Spring Harbor Laboratory Press, 2001); and Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999).

[0088] In one aspect the present invention provides methods for treating a subject for a disease or condition responsive to enhancing, stimulating, or eliciting an immune response. As used herein, the terms "treatment" or "treating" refers to both therapeutic treatment and prophylactic or preventive measures. Subjects in need of treatment include those subjects that already have the disease or condition, as well as those that may develop the disease or condition and in whom the object is to prevent, delay, or diminish the disease or condition. As used herein, the term "subject" denotes a mammal, such as a rodent, a feline, a canine, and a primate. Preferably, a subject according to the invention is a human.

[0089] The term "therapeutically effective amount," as used herein, refers to the amount of a compound or composition that is necessary to provide a therapeutic and/or preventative benefit to the subject.

[0090] In one aspect, the antibodies and antigen binding fragments thereof are useful in the treatment of solid or non-solid tumors. Thus, in one aspect, the present invention provides methods for treatment of cancer. "Cancer" as used herein refers to the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to carcinoma, lymphoma, blastoma, sarcoma (including liposarcoma, osteogenic sarcoma, angiosarcoma, endotheliosarcoma, leiomyosarcoma, chordoma, lymphangiosarcoma, lymphangioendotheliosarcoma, rhabdomyosarcoma, fibrosarcoma, myxosarcoma, chondrosarcoma), neuroendocrine tumors, mesothelioma, synovioma,

schwanoma, meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), Hodgkin's lymphoma; non-Hodgkin's lymphomas (Burkitt's lymphoma, small lymphocytic lymphoma/chronic lymphocytic leukemia, mycosis fungoides, mantle cell lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, marginal zone lymphoma, hairy cell leukemia and lymphoplasmacytic leukemia), tumors of lymphocyte precursor cells, including B-cell acute lymphoblastic leukemia/lymphoma, and T-cell acute lymphoblastic leukemia/lymphoma, thymoma, tumors of the mature T and NK cells, including peripheral T-cell leukemias, adult T-cell leukemia/T-cell lymphomas and large granular lymphocytic leukemia, Langerhans cell histocytosis, myeloid neoplasias such as acute myelogenous leukemias, including AML with maturation, AML without differentiation, acute promyelocytic leukemia, acute myelomonocytic leukemia, and acute monocytic leukemias, myelodysplastic syndromes, and chronic myeloproliferative disorders, including chronic myelogenous leukemia,B-cell acute lymphoblastic leukemia/lymphoma, T-cell acute lymphoblastic leukemia/lymphoma, lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, small cell lung carcinoma, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, testicular cancer, esophageal cancer, tumors of the biliary tract, Ewing's tumor, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, testicular tumor, lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemia, lymphoma, multiple myeloma, Waldenstrom's macroglobulinemia, myelodysplastic disease, heavy chain disease, neuroendocrine tumors, Schwanoma, and other carcinomas, as well as head and neck cancer.

[0091] In one embodiment, the antibodies and fragments thereof provided herein are useful in the treatment of diseases caused by infectious agents. Infectious agents include, but are not limited to, bacterial, mycological, parasitic, and viral agents. Examples of such infectious agents include the following: staphylococcus, methicillin-resistant staphylococcus aureus, Escherichia coli, streptococcaceae, neisseriaaceae, cocci, enterobacteriaceae, enterococcus, vancomycin-resistant enterococcus, cryptococcus, histoplasmosis, aspergillus, pseudomonadaceae, vibrionaceae, campylobacter, pasteurellaceae, bordetella, francisella, brucella, legionellaceae, bacteroidaceae, gram-negativebacilli, clostridium, corynebacterium, propionibacterium, gram-positive bacilli, anthrax, actinomyces, nocardia, mycobacterium, treponema, borrelia, leptospira, mycoplasma, ureaplasma, rickettsia, chlamydiae, candida, systemic mycoses, opportunistic mycoses, protozoa, nematodes, trematodes, cestodes, adenoviruses, herpesviruses (including, for example, herpes simplex virus and Epstein Barr virus, and herpes zoster virus), poxviruses, papovaviruses, hepatitis viruses, (including, for example, hepatitis B virus and hepatitis C virus), papilloma viruses, orthomyxoviruses (including, for example, influenza A, influenza B, and influenza C), paramyxoviruses, coronaviruses, picornaviruses, reoviruses, togaviruses, flaviviruses, bunyaviridae, rhabdoviruses, rotavirus, respiratory syncitial virus, human immunodeficiency virus and retroviruses. Exemplary infectious diseases include but are not limited to candidiasis, candidemia, aspergillosis, streptococcal pneumonia, streptococcal skin and oropharyngeal conditions, gram negative sepsis, tuberculosis, mononucleosis, influenza, respiratory illness caused by Respiratory Syncytial Virus, malaria, schistosomiasis, and trypanosomiasis.

[0092] In one embodiment, the antibodies and fragments thereof provided herein are useful in the treatment of diseases mediated by T-helper type 2 (Th2) T cells, such as, for example, asthma, allergy, or graft versus host disease.

[0093] In one embodiment, the antibodies and fragments thereof provided herein are useful in for the stimulation of an immune response in a subject in need thereof. For example, in one embodiment, the anti-PD-1 antibodies and fragments thereof may be administered in conjunction with an antigen of interest for the purpose of eliciting an immune response to said antigen. An antigen of interest may be an antigen associated with a pathogen such as a virus or bacterium. Thus, in one embodiment, the present invention provides a vaccine comprising an anti-PD-1 antibody and an antigen, wherein the vaccine elicits an antigen-specific immune response.

[0094] In one embodiment, the anti-PD-1 antibodies provided herein modulate regulatory T cell function. CD4+ CD25+ regulatory T cells are lymphocytes that suppress or reduce the effects of effector T cell functions. The terms "regulatory T cell" and "Treg" are used interchangeably herein. In one embodiment, the anti-PD-1 antibodies provided herein prevent or reverse the inhibitory effects of regulatory T cells on effector T cell cytokine production. For example, in one embodiment, the anti-PD-1 antibodies provided herein restore the capacity for IFNγ production to effector T cells in contact with regulatory T cells.

[0095] In one embodiment, the antibodies and fragments thereof disclosed herein may be administered to the subject by at least one route selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intratympanic, intrauterine, intravesical, intravitreal, bolus, subconjunctival, vaginal, rectal, buccal, sublingual, intranasal, intratumoral, and transdermal.

[0096] In one embodiment, the antibodies and fragments thereof disclosed herein may be administered to a subject in need thereof in combination with one or more additional therapeutic agent. In one embodiment, the antibodies and fragments thereof may be administered to a subject before, during, and/or after administration to the subject of the additional therapeutic agent. In one embodiment, the additional therapeutic agent is a chemotherapeutic agent, radiotherapeutic agent, cytokine, antibody or fragment thereof, or any other additional therapeutic that is indicated for the disease to be treated. In one embodiment, the anti-PD-1 antibody and the additional therapeutic agent exhibit therapeutic synergy when administered together, whether concurrently or sequentially. In one embodiment, the anti-PD-1 antibody and the additional therapeutic agent are administered in separate formulations. In another embodiment, the anti-PD-1 antibody and the additional therapeutic agent are administered in the same formulation. In one embodiment, the anti-PD-1 antibodies and fragments provided herein enhance the immune modulating effect of the one or more additional therapeutic agent. In another embodiment, the one or more additional therapeutic agent enhances the effect of the anti-PD-1 antibody or fragment thereof.

[0097] The present invention provides isolated antibodies and antigen binding fragments thereof, and nucleic acids encoding such antibodies and fragments, as well as compositions comprising such isolated antibodies, fragments, and nucleic acids. The term "isolated" refers to a compound of interest (e.g., an antibody or nucleic acid) that has been separated from its natural environment. The present invention further provides pharmaceutical compositions comprising the isolated antibodies or fragments thereof, or nucleic acids encoding such antibodies or fragments, and further comprising one or more pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers include, for example, excipients, diluents, encapsulating materials, fillers, buffers, or other agents.

[0098] The use of the singular includes the plural unless specifically stated otherwise. The word "a" or "an" means "at least one" unless specifically stated otherwise. The use of "or" means "and/or" unless stated otherwise. The meaning of the phrase "at least one" is equivalent to the meaning of the phrase "one or more." Furthermore, the use of the term "including," as well as other forms, such as "includes" and "included," is not limiting. Also, terms such as "element" or "component" encompass both elements or components comprising one unit and elements or components comprising more than one unit unless specifically stated otherwise.

[0099] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims. The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of non-critical parameters that could be changed or modified to yield essentially similar results.

EXAMPLES

Example 1 –Mouse Immunization and Production of Mouse Antibodies Against human PD-1

[00100] To generate antibodies against the human PD-1, cDNAs encoding the open reading frame of the extracellular domain of hPD-1 fused with a histidine tag (hPD-1-HisTag, SEQ ID NO:1), mouse Fc (hPD-L1-mFc, SEQ ID NO:13), and human Fc tag (hPD-1-hFc, SEQ

ID NO:5) were obtained by PCR and subcloned into expression vector pcDNA3.1 (Invitrogen CAT#:V-790), respectively. After transient expression in freestyle 293 cells, hPD-1-HisTag was purified with NTA column (GE healthcare), hPD-1-mFc and hPD-1-hFc were purified with Protein G column (GE healthcare).

[00101] To immunize mice necessary for generating hybridoma cell lines, 100 μg of human PD-1-mouse Fc fusion protein or and a complete Freund's adjuvant in the same amount were mixed, and the mixture was administered via an subcutaneous injection to each of five 6 to 7-week-old BALB/c mice. After two weeks, the antigen (half the previously injected amount) was mixed with an incomplete Freund's adjuvant using the same method as described above, and the mixture was administered to each mouse via subcutaneous injection. After one week, final boosting was performed, and blood was collected from the tail of each mouse after three days to obtain serum. Then, serum was diluted at 1/1000 with PBS, and an ELISA was performed to analyze whether the titer of the antibody recognizing human PD-1-mFc increased. Afterwards, mice in which a sufficient amount of the antibody was obtained were selected, and a cell fusion process was performed on the selected mice.

[00102] Three days before a cell fusion experiment, a mixture of 50 μg of PBS and human PD-1-mFc fusion protein was administered via an intraperitoneal injection to each mouse. Each immunized mouse was anesthetized, and its spleen located on the left side of the body was then extracted and ground with a mesh to isolate cells, which were mixed with a culture medium (RPMI1640) to prepare a spleen cell suspension. The suspension was centrifuged to collect a cell layer. The obtained 1×10^8 of spleen cells were mixed with 1.5×10^7 of myeloma cells (Sp2/0), and the mixture was centrifuged to precipitate the cells. The precipitate was slowly dispersed and treated with PEG Hybri-Max (Sigma Inc., CAT#:7181). The mixed cells were distributed into 96-well plates at 0.1 ml per well and incubated at 37°C, 5% CO_2 incubator. On day 1, the cells were fed by the addition of an additional 0.1 ml media containing serum and HAT plus 2×methotrexate for each well. On day 3 and day 7, 0.1 ml of medium from each well was replaced with 0.1 ml of fresh HT medium. The screening typically occurred between days 9-14.

Example 2 -Selection of the Hybridoma Cells that Produce Monoclonal Antibodies Against human PD-1 Protein based on ELISA and FACS analyses.

[00103] ELISA binding analysis was conducted based on human PD-1-hFc Protein. 96-well plates (Costar, Cat No:9018) were coated with 100 μL of 2 μg/ml PD1-hFc (CrownBio) in coating buffer (PBS, Hyclone, Cat No:SH30256.01B) overnight at 4° C. The wells were aspirated and non-specific binding sites were blocked by adding 200 μL of blocking buffer with 1% (w/v) of bovine serum albumin (BSA, Roche, Cat No: 738328) and incubating for 1 hour at 37° C. After the plates are washed three times with wash buffer (PBS with 0.05%(v/v) Tween20 (Sigma, Cat No:P1379), 100 μL/well of a suitable dilutions of hybridoma supernatant in blocking buffer were added and incubated at room temperature for 1 hour. The plates were washed and incubated with 100 μL/well of Goat anti-Mouse IgG (H+L) (Thermo, Cat No: 31432) in blocking buffer for 60 min. After the plates were washed, 100 μL/well of substrate solution (TMB(eBioscience, Cat No:00-4201-56) was added and the plates were incubated for 2min at room temperature. 100 μL/well of stop solution (2N H2SO4) was added to stop the reaction. The colorimetric signals were developed and read at 450 nm using a Auto Plate SpectraMax Plus (Supplier: Molecular Devices; Model: MNR0643; Software: SoftMax Pro v5.4). Through this method, hybridoma cell lines that produce antibodies highly specifically binding to the human PD-1 protein were repeatedly selected.

[00104] ELISA based ligand blockage analysis was conducted via blocking biotinylated human PD-L1-mFcs from binding to human PD-1-hFc. PD-1-mFc antigen (CrownBio) was suspended in PBS (Hyclone,Cat No:SH30256.01B) buffer (2ug/ml, 100ul/well) and coated on the 96 well plate (costar, Cat No.:9018) 4℃ overnight. Plates were washed 3 times using washing buffer: PBS+0.05% Tween 20(Sigma, Cat No.:P1379). 200ul of blocking buffer (PBS+1%BSA(Roche, Cat No.:738328)), was added to each well, incubated at 37℃ for 1 hour, and washed 3 times. Various concentrations (suitable dilutions of hybridoma supernatant in PBS) of the anti-PD-1 Abs were added to the wells (100 μl/well) and incubated at 37℃ for 1 hour. Ligand was added (0.1ug/ml PDL-1-mFc-biotin, 100 μl/well), incubated at 37℃ for 2h, and washed 3 times. Secondary antibody (Avidin HRP eBioscience cat No.:E07418-1632, 1:500, 100 ul/well) was added, incubated at 37 ℃ for 0.5 hour, and washed 3 times. TMB (Sigma, Cat No.: T0440, 100ul/well) was added, and incubated for 3 min at RT. To stop the reaction, 2N H2SO4 (100ul/well), was added. The colorimetric signals were developed and read at 450 nm using a Auto Plate SpectraMax Plus (Supplier: Molecular Devices; Model: MNR0643; Software: SoftMax Pro v5.4).

[00105] Cell binding analysis of antibodies was performed based on hPD-1-293T cell line. $2x10^5$ 293T-PD-1 cells were used for each reaction by putting them into each well of 96-well culture plates. The cells were incubated with the indicated antibody (20ug/ml with the dilution of 1/5) at 4°C for 1 h. Cells were washed three times with FACS buffer. A secondary antibody (PE Goat anti-mouse: 1:200; PE mouse anti-human: 1:10) was added to the cells at 100ul/well, and incubated at 4°C for 40min. Cells were washed three times with FACS buffer and analyzed by FACS Array.

[00106] FACS based ligand blockage analysis was conducted to determine the anti-PD-1 hybridoma antibodies in the blockage of biotinylated human PD-L1 and PD-L2 binding to hPD-1-293T cells using a flow cytometry assay. PD-1 expressing 293T cells were suspended in FACS buffer (PBS with 3% fetal calf serum). Various concentrations of the testing hybridoma antibodies were added to the cell suspension and incubated at 4^0C for 60 minutes in 96 well plates. Biotin-labeled PD-L1 protein or Biotin-labeled PD-L2 protein was added into the wells and incubated at 4^0C for 60 minutes. Plates were washed 3 times, and mouse anti-biotin PE antibody (Biolgend, cat# 409004) was added. Flow cytometric analyses were performed using a FACS Array. The results of the study are depicted in **Figure 1A** (PD-L1) and **Figure 1B** (PD-L2). The anti- PD-1 monoclonal antibodies blocked binding of PD-L1 or PD-L2 to 293T cells transfected with human PD-1, as measured by the mean fluorescent intensity (MFI) of staining. These data demonstrated that the anti-PD-1 antibodies block binding of ligand PD-L1 and PD-L2 to cell surface PD-1.

Example 3 - Subcloning to Obtain Monoclonal Antibody Clones and Purification of Anti-hPD-1 Antibodies

[00107] Subcloning is based on the procedure of limited dilution, and is designed to obtain individual hybridoma clones producing monoclonal antibodies. Each of the hybridomas was subjected to multiple rounds (4 rounds) of limiting dilution. For each round of subcloning, the clones were tested by ELISA and FACS based blockage analyses.

[00108] Antibody purification was conducted for a total of twenty two anti-hPD-1 hybridoma antibodies. The hybridoma cells were cultured in Dulbecco's Modified Eagle's medium (GIBCO; Invitrogen Corporation, Carlsbad, Calif.) containing 10% fetal calf serum, 1% penicillin/streptomycin, 2% L-glutamine, and 1% adjusted $NaHCO_3$ solution. The selected hybridoma cells were then adapted in serum free culture medium and the antibody was purified

from the supernatant using Protein-G column (GE healthcare). After washing with PBS, bound antibodies were eluted using 0.1 M Glycine pH3.0, followed by pH neutralization using 2.0 M Tris. Ultra-15 centrifugal concentrators (Amicon) were used for buffer exchanging and antibody concentrating.

Example 4 - Characterization of the purified murine anti-hPD-1 antibodies in binding and ligand blockage activities based on ELISA and FACS analyses

[00109] The purified hybridoma antibodies were characterized further based on ELISA and FACS analyses. The methods used were similar to those described above in Example 2 except that in these cases, purified antibodies were measured in amount and concentration, and the results were used to calculate EC50 and IC50 values. The following tables, **Tables 1-5**, show the results of 10 antibodies.

Table 1. ELISA based binding EC50 of 10 murine anti-PD-1 antibodies

ng/ml	15H5	22A5	14A6	6E1	7D3	10D1	4C10	13F1	5A8	7A4
EC50	13.35	19.38	7.07	15.67	24.36	17.18	13.25	17.33	11.25	9.32

Table 2. ELISA based blockage IC50 of 10 murine anti-PD-1 antibodies

ng/ml	15H5	22A5	14A6	6E1	7D3	10D1	4C10	13F1	5A8	7A4
IC50	873.2	1114.8	923	961.2	982.0	1409	1464.3	701.0	1128.7	698.8

Table 3. FACS based binding EC50 of 10 murine anti-PD-1 antibodies

ng/ml	15H5	22A5	14A6	6.00E+01	7D3	10D1	4C10	13F1	5A8	7A4
EC50	45.37	77.45	NA	49.09	52.66	NA	108.9	30.79	NA	38.93

Table 4. FACS based PD-L1 blockage IC50 of 10 murine anti-PD-1 antibodies selected.

ng/ml	15H5	22A5	14A6	6E1	7D3	10D1	4C10	13F1	5A8	7A4
IC50	35.64	56.22	21.13	26.85	38	36.9	83.32	19.86	28.22	28.85

Table 5. FACS based PD-L2 blockage IC50 of 10 murine anti-PD-1 antibodies selected

ng/ml	15H5	22A5	14A6	6E1	7D3	10D1	4C10	13F1	5A8	7A4
IC50	63.04	210.1	91.98	113.7	140.5	150	250.7	52.93	18.84	4.45

Example 5: Biacore analysis of the murine anti-PD-1 antibodies

[00110] To further characterize the binding characteristics of the antibodies, 10 hybridoma antibodies were profiled using Biacore (Biacore 3000, GE) to elucidate binding kinetics and calculate equilibrium binding constants. This assay was performed by capture method, using the mouse antibody capture kit (BR-1008-38, GE). After diluting anti-mouse Fc mab to 25 µg/ml in pH 5.0 immobilization buffer, immobilization was conducted with the parameters shown in **Table 6** at a flow rate of 5 µl/min. The kinetic runs were done by 1) injecting ligand for typical 0.5-1 min at flow rate of 10 µl/min.; 2) injecting analytes of choice for typical 3 min followed by dissociation in running buffer (1X PBS-P20) for typical 5-10 min at flow rate of 30 µl/min.; and 3) injecting regeneration solution 10mM Glycine pH1.7 for typical 1-2 min at flow rate of 10 µl/min.

Table 6. Biacore parameters.

Event	**Injection**	**Conditions**
Activation	EDC/NHS (1:1 Mix)	7 minutes
Immobilization	Diluted Anti-human Fc mAb	4 minutes to achieve ~7000 RU Immobilization level
Deactivation	Ethanolamine-HCl	7 minutes

[00111] The results of the study are shown in **Table 7**. Each of the anti-human PD1 antibodies exhibited an association rate (ka) in the range of 1.11E+05 1/Ms to 8.40E+05 1/Ms; a dissociation rate (kd) in the range of 2.83E-05 1/s to 7.55E-05 1/s; an equilibrium association constant (KA) in the range of 1.60E+10 1/M to 5.44E+10 1/M; and an affinity (KD) in the range of 1.84E-11 M to 6.23E-11 M (0.0184 nM to 0.0623 nM).

Table 7. KD values of anti-PD-1 hybridoma antibodies.

	ka (1/Ms)	kd (1/s)	KA (1/M)	KD (M)
10D1	8.40E+05	5.24E-05	1.60E+10	6.23E-11
14A6	1.51E+06	4.52E-05	3.33E+10	3.00E-11
22A5	1.49E+06	2.88E-05	5.17E+10	1.93E-11
4C10	7.91E+05	2.63E-05	3.01E+10	3.32E-11
7A4	1.96E+06	4.82E-05	4.06E+10	2.46E-11
6E1	1.11E+06	2.83E-05	3.92E+10	2.55E-11
13F1	1.41E+06	3.92E-05	3.60E+10	2.78E-11
15H5	2.00E+06	3.67E-05	5.44E+10	1.84E-11
5A8	1.29E+06	7.55E-05	1.70E+10	5.87E-11
7D3	1.14E+06	2.83E-05	4.02E+10	2.49E-11

Example 6: Cross-reactivity among species and among similar molecules

[00112] To assess the species cross-reactivity of the antibodies, the mouse and cynomolgus macaque PD-1 receptors were cloned by PCR and stably transfected 293T-PD-1 cells were generated. The antibodies were tested for binding to the cynomolgus receptor using protein based ELISA. The results of the study showed that the antibodies bind with equal affinity to human and cynomolgus PD-1 and block binding of hPD-Ll/Fc and hPD-L2/Fc to cynomolgous PD-1 with similar efficacy as compared to human PD-1. None of the antibodies selected bound mouse PD-1 with detectable affinity in any of the assays used. None cross reacts with human CTLA4, ICOS and CD28 (see **Table 8**).

Example 7: Effect of the anti-PD-1 hybridoma antibodies on cytokine production in a Mixed Lymphocyte Reaction.

[00113] A mixed lymphocyte reaction was used to demonstrate the effect of blocking the PD-1 pathway on lymphocyte effector cells. T cells in the assay were tested for proliferation, IFN-γ secretion and IL-2 secretion in the presence or absence of a murine anti-human PD-1 monoclonal antibody. In the assay, human CD4+ T-cells were purified from PBMC using a CD4+ negative selection (Miltenyi Biotech, cat# 130-091-155). Mature Dendritic cells (DC) were derived from purified monocytes (Miltenyi, Mo-DC Generation Toolbox, cat#130-093-568) culture with Mo-DC Differentiation Medium for 7 days; then, DC maturation was induced with Mo-Dc Maturation for 2 days. Each culture contained 10^5 purified T-cells and 10^4 allogeneic dendritic cells in a total volume of 200 µl. Anti-PD-1 monoclonal antibody 4C10, 5A8, 6E1, 7D3, 7A4, 10D1, 13F1, 14A6, 15H5, or 22A5 was added to each culture at different antibody concentrations. Either no antibody or an isotype control antibody was used as a

negative control. The cells were cultured for 5 days at 37° C. On 5 day, 50 µl of medium was collected for measurement of IL-2 and IFN-γ. The levels of IFN-γ and IL-2 in the culture fluid were measured using an EIA hIFN-γ ELISA kit (R&D, cat#DY285) and IL-2 ELISA kit (eBioscience) The results of the study are provided in **Figure 2** (IL-2 secretion) and **Figure 3** (IFN-γ secretion) and show that the anti-human PD-1 monoclonal antibodies promoted T-cell proliferation, IFN-γ secretion and IL-2 secretion in a concentration dependent manner. In contrast, cultures containing the isotype control antibody did not show an increase in T cell proliferation, IFN-γ or IL-2 secretion.

Example 8: Features of 10 Murine anti-hPD-1 antibodies

[00114] Characteristics of 10 anti-PD1 monoclonal antibodies that were purified and characterized are summarized in **Table 8**. These antibodies bound tightly to PD-1 (with dissociation constants in the 20 uM to 3 nM range) and were capable of blocking the interaction with both PD-L1 and PD-L2 with varying IC50 values. Each of the antibodies induced IL2 and IFNγ production. None of the 10 antibodies crossreacted with CTLA4, ICOS, or CD28. Each of the antibodies bound cynomolgous PD-1. Each of the antibodies, when added in solution acted as receptor antagonists, ultimately enhanced T cell responses (see Example 5).

Table 8. Summary of characterized features of 10 Murine anti-hPD-1 antibodies.

No.	Selected Abs	KD (Biacore)	ELISA Binding EC50 (ng/ml)	FACS at 20ug/ml	Blockage ELISA EC50 (ug/ml)	PD-L1 Blockage FACS EC50 (ng/ml)	PD-L2 Blockage FACS EC50 (ng/ml)	Tcell activation IL2	Tcell activation IFN-g	Interaction with CTLA4, ICOS CD28	Interaction with cyno-PD-1
1	15H5	1.84E-11	13.35	+++	873.2	35.64	63.04	+++	++	-	+++
2	22A5	1.93E-11	19.38	+++	1114.8	56.22	210.10	+++	++	-	+++
3	14A6	3.00E-11	7.07	+++	923	21.13	91.98	+++	+++	-	+++
4	6E1	2.55E-11	15.67	+++	961.2	26.85	113.70	+++	++	-	+++
5	7D3	2.49E-11	24.36	+++	982	38	140.50	++	+++	-	+++
6	10D1	6.23E-11	17.18	+++	1409	36.9	150.00	+++	+++	-	+++
7	4C10	3.32E-11	13.25	+++	1464.3	83.32	250.70	+++	+++	-	+++
8	13F1	2.78E-11	17.33	+++	701	19.86	52.93	+++	++++	-	+++
9	5A8	5.87E-11	11.25	+++	1128.7	28.22	18.84	+++	+++	-	+++
10	7A4	NA	9.32	+++	698.8	28.85	4.45	++++	+++	-	+++

Example 9: Anti-PD-1 antibody cDNA sequences cloning and humanization

[00115] Cloning of Immunoglobulin cDNAs

[00116] Total RNA isolated from the hybridoma cell line producing hPD-1 antibody by RNeasy Mini Kit (Qiagen, CAT#:74104) was used as the template to synthesize first-strand cDNA with SuperScript® II Reverse Transcriptase (Life Technology, CAT#:18064-14) according to the manufacturer's instructions. The cDNA product was then subjected to PCR in a 50 µl volume reaction mixture using degenerate mouse IgG primers (Kettleborough CA, et al, European Journal of Immunology 23: 206-211 (1993), Strebe N, et al, Antibody Engineering 1:3-14 (2010)). The reaction was carried out in a S1000™ Thermal Cycler (Bio-Rad, CAT#:184-2000) with 30 cycles of: 94° C, 1.5 minutes for denaturation; 50° C, 1 minutes for annealing; and 72° C, 1 minute for synthesis. At the end of the 30th cycle, the reaction mixture was incubated another 7 minutes at 72° C for extension.

[00117] The PCR mixture was subjected to electrophoresis in a 1% agarose/Tris-Borate gel containing 0.5 µg/ml ethidium bromide. DNA fragments having the expected sizes (approximately 400 bp for the heavy chain and the light chain) were excised from the gel and purified.3 µl of purified PCR product were cloned into the pMD-18T vector (Takara, CAT#:D101A) and transformed into One Shot® TOP10 Chemically Competent E. coli (Invitrogen, CAT#:C4040-03). Clones were screened by colony PCR using universal M13 forward and reverse primers, and 10 positive clones from each reaction were chosen for DNA sequencing in both directions using M13 forward and M13 reverse primers.

[00118] The variable region sequences of antibodies 4C10 (SEQ ID NOs: 28, 33), 5A8 (SEQ ID NOs: 99, 104), 6E1 (SEQ ID NOs: 89, 94), 7D3 (SEQ ID NOs: 39, 44), 7A4 (SEQ ID NOs: 109, 114), 10D1 (SEQ ID NOs: 18, 23), 13F1 (SEQ ID NOs: 49, 54), 14A6 (SEQ ID NOs:69, 74), 15H5 (SEQ ID NOs: 59, 64) and 22A5 (SEQ ID NOs: 79, 84) were amplified from the corresponding hybridoma clones. These antibodies showed desired functions, such as blocking PD-1 binding to PD-L1 and enhanced T cell activation and cytokine release.

[00119] Construction and Expression of Chimeric 7A4 and 13F1 Antibody

[00120] 7A4 and 13F1 chimeric light chains (SEQ ID NOs: 123 and 129, respectively) were constructed by linking the PCR-cloned cDNAs of mouse VL regions to human kappa and IgG1, respectively. 7A4 and 13F1 chimeric IgG1 heavy chains (SEQ ID NOs: 119 and 125, respectively) were constructed by linking the PCR-cloned cDNAs of mouse VH regions to human IgG1 constant region. 7A4 and 13F1 chimeric IgG4 heavy chains (SEQ ID NOs: 121 and 127, respectively) were constructed by linking the PCR-cloned cDNAs of mouse VH regions to

human IgG4 constant region The 5′ends of the mouse cDNA sequences were modified using PCR primers designed to add a leader sequence to both light chain and heavy chain.

[00121] Freestyle 293 cells (200 mL at 10^6/mL) were transfected with 100μg of each of the chimeric heavy and light chain expression plasmids and cultured for 6 days. The chimeric antibody in the supernatant was then purified with Protein-G column (GE healthcare). Binding of the chimeric antibodies to PD-1 was measured by ELISA and Biacore as described above in Examples 2 and 5, and was shown to bind to PD-1 with comparable affinity to that of the murine parent antibody. **Table 9** shows the binding EC50 of each of the chimeric anti-PD-1 antibodies as measured by ELISA. **Table 10** shows the PD-L1 blockage IC50 of each of the chimeric anti-PD-1 antibodies as measured by ELISA. **Table 11** shows the binding EC50 of each of the chimeric anti-PD-1 antibodies as measured by FACS. **Table 12** shows the PD-L1 blockage IC50 of each of the chimeric anti-PD-1 antibodies as measured by FACS.

Table 9. ELISA based binding EC50 of chimeric anti-PD-1 antibodies

ng/ml	15H5 hIgG4	22A5 hIgG4	14A6 hIgG4	6E1 hIgG4	7D3 hIgG4	10D1 hIgG4	4C10 hIgG4	13F1 hIgG4	7A4 hIgG4
EC50(ug/ml)	81.8	41.3	64.6	32.54	51.7	58.8	94.56	58.73	62

Table 10. ELISA based blockage IC50 of chimeric anti-PD-1 antibodies

ng/ml	15H5 hIgG4	22A5 hIgG4	14A6 hIgG4	6E1 hIgG4	7D3 hIgG4	10D1 hIgG4	4C10 hIgG4	13F1 hIgG4	7A4 hIgG4
IC50	1367.0	1010.9	823.4	868.6	948.1	1034.5	977.6	856.2	871.1

Table 11. FACS based binding EC50 of chimeric anti-PD-1 antibodies

ng/ml	15H5 hIgG4	22A5 hIgG4	14A6 hIgG4	6E1 hIgG4	7D3 hIgG4	10D1 hIgG4	4C10 hIgG4	13F1 hIgG4	7A4 hIgG4
EC50	243.6	145.3	103.4	143.1	130.9	218.8	220.6	113.1	91.6

Table 12. FACS based PD-L1 blockage IC50 of chimeric anti-PD-1 antibodies

ng/ml	15H5 hIgG4	22A5 hIgG4	14A6 hIgG4	6E1 hIgG4	7D3 hIgG4	10D1 hIgG4	4C10 hIgG4	13F1 hIgG4	7A4 hIgG4
IC50	100.5	84.26	61.37	54.01	NA	40.33	129	52.13	70.55

[00122] Mixed lympohcyte reactions as described above in Example 7 were used to determine the effect of the chimeric anti-PD-1 antibodies on IL-2 secretion (**Figure 4**) and IFN-γ secretion (**Figure 5**) from T cells. Each of the chimeric anti-PD-1 monoclonal antibodies promoted IL-2 secretion and IFNγ secretion in a concentration dependent manner in the MLR assay. In contrast, the isotype control antibody (hIgG4) did not elicit IL-2 secretion or IFNγ secretion at any concentration tested.

[00123] Antibody Humanization Design

[00124] 7A4 and 13F1 antibody were humanized using a CDR grafting approach (U.S. Pat. No.5,225,539, incorporated herein by reference in its entirety). The light chain and heavy chain variable chain sequences of the murine antibody 7A4 and 13F1 were compared to those available in the Research Collaboratory for Structural Bioinformatics (RCSB) protein databank by searching the NCBI database, http://www.ncbi.nlm.nih.gov/igblast/igblast.cgi. The model of 7A4 and 13F1 were generated respectively based on the VH and VL structure with the highest sequence homology.

[00125] The template human antibodies to be grafted with the complementary determining regions (CDRs) in the VH and VL of mouse 7A4 and 13F1 antibody were selected from human antibody germlines having an amino acid sequence with high homology with the mouse 7A4 and 13F1 antibody by searching the IMGT/Domain Gap Align 3D structure database, http://www.imgt.org/3Dstructure-DB/cgi/DomainGapAlign.cgi. For 7A4, the template human VH selected was a combination of IGHV2-5*10 and IGHJ4*01, and template human VL selected was a combination of IGKV1-33*01 and IGKJ2*01. For 13F1, the template human VH selected was a combination of IGHV3-21*04 and IGHJ4*01, and template human VL selected was a combination of IGKV7-3*01 and IGKJ2*01.

[00126] CDR amino acid sequences of the aforementioned template human antibodies were substituted by the amino acid sequence of CDRs of mouse 7A4 and 13F1 antibody. In addition, the frameworks of the above-mentioned template human antibody VH and VL were grafted with the necessary amino acid sequences from VH and VL of mouse 7A4 and 13F1 antibody to give a functional humanized antibody. As for VH and VL of 7A4 and 13F1, several sites of framework amino acid of the aforementioned template human antibody were back mutated to the corresponding amino acid sequences in mouse 7A4 and 13F1 antibody. For the light chain variable region of humanized 7A4 antibody, the amino acid at position 40 was

mutated from Tyr (Y) to Phe (F) and the amino acid at position 72 was mutated from Gly (G) to Arg (R); and for the heavy chain variable region of humanized 7A4 antibody, the amino acid at position 2 was mutated from Val (V) to Ile (I), the amino acid at position 46 was mutated from Glu (E) to Lys (K), and the amino acid at position 70 was mutated from Phe (F) to Ile (I). For the light chain variable region of humanized 13F1 antibody, the amino acid at position 45 was mutated from Leu (L) to Pro (P) and the amino acid at position 70 was mutated from Phe (F) to Tyr (Y); and for the heavy chain variable region of humanized 13F1 antibody, the amino acid at position 26 was mutated from Gly (G) to Tyr (Y), the amino acid at position 48 was mutated from Ile (I) to Met (M), the amino acid at position 49 was mutated from Gly (G) to Ala (A), the amino acid at position 67 was mutated from Val (V) to Ile (I), and the amino acid at position 71 was mutated from Val (V) to Arg (R).

[00127] The amino acid sequences of the variable light and variable heavy chains of humanized 13F1 antibody were designated SEQ ID NOs: 143 and 141, respectively. The base sequences of DNAs encoding the amino acid sequences were designed (SEQ ID NO: 140 and 142, respectively).The amino acid sequences of the variable light and variable heavy chains of humanized 7A4 antibody were designated SEQ ID NOs: 133 and 131, respectively. The base sequences of DNAs encoding the amino acid sequences were designed (SEQ ID NO: 130 and 132, respectively).

[00128] IgG1 and IgG4 versions of the humanized 7A4 and 13F1 antibodies were produced (h13F1-IgG1, h13F1-IgG4, h7A4-IgG1 and h7A4-IgG4). The IgG1 constant region carries D265A mutation (Clynes R, et al, Nature Medicine 6: 443-446 (2000)) while IgG4 constant region has F234A and L235A double mutation (Xu D, et al, Cellular Immunology 200: 16-26 (2000)). The constant region sequences are disclosed in SEQ ID NOS: 150 and 151. The full light and heavy chain amino acid sequences for h13F1-IgG1 (SEQ ID NOs: 149 and 145), h13F1-IgG4 (SEQ ID NOs: 149 and 147), h7A4-IgG1 (SEQ ID NOs: 139 and 135), and h7A4-IgG4 (SEQ ID NOs: 139 and 137) are provided above in Table 3. To remove the potential deamidation site in the light chain of 7A4, Asn85 is mutated to Asp (h7A4D). The light chain variable region (SEQ ID NO: 152) and full light amino acid sequences (SEQ ID NO: 153) are also provided above in Table 3.

[00129] Construction and Expression of Humanized 7A4, 7A4D and 13F1 Antibodies

[00130] DNA encoding humanized 7A4, 7A4D and 13F1 antibody light chain and heavy chain was synthesized and cloned to the expression vector pcDNA3.1 (Invitrogen, CAT: #V-790). Freestyle 293 cells (200 mL at 10^6/mL) were transfected with 100μg of each of the humanized heavy and light chain expression plasmids and cultured for 6 days. The humanized antibody in the supernatant was then purified with Protein-G column (GE healthcare).

Example 10: Characterization of Humanized Anti-PD-1 Antibodies in Binding Activity and Specificity, and Ligand (PD-L1) Blockage Activity

[00131] After generation and purification of humanized 13F1-hIgG1, 13F1-hIgG4, 7A4-IgG1 and7A4-hIgG4 antibodies, the binding and specificity of the antibodies were determined based on ELISA-based binding and PD-1 blockage analyses, as well as FACS-based binding and PD-L1 blockage analyses. The methods used were similar to those described above in Examples 2 and 4.

[00132] In the ELISA-based binding assays, humanized 13F1 antibodies hu-13F1-hIgG1 and hu-13F1-hIgG4 exhibited similar binding to PD-1 compared to the chimeric antibody 13F1-chimeric (**Figure 6A**, top panel); and humanized 7A4 antibodies hu-7A4-D265A-hIgG1 and 7A4-huIgG4 exhibited similar binding to PD-1 compared to the chimeric 7A4 antibodies (**Figure 6B**, top panel). In contrast, the isotype control hIgG4 antibody did not exhibit PD-1 binding. The bottom panels of **Figure 6A** and **Figure 6B** show the EC50 for each of the antibodies tested, calculated from the ELISA binding data, and demonstrates that the humanized 13F1 and 7A4 antibodies exhibited PD-1 binding.

[00133] Similarly, in the FACS-based binding assays, humanized 13F1 antibodies hu-13F1-hIgG1 and hu-13F1-hIgG4 (**Figure 7A**, top panel) and humanized 7A4 antibodies hu-7A4-D265A-hIgG1 and 7A4-huIgG4 (**Figure 7B**, top panel) exhibited binding to PD-1. The EC50 calculated from the FACS binding data for humanized 13F1 and 7A4 antibodies are shown in **Figure 7A** and **Figure 7B**, respectively.

[00134] **Figure 8** shows the results of the ELISA-based ligand blocking assays for humanized 13F1 and humanized 7A4 antibodies. As shown in **Figure 8A** and **Figure 8B**, the humanized 13F1 and 7A4 antibodies, respectively, exhibited similar ligand blockage activity relative to the corresponding chimeric antibody. Quantification of the IC50 for each of the humanized and chimeric antibodies is shown in **Figure 8C**.

[00135] **Figure 9** shows that each of the humanized 13F1 and humanized 7A4 antibodies blocked PD-L1 binding as measured by FACS-based ligand blockage assay. The bottom panel of **Figure 9** provides the IC50 for each of the humanized antibodies.

Example 11: Biacore Kinetic analysis of the Humanized 13F1, 7A4 and 7A4D Anti-PD-1 Antibodies

[00136] To characterize the binding characteristics of the humanized antibodies, the binding kinetics between PD-1 and PD-1 antibodies were measured by Biacore3000 and recorded with a data collection rate of 1 Hz. The polyclonal rabbit anti-mouse IgG (GE, BR-1008-38) was diluted with 10 mM pH 5.0 sodium acetate and immobilized onto reference and experiment flow cells of a CM5 biosensor chip to around 15000RU using an amine coupling kit (GE, BR10050). In the beginning of each cycle, diluted test antibody (1.5 μg/mL) was injected over experiment flow cell for 1 minute to be captured. PD-1 analyte series were prepared by diluting the stocks with running buffer to 100nM followed by 2X serial dilution in the same buffer down to 0.78nM. Analytes were injected in series over the reference and experiment flow cells for 3 minutes at a flow rate of 30 μL/minute. Then running buffer (PBS with 0.05% P20) was allowed to flow over for 10 minutes at a flow rate of 30 μL/minute. At the end of each cycle, the biosensor surface was regenerated with 3 minutes injection of 10 mM pH1.7 Glycine-HCl buffer at a flow rate of 10 μL/minute. For each analyte sample injection (i.e. each cycle), binding responses obtained from the experimental biosensor surface were double referenced by subtracting simultaneously recorded responses from the reference surface followed by additional subtraction of responses from a single referenced running buffer sample. The association and dissociation rate constants (ka and kd) were determined simultaneously by fitting double-referenced sensorgrams of the entire titration series to Langmuir model (1:1) using Biaevaluation 4.0 software. The dissociation constant, KD, was calculated from the determined rate constants by the relation KD = kd/ka. As shown in **Figures 10** and **17A**, the humanized anti-PD-1 antibodies 13F1, 7A4, and 7A4D bound human PD-1 with high affinity. The Biocore binding curves are shown in **Figures 10** and **17A**, top panel, and the quantified binding data are summarized in **Figures 10** and **17A,** bottom panel. **Figure 17B** indicates the blockage IC50 of PD-L1's binding to 293T-PD1 cells by 7A4D-hIgG4 antibody.

Example 12: Effect of humanized anti-PD-1 antibodies on cytokine production in a Mixed Lymphocyte Reaction (MLR)

[00137] Mixed Lymphocyte Reactions (MLR) were employed to demonstrate the ability of the humanized antibodies to block the PD-1 pathway in lymphocyte effector cells. T cells in the assay were tested for IFNγ and IL-2 secretion in the presence or absence of anti-PD-1 antibodies. Human $CD4^+$ T-cells were purified from human PBMC using a CD4 negative selection isolation kit (Mitenyi Biotech, cat# 130-091-155). Immature dendritic cells (DC) were derived from monocytes isolated from human PBMC using the Mo-DC Generation Toolbox (Miltenyi, cat#130-093-568). The cells were cultured with Mo-DC Differentiation Medium for 7 days, and were then induced to be mature DC with Mo-Dc Maturation medium for 2 days. To set up the MLR, 10^5 purified T-cells and 10^4 allogeneic mature DC cells in a total volume of 200 µl were added to each well. The testing antibody was assayed at a range of concentrations from 20 µg/ml to 0.002 µg/ml. Either no antibody or an isotype control antibody (hIgG4) was used as a negative control. The cells were cultured for 5 days at 37 ^{0}C. On day 6^{th}, the levels of IFN-γ and IL-2 in the culture medium were measured using the IL-2 ELISA kit (eBioscience) and hIFN-γ ELISA kit (R&D, cat#DY285). For humanized 13F1 antibodies, the results are shown in **Figure 11** (IL-2 production) and **Figure 12** (IFNγ production). Each of the humanized 13F1 antibodies promoted IL-2 and IFNγ production in a concentration dependent manner. Similarly, humanized 7A4 and 7A4D antibodies promoted IL-2 (**Figures 13 and 18**) and IFNγ (**Figures 14 and 19**) production in a concentration dependent manner. Cultures containing the isotype control antibody did not show increase in IFN-γ and IL-2 secretion. Thus, the results of the study showed that the humanized PD-1 antibodies block the PD-1 pathway, stimulating T cell immune responses.

Example 13: Human recall T cell response to Tetanus Toxoid challenge is enhanced by humanized anti-PD-1 antibody

[00138] To investigate whether the antigen-specific T cell receptor triggering was modulated by blocking PD-1/PD-L1 pathway with anti-PD-1 antibodies, the human T-cell recall assay was employed using tetanus toxoid (TT) antigen to stimulate pre-existing memory T cells in the blood of healthy TT immunized donors. To this end, fresh PBMC recently collected (samples collected within less than 1 year) from TT immunized donors were plated into 96-well

round bottom plates (costar, cat#3799) at 4 x 10^5 cells/well using RPMI1640 (Invitrogen, cat# A10491-01) supplemented with 80 U/ml penicillin, 80 g/ml streptomycin and 30% autologous serum. Humanized 13F1 or 7A4 antibodies were added at various concentrations, and stimulated with 0.1ug/ml SEB and 1ug/ml TT (Astarte Biologies). After co-culture for 7 days at 37°C, 5% CO_2, the supernatant was harvested and the concentration of IFN-γ was measured. The results of the study are shown in **Figure 15**, and demonstrate that, compared to TT antigen alone, PD-L1 blockage with anti-PD-1 antibodies resulted in enhanced IFN-γ secretion by memory T cells.

Example 14: Effect of Humanized Anti-PD-1 Antibody on Autologous T Cell Activation

[00139] In this example, the effect of blocking PD-/PD-L1 pathway by humanized anti-PD-1 antibody on T cell activation was examined. Purified human CD4+ T cells (Mitenyi Biotech, cat# 130-091-155) were activated with 1μg/ml soluble anti-CD3 antibody (R&D, cat#MAB100) in the presence of autologous monocyte-derived dendritic cells (DCs). After three days of activation in the presence or absence of titrated anti-PD-1 antibody, culture medium was harvested and the concentration of IFNγ was measured with ELISA. The results are shown in **Figure 16** and indicate that PD-L1 blockage by anti-PD-1 antibody enhanced IFN-γ secretion by T cells.

CLAIMS

1. An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody or fragment thereof comprises a light chain CDR1 sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 34, 45, 55, 65, 75, 85, 95, 105, and 115; a light chain CDR2 sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 25, 35, 46, 56, 66, 76, 86, 96, 106, and 116; a light chain CDR3 sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 26, 36, 47, 57, 67, 77, 87, 97, 107, and 117; a heavy chain CDR1 sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 29, 40, 50, 60, 70, 80, 90, 100, and 110; a heavy chain CDR2 sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 30, 41, 51, 61, 71, 81, 91, 101, and 111; and a heavy chain CDR3 sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 21, 31, 42, 52, 62, 72, 82, 92, 102, and 112.

2. The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof comprises a light chain CDR1 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 34, 45, 55, 65, 75, 85, 95, 105, and 115; a light chain CDR2 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 25, 35, 46, 66, 76, 86, 96, 106, and 116; a light chain CDR3 consisting of an amino acid sequences selected from the group consisting of SEQ ID NOs: 26, 36, 47, 57, 67, 77, 87, 97, 107, and 117; a heavy chain CDR1 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 29, 40, 50, 60, 70, 80, 90, 100, and 110; a heavy chain CDR2 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 30, 41, 51, 61, 71, 81, 91, 101, and 111; and a heavy chain CDR3 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 21, 31, 42, 52, 62, 72, 82, 92, 102, and 112.

3. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 24, 25, and 26, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 19, 20, and 21, respectively.

4. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 34, 35, and 36, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 29, 30, and 31, respectively.

5. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 45, 46, and 47, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 40, 41, and 42, respectively.

6. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 55, 56, and 57, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 50, 51, and 52, respectively.

7. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 65, 66, and

67, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 60, 61, and 62, respectively.

8. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 75, 76, and 77, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 70, 71, and 72, respectively.

9. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 85, 86, and 87, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 80, 81, and 82, respectively.

10. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 95, 96, and 97, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 90, 91, and 92, respectively.

11. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 105, 106, and 107, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 100, 101, and 102, respectively.

12. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 115, 116, and 117, respectively; and a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology to an amino acid sequence according to SEQ ID NOs: 110, 111, and 112, respectively.

13. The isolated antibody or fragment thereof of any one of claims 1-12, wherein the antibody or fragment thereof is chimeric or humanized.

14. An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 23, 33, 44, 54, 64, 74, 84, 94, 104, 114, 133, and 143; and a heavy chain variable region comprising an amino acid sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 28, 39, 49, 59, 69, 79, 89, 99, 109, 131, and 141.

15. The isolated antibody or fragment thereof of claim 14, wherein the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 23, 33, 44, 54, 64, 74, 84, 94, 104, 114, 133, and 143; and a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 28, 39, 49, 59, 69, 79, 89, 99, 109, 131, and 141.

16. An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody or fragment thereof comprises a light chain variable region according to SEQ ID NO: 133 or 152 and a heavy chain variable region according to SEQ ID NO: 131.

17. An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody or fragment thereof comprises a light chain variable region according to SEQ ID NO: 143 and a heavy chain variable region according to SEQ ID NO: 141.

18. An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody or fragment thereof comprises a light chain according to SEQ ID NO: 139 or 153 and a heavy chain according to SEQ ID NO: 135.

19. An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody or fragment thereof comprises a light chain according to SEQ ID NO: 139 or 153 and a heavy chain according to SEQ ID NO: 137.

20. An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody or fragment thereof comprises a light chain according to SEQ ID NO: 149 and a heavy chain according to SEQ ID NO: 145.

21. An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody or fragment thereof comprises a light chain according to SEQ ID NO: 149 and a heavy chain according to SEQ ID NO: 147.

22. An isolated antibody or fragment thereof, wherein the antibody or fragment thereof binds to the same epitope as an antibody or fragment thereof according to any one of claims 1 to 21.

23. An isolated antibody or fragment thereof, wherein the antibody or fragment thereof competes for binding to PD-1 with an antibody or fragment thereof of any one of claims 1 to 21, wherein the competition is measured by ELISA, flow cytometry, or surface plasmon resonance (SPR) assay.

24. The isolated antibody or fragment thereof of any one of claims 1-21, wherein the antibody or fragment thereof is selected from the group consisting of a monoclonal antibody, an scFv, a Fab fragment, an Fab' fragment, and an F(ab)' fragment.

25. An antibody or fragment thereof according to any one of claims 1-21, wherein the antibody or fragment thereof is linked or conjugated to a therapeutic agent.

26. The antibody or fragment thereof according to claim 25, wherein the therapeutic agent is a cytotoxic drug, a radioactive isotope, an immunomodulator, or an antibody.

27. An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody or fragment thereof has an affinity for PD-1 of about 1 nM to about 0.01 nM.

28. The isolated antibody or fragment thereof according to claim 27, wherein the antibody or fragment thereof as an affinity for PD-1 of about 1 nM or less.

29. The isolated antibody or fragment thereof according to claim 27, wherein the antibody or fragment thereof as an affinity for PD-1 of about 0.1 nM or less.

30. An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody has a binding EC50 of about 5 ng/mL to about 1000 ng/mL.

31 An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody blocks binding of PD-1 to PD-L1.

32. An isolated antibody or fragment thereof of claim 31, wherein the antibody or fragment thereof blocks the binding of PD-1 to PD-L1 or PD-L2 at an IC50 of about 5 ng/mL to about 1000 ng/mL.

33. An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody or fragment increases T cell activation as measured by inflammatory cytokine production.

34. The isolated antibody or fragment thereof according to claim 28, wherein the antibody or fragment thereof increases T cell production of IL-2 and IFNγ.

35. An isolated antibody or fragment thereof that binds to PD-1, wherein the antibody or fragment thereof is produced by a hybridoma selected from the group consisting of 10D1, 4C10, 7D3, 13F1, 15H5, 14A6, 22A5, 6E1, 5A8, 7A4, and/or 7A4D.

36. A composition comprising the antibody or fragment thereof according to any one of claims 1-35 and a pharmaceutically acceptable carrier.

37. An isolated polynucleotide encoding the antibody or fragment thereof according to any one of claims 1-35.

38. An expression vector comprising the isolated polynucleotide according to claim 37.

39. A host cell comprising the expression vector according to claim 38.

40. An isolated hybridoma cell line selected from the group consisting of 10D1, 4C10, 7D3, 13F1, 15H5, 14A6, 22A5, 6E1, 5A8, 7A4 and 7A4D.

41. A method for increasing T cell activation, the method comprising contacting T cells with an antibody or fragment thereof according to any one of claims 1-35.

42. A method for reducing tumors or inhibiting the growth of tumor cells in a subject, the method comprising administering to the subject a therapeutically effective amount of the isolated antibody or fragment thereof according to any one of claims 1-35.

43. A method for treating a cancer in a subject in need thereof, the method comprising administering a therapeutically effective amount of the isolated antibody or fragment thereof according to any one of claims 1-35 to the subject.

44. The method according to claim 43, wherein the cancer is selected from the group consisting of lymphoma, leukemia, melanoma, glioma, breast cancer, lung cancer, colon cancer, bone cancer, ovarian cancer, bladder cancer, kidney cancer, liver cancer, stomach cancer, rectal cancer, testicular cancer, salivary cancer, thyroid cancer, thymic cancer, epithelial cancer, head or neck cancer, gastric cancer, pancreatic cancer, or a combination thereof.

45. A method for treating an infectious disease in a subject in need thereof, the method comprising administering a therapeutically effective amount of the isolated antibody or fragment thereof according to any one of claims 1-35 to the subject.

46. The method according to claim 45, wherein the infectious disease is selected from the group consisting of candidiasis, candidemia, aspergillosis, streptococcal pneumonia, streptococcal skin and oropharyngeal conditions, gram negative sepsis, tuberculosis, mononucleosis, influenza, respiratory illness caused by Respiratory Syncytial Virus, malaria, schistosomiasis, and trypanosomiasis.