

ANTI-PD-L1 ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to International Application No. PCT/CN2014/083715, filed August 5, 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-L1, 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_007_01WO_SeqList_ST25.txt); date recorded: August 4, 2015; file size 153 KB).

BACKGROUND

[0004] Programmed death receptor Ligand 1 (PD-L1) is a ligand of programmed death receptor 1 (PD-1). PD-1 is primarily expressed on lymphocytes and has two ligands, PD-L1 and PD-L2. PD-L2 is not as common as PD-L1. PD-L1 is also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1) and is a 40kDa type 1 transmembrane protein which is encoded by the CD274 gene. Both PD-L1 and PD-1 belong to immunoglobulin superfamily and consist of two extracellular Ig domains, an N-terminal V domain, and a C-terminal constant domain. The binding interface of PD-L1 to programmed death 1 (PD-1) and B7-1 (CD80) is on the IgV-like domain (Lin et al. (2008) PNAS 105:3011-3016). While PD-L1 contains a conserved short intracellular tail (about 30 amino acids), 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 (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).

[0005] PD-L1 is not only widely distributed on leukocytes and nonhematopoietic cells in lymphoid and nonlymphoid tissues, but also in various cancer cells. Clinical data suggest that high tumor expression of PD-L1 is associated with increased tumor aggressiveness and poorer prognosis. The formation of PD-1/PD-L1 complex transmits an inhibitory signal and negatively regulates T cell immune responses; it inhibits TCR-mediated T cell activation, cytokine production and T cell proliferation (Fife et al. (2011) *Nature Immunology* 10:1185-1193); induces exhaustion or anergy among cognate antigen-specific T cells (Hofmeyer et al. (2011) *Journal of Biomedicine and Biotechnology* 2011:1-9); promotes the differentiation of Th1 cells into Foxp3⁺ regulatory T cells (Armanath et al. (2011) *Science TransMed* 3:1-13; Francisco et al. (2009) *J. Exp. Med.* 206:3015-3029); and induces apoptosis of effector T cells. Disruption of the PD-L1 gene leads to up-regulated T cell responses and the generation of self-reactive T cells (Latchman et al. (2004) *PNAS* 101:10691–10696). Antibody blockade of either PD-1 or PD-L1 leads to increased antitumor immunity (Iwai et al. (2002) *PNAS* 99:12293–12297).

[0006] 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/PD-L1 functions mainly in inducing and regulating peripheral tolerance. Thus, therapeutic blockade of the PD-1/PD-L1 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/PD-L1 pathway.

SUMMARY OF THE INVENTION

[0007] In one aspect, the present invention provides antibodies and antigen-binding fragments thereof that bind to programmed death-1 ligand 1 (PD-L1). In some embodiments, the antibodies and antigen-binding fragments thereof bind to human PD-L1. In some embodiments, the antibodies and antigen-binding fragments thereof bind to PD-L1 and block binding of PD-1 and/or CD80 to PD-L1. In further embodiments, the anti-PD-L1 antibodies and fragments thereof bind to PD-L1 and disrupt the PD-L1/PD-1 or PD-L1/CD80 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-L1 antibody or

fragment thereof is a monoclonal antibody, scFv, Fab fragment, Fab' fragment, F(ab)' fragment, bispecific antibody, immunoconjugate, or a combination thereof

[0008] 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: 81-140.

[0009] 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: 81, 87, 93, 99, 105, 111, 117, 123, 129, and 135.

[0010] 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: 82, 88, 94, 100, 106, 112, 118, 124, 130, and 136.

[0011] 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: 83, 89, 95, 101, 107, 113, 119, 125, 131, and 137.

[0012] 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: 84, 90, 96, 102, 108, 114, 120, 126, 132, and 138.

[0013] 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: 85, 91, 97, 103, 109, 115, 121, 127, 133, and 139.

[0014] 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: 86, 92, 98, 104, 110, 116, 122, 128, 134, and 140.

[0015] In one embodiment, the antibody or fragment thereof comprises a heavy chain CDR1 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 81, 87, 93, 99, 105, 111, 117, 123, 129, and 135; a heavy chain CDR2 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 82, 88, 94, 100, 106, 112, 118, 124, 130, and 136; a heavy chain CDR3 consisting of an amino acid sequences selected from the group consisting of SEQ ID NOs: 83, 89, 95, 101, 107, 113, 119, 125, 131, and 137; a light chain CDR1 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 84, 90, 96, 102, 108, 114, 120, 126, 132, and 138; a light chain CDR2 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 85, 91, 97, 103, 109, 115, 121,

127, 133, and 139 and a light chain CDR3 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 86, 92, 98, 104, 110, 116, 122, 128, 134, and 140.

[0016] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises 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: 81, 82, and 83, respectively; and 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: 84, 85, and 86, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 81, 82, and 83, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 84, 85, and 86, respectively.

[0017] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises 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: 87, 88, and 89, respectively; and 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: 90, 91, and 92, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 87, 88, and 89, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 90, 91, and 92, respectively.

[0018] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises 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: 93, 94, and 95, respectively; and 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: 96, 97, and 98, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 93, 94, and 95, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 96, 97, and 98, respectively.

[0019] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises 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: 99, 100, and 101, respectively; and 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: 102, 103, and 104, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 99, 100, and 101, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 102, 103, and 104, respectively.

[0020] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises 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: 105, 106, and 107, respectively; and 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: 108, 109, and 110, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 105, 106, and 107, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 108, 109, and 110, respectively.

[0021] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises 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: 111, 112, and 113, respectively; and 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: 114, 115, and 116, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 111, 112, and 113, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 114, 115, and 116, respectively.

[0022] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises 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: 117, 118, and 119, respectively; and 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: 120, 121, and 122, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 117, 118, and 119, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 120, 121, and 122, respectively.

[0023] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises 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: 123, 124, and 125, respectively; and 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: 126, 127, and 128, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 123, 124, and 125, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 126, 127, and 128, respectively.

[0024] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises 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: 129, 130, and 131, respectively; and 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: 132, 133, and 134, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 129, 130, and 131, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 132, 133, and 134, respectively.

[0025] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises 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: 135, 136, and 137, respectively; and 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: 138, 139, and 140, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 135, 136, and 137, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 138, 139, and 140, respectively.

[0026] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises 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: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, and 46; and 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: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48. In a further embodiment, the isolated antibody or fragment thereof binds

PD-L1 and comprises 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: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, and 46; and 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: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48.

[0027] In one embodiment, the invention provides anti-PD-L1 antibodies that comprise a variable heavy chain of an antibody selected from the group consisting of 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and 15F1 and a variable light chain of an antibody selected from the group consisting of 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and 15F1. Thus, in one embodiment, the invention provides an antibody or fragment thereof comprising a heavy chain variable region comprising SEQ ID NO: 2 and a light chain variable region comprising SEQ ID NO: 4; a heavy chain variable region comprising SEQ ID NO: 6 and a light chain variable region comprising SEQ ID NO: 8; a heavy chain variable region comprising SEQ ID NO: 10 and a light chain variable region comprising SEQ ID NO: 12; a heavy chain variable region comprising SEQ ID NO: 14 and a light chain variable region comprising SEQ ID NO: 16; a heavy chain variable region comprising SEQ ID NO: 18 and a light chain variable region comprising SEQ ID NO: 20; a heavy chain variable region comprising SEQ ID NO: 22 and a light chain variable region comprising SEQ ID NO: 24; a heavy chain variable region comprising SEQ ID NO: 26 and a light chain variable region comprising SEQ ID NO: 28; a heavy chain variable region comprising SEQ ID NO: 30 and a light chain variable region comprising SEQ ID NO: 32; a heavy chain variable region comprising SEQ ID NO: 34 and a light chain variable region comprising SEQ ID NO: 36; or a heavy chain variable region comprising SEQ ID NO: 38 and a light chain variable region comprising SEQ ID NO: 40.

[0028] In one embodiment, the present invention provides a chimeric anti-PD-L1 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: 50, 54, 58, 60, 64, and 66; 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: 52, 56, 62 and 68.

[0029] In one embodiment, the present invention provides a humanized anti-PD-L1 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: 42 and 46. In another embodiment, the present invention provides a humanized anti-PD-L1 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: 44 and 48.

[0030] In another embodiment, the present invention provides a humanized anti-PD-L1 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: 42 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: 44. In another embodiment, the present invention provides a humanized anti-PD-L1 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: 46 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: 48.

[0031] In one embodiment, the present invention provides a humanized anti-PD-L1 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: 70, 72, 76, and 78. In another embodiment, the present invention provides a humanized anti-PD-L1 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: 74 and 80.

[0032] In one embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 70 and a light chain according to SEQ ID NO: 74. In another embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 72 and a light chain according to SEQ ID NO: 74. In another embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 76 and a light chain according to SEQ ID NO: 80. In another embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 78 and a light chain according to SEQ ID NO: 80.

[0033] In one embodiment, the present invention provides anti-PD-L1 antibodies or fragments thereof that bind to the same epitope on PD-L1 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-L1. Binding to PD-L1 may be measured by ELISA, flow cytometry, surface plasmon resonance (SPR) assay, or any other method known in the art.

[0034] In one embodiment, the present invention provides anti-PD-L1 antibodies and fragments thereof that bind to PD-L1 with a binding affinity K_D of about 10 nM to about 0.01 nM. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of from about 10 nM to about 0.05 nM. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of from about 8 nM to about 0.1 nM. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of from about 5nM to about 0.2 nM. In another embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 10 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 6nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 4nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 2 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 1nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 0.75 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 0.5 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 0.25 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 0.2 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 0.15 nM or less. In a further embodiment, the anti- PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 0.1 nM or less. In a further embodiment, the anti- PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 0.075 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 0.05 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 0.025 nM or less. In a

further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 0.02 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 0.015 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity K_D of about 0.01 nM or less. In one embodiment, the binding affinity K_D of the anti-PD-L1 antibodies and fragments provided herein is measured by Biacore assay.

[0035] In one embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC_{50} for PD-L1 of about 1 ng/mL to about 2000 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC_{50} for PD-L1 of about 1 ng/mL to about 1500 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC_{50} for PD-L1 of about 1 ng/mL to about 1000 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC_{50} for PD-L1 of about 2 ng/mL to about 500 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC_{50} for PD-L1 of about 2 ng/mL to about 250 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC_{50} for PD-L1 of about 5 ng/mL to about 200 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC_{50} for PD-L1 of about 5 ng/mL to about 50 ng/mL. In one embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC_{50} for PD-L1 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. In one embodiment, the EC_{50} of the anti-PD-L1 antibodies and fragments provided herein is measured by ELISA or FACS.

[0036] In one embodiment, the anti PD-L1 antibodies and fragments thereof provided herein inhibit PDL1/PD-1 binding with an IC_{50} of about 1 ng/mL to about 1500 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein inhibit PDL1/PD-1 binding with an IC_{50} of about 2 ng/mL to about 1200 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein inhibit PDL1/PD-1 binding with an IC_{50} of about 5 ng/mL to about 500 ng/mL. In a further embodiment, the anti

PD-L1 antibodies and fragments thereof provided herein inhibit PDL1/PD-1 binding with an IC50 of about 5 ng/mL to about 100 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein inhibit PDL1/PD-1 binding with an IC50 of about 10 ng/mL to about 50 ng/mL. In one embodiment, the anti PD-L1 antibodies and fragments thereof provided herein inhibit PDL1/PD-1 binding with an IC50 of about 1200 ng/mL or less, about 1000 ng/mL or less, 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, about 30 ng/mL or less, about 20 ng/mL or less, or about 10 ng/mL or less. In one embodiment, the IC50 of the anti-PD-L1 antibodies and fragments provided herein is measured by ELISA or FACS.

[0037] In one embodiment, the anti-PD-L1 antibody provided herein is a humanized antibody having a heavy chain variable region amino acid sequence according to SEQ ID NO: 42 and a light chain variable region amino acid according to SEQ ID NO: 44; or having a heavy chain variable region amino acid sequence according to SEQ ID NO: 46 and a light chain variable region amino acid sequence according to SEQ ID NO: 48; wherein the anti-PD-L1 antibody has a PD-L1 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 or about 50 ng/mL or less, as measured by ELISA or FACS. In another embodiment, the anti-PD-L1 antibody provided herein is a humanized antibody having a heavy chain variable region amino acid sequence according to SEQ ID NO: 42 and a light chain variable region amino acid according to SEQ ID NO: 44; or having a heavy chain variable region amino acid sequence according to SEQ ID NO: 46 and a light chain variable region amino acid sequence according to SEQ ID NO: 48; wherein the anti-PD-L1 antibody has a PDL1/PD-1 blockage IC50 of about 1200 ng/mL or less, or 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-L1 antibody provided herein is a humanized antibody having a heavy chain variable region amino acid sequence according to SEQ ID NO: 42 and a light chain variable region amino acid according to SEQ ID NO: 44; or having a heavy

chain variable region amino acid sequence according to SEQ ID NO: 46 and a light chain variable region amino acid sequence according to SEQ ID NO: 48; wherein the anti-PD-L1 antibody has a binding affinity K_D for PD-L1 of about 10 nM or less, or about 5 nM or less, or about 2 nM or less, or about 1 nM or less, or about 0.5 nM or less, or about 0.1 nM or less, or about 0.05 nM or less, as measured by Biacore assay. In one embodiment, the humanized anti-PD-L1 antibody has a binding affinity K_D for PD-L1 of about 2 nM. In another embodiment, the humanized anti-PD-L1 antibody has a binding affinity K_D for PD-L1 of about 1 nM. In another embodiment, the humanized anti-PD-L1 antibody has a binding affinity K_D for PD-L1 of about 0.5 nM. In another embodiment, the humanized anti-PD-L1 antibody has a binding affinity K_D for PD-L1 of about 0.1 nM.

[0038] In one embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1, 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-L1 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-L1 and result in an increase of one or more cytokines selected from the group consisting of IL-2, IFN γ , 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 and antigen presenting cells with the anti-PD-L1 antibody or fragment thereof. In one embodiment, the modulation of an immune response by the anti-PD-L1 antibodies and fragments provided herein may be measured in a mixed lymphocyte (MLR) reaction. In one embodiment, the anti-PD-L1 antibodies provided herein increase the level of cytokine production from lymphocytes in an MLR. In a further embodiment, the anti-PD-L1 antibodies increase the level of IL-2 production and/or IFN γ production in an MLR. In a yet further embodiment, the anti-PD-L1 antibodies increase the level of IL-2 production and IFN γ production in an MLR. In one embodiment, the anti-PD-L1 antibodies enhance memory T cell responses. In a further embodiment, the anti-PD-L1 antibodies enhance memory T cell responses as measured by an increase in IFN γ production from memory T cells.

[0039] In one embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein inhibit regulatory T cell function. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof inhibit the suppression of effector T cells by regulatory T cells. In another embodiment, the anti-PD-L1 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-L1 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.

[0040] In one aspect, an isolated antibody or fragment thereof that binds to PD-L1 is provided, wherein the antibody is produced by a hybridoma selected from the group consisting of the hybridomas herein termed 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and 15F1. Thus, the present invention also encompasses the hybridomas 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and 15F1, 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.

[0041] In one embodiment, the present invention provides anti-PD-L1 antibody immunoconjugates. Thus, the present invention provides an antibody or fragment thereof that binds to PD-L1 and that is linked or conjugated to a therapeutic agent. Therapeutic agents that may be linked or conjugated to the anti-PD-L1 antibody may include, but are not limited to, cytotoxic drugs, radioactive isotopes, immunomodulators, or antibodies.

[0042] In one aspect, the present invention provides compositions comprising one or more anti-PD-L1 antibody or fragment thereof provided herein, and a pharmaceutically acceptable carrier.

[0043] 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-L1 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-L1 antibody or fragment thereof provided herein.

[0044] 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-L1 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-L1 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-L1 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.

[0045] 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-L1 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 DESCRIPTION OF THE FIGURES

[0046] **Figure 1a-d** is set of graphs showing the binding of the murine hybridoma anti-PD-L1 antibodies to PD-L1 over a range of antibody concentrations as measured by ELISA. Binding of hybridoma antibodies 8H3-mIgG (m8H3), 15F1-mIgG (m15F1), 5G9-mIgG (m5G9), and 4A8-mIgG (m4A8) is shown in **Figure 1a**. Binding of hybridoma antibodies 5G11-mIgG (m5G11), 7B4-mIgG (m7B4), 4D1-mIgG (m4D1), and 8H4-mIgG (m8H4) is shown in **Figure 1b**. Binding of hybridoma antibody 8C6-mIgG (m8C6) is shown in **Figure 1c**. Binding of hybridoma antibody 13C5-mIgG (m13C5) is shown in **Figure 1d**. In each of Figures 1a-1d, binding of mIgG1 is shown as a negative control.

[0047] **Figure 2a-c** is set of graphs showing the binding of chimeric anti-PD-L1 antibodies to PD-L1 over a range of concentrations as measured by ELISA. Binding of chimeric antibodies ch5G11-hIgG4 and ch5G11-hIgG1 is shown in **Figure 2a**. Binding of chimeric antibodies ch13C5-hIgG4, ch13C5-hIgG1, and ch8H4-hIgG4 is shown in **Figure 2b**. Binding of chimeric

antibody ch8C6-hIgG4 is shown in **Figure 2c**. In each of Figures 2a-2c, binding of hIgG4 is shown as a negative control.

[0048] Figure 3a-b is set of graphs showing the binding of humanized anti-PD-L1 antibodies to PD-L1 over a range of antibody concentrations as measured by ELISA. Binding of control hIgG4 and humanized antibodies hu5G11-hIgG1 and hu5G11-hIgG4 is shown in **Figure 3a**. Binding of control hIgG4 and humanized antibodies hu13C5-hIgG1 and hu13C5-hIgG4 is shown in **Figure 3b**.

[0049] . Figure 4a-c is a set of graphs showing the blockage of the PD-1/PD-L1 interaction by hybridoma anti-PD-L1 antibodies over a range of antibody concentrations as measured by ELISA. Blockage of PD-1/PD-L1 binding by hybridoma antibodies 13C5-mIgG (m13C5), 8C6-mIgG (m8C6), 5G9-mIgG (m5G9), and 4A8-mIgG (m4A8) as compared to control mIgG1 is shown in **Figure 4a**. Blockage of PD-1/PD-L1 binding by hybridoma antibodies 5G11-mIgG (m5G11), 7B4-mIgG (m7B4), 4D1-mIgG (m4D1), and 8H4-mIgG (m8H4) as compared to control mIgG1 is shown in **Figure 4b**. Blockage of PD-1/PD-L1 binding by hybridoma antibodies 8H3-mIgG (m8H3) and 15F1-mIgG (m15F1) as compared to control mIgG1 is shown in **Figure 4c**.

[0050] Figure 5a-c is a set of graphs showing the blockage of the PD-1/PD-L1 interaction by chimeric anti-PD-L1 antibodies over a range of antibody concentrations as measured by ELISA. Blockage of PD-1/PD-L1 binding by chimeric antibodies ch5G11 hIgG4 and ch5G11 hIgG1 as compared to control hIgG4 is shown in **Figure 5a**. Blockage of PD-1/PD-L1 binding by chimeric antibody ch8C6-hIgG4 as compared to control hIgG4 is shown in **Figure 5b**. Blockage of PD-1/PD-L1 binding by chimeric antibodies ch8H4-hIgG4, ch13C5-hIgG1, and ch13C5-hIgG4 as compared to control hIgG4 is shown in **Figure 5c**.

[0051] Figure 6a-b is a set of graphs showing the blockage of the PD-1/PD-L1 interaction by humanized anti-PD-L1 antibodies over a range of antibody concentrations as measured by ELISA. Blockage of PD-1/PD-L1 binding by control hIgG4 and humanized antibodies 5G11-hIgG1 and 5G11-hIgG4 is shown in **Figure 6a**. Blockage of PD-1/PD-L1 binding by control hIgG4 and humanized antibodies 13C5-hIgG1 and 13C5-hIgG4 is shown in **Figure 6b**.

[0052] Figure 7a and 7b show the binding of the hybridoma anti-PD-L1 antibodies to PD-L1 over a range of antibody concentrations as measured by FACS. Binding (as measured by the mean fluorescence intensity) of hybridoma antibodies 4A8, 15F1, 4D1, 13C5, 8H4, and 8H3 as

compared to control antibody mIgG1 is shown in **Figure 7a**. Binding (as measured by the mean fluorescence intensity) of hybridoma antibodies 5G11, 8C6, 5G9, or 7B4 as compared to control antibody mIgG1 is shown in **Figure 7b**.

[0053] **Figure 8** shows the binding of the chimeric anti-PD-L1 antibodies to PD-L1 over a range of antibody concentrations as measured by FACS. Binding of control antibody hIgG4, and chimeric antibodies ch13C5-hIgG1, ch5G11-hIgG1, and ch5G11-hIgG4 are shown.

[0054] **Figure 9** shows the binding of humanized anti-PD-L1 antibodies to PD-L1 over a range of antibody concentrations as measured by FACS. Binding of control antibody hIgG4 and humanized antibodies hu13C5-hIgG1, hu13C5-hIgG4, hu5G11-hIgG1, and hu5G11-hIgG4 are shown.

[0055] . **Figure 10a** and **10b** show the blockage of the PD-1/PD-L1 interaction by hybridoma anti-PD-L1 antibodies over a range of antibody concentrations as measured by FACS. Blockage of PD-1/PD-L1 binding by control antibody mIgG1 and hybridoma antibodies m4D1, m5G11, m13C5, m7B4, and m8H4 is shown in **Figure 10a**. Blockage of PD-1/PD-L1 binding by control antibody mIgG1 and hybridoma antibodies m4A8, m5G9, m8C6, m8H3, and m15F1 is shown in **Figure 10b**.

[0056] **Figure 11** shows the blockage of the PD-1/PD-L1 interaction over a range of concentrations of control antibody hIgG4 or chimeric anti-PD-L1 antibodies ch8C6-hIgG4, ch5G11-hIgG1, ch5G11-hIgG4, ch13C5-hIgG1, ch13C5-hIgG4, or ch8H4-hIgG4, as measured by FACS.

[0057] **Figure 12** shows the blockage of the PD-1/PD-L1 interaction over a range of concentrations of control antibody hIgG4 or humanized antibodies hu 13C5-hIgG1, hu13C5-hIgG4, hu5G11-hIgG1, or hu5G11-hIgG4, as measured by FACS.

[0058] **Figure 13a** is a graph showing IL-2 (pg/mL) production in an MLR in response to different concentrations of hybridoma anti-PD-L1 antibodies. **Figure 13b** is a graph showing IFN γ (pg/mL) production in an MLR in response to different concentrations of hybridoma anti-PD-L1 antibodies. For both **Figure 13a** and **13b**, the antibodies tested were, from left to right, control mIgG1, m8C6, m4D1, m5G11, m7B4, m8H4, m5G9, m13C5, m8H3, and m15F1. T cell only and/or DC only wells were also included as negative controls. As shown on the x-axis for both **Figure 13a** and **13b**, each antibody was tested at 20 μ g/mL, 2 μ g/mL, 0.2 μ g/mL, 0.02 μ g/mL, and 0.002 μ g/mL.

[0059] Figure 14a is a graph showing IL-2 (pg/mL) production in an MLR in response to different concentrations of chimeric anti-PD-L1 antibodies. **Figure 14b** is a graph showing IFN γ (pg/mL) production in an MLR in response to different concentrations of chimeric anti-PD-L1 antibodies. For both **Figure 14a** and **14b**, the antibodies tested were, from left to right, control hIgG4, chimeric 8C6-hIgG4, chimeric 8H4-hIgG4, chimeric 5G11-hIgG4, and chimeric 13C5-hIgG1. As shown on the x-axis for both **Figure 14a** and **14b**, each antibody was tested at 20 μ g/mL, 2 μ g/mL, 0.2 μ g/mL, 0.02 μ g/mL, and 0.002 μ g/mL.

[0060] Figure 15a is a graph showing IL-2 (pg/mL) production in an MLR in response to different concentrations of humanized anti-PD-L1 antibodies. **Figure 15b** is a graph showing IFN γ (pg/mL) production in an MLR in response to different concentrations of humanized anti-PD-L1 antibodies. For both **Figure 15a** and **15b**, the antibodies tested were, from left to right, control hIgG4, hu13C5-hIgG1, hu13C5-hIgG4, hu5G11-hIgG1, and hu5G11-hIgG4. As shown on the x-axis for both **Figure 15a** and **15b**, each antibody was tested at 20 μ g/mL, 2 μ g/mL, 0.2 μ g/mL, 0.02 μ g/mL, and 0.002 μ g/mL.

[0061] Figure 16 shows the effects of chimeric (ch) or humanized (hu) anti-PD-L1 antibodies on Treg-mediated inhibition of IFN γ production (pg/mL), in an allogeneic MLR with CD4⁺ CD25⁺ Treg cells, CD4⁺CD25⁻ T cells, and dendritic cells. The antibodies tested were, from left to right, control hIgG4, ch13C5-hIgG1, ch13C5-hIgG4, hu13C5-hIgG1, hu13C5-hIgG4, ch5G11-hIgG1, ch5G11-hIgG4, hu5G11-hIgG1, and hu5G11-hIgG4.

[0062] Figure 17 shows IFN- γ production (pg/mL) from T cells in response to costimulation with autologous DCs and anti-CD3 antibody, in the presence of humanized anti-PD-L1 antibody (hu13C5-hIgG1, hu13C5-hIgG4, hu5G11-hIgG1, or hu5G11-hIgG4), isotype control (hIgG4) antibody, or no antibody.

[0063] Figures 18a and 18b show the effect of humanized anti-PD-L1 antibodies on memory T cell responses recalled by tetanus toxin, as measured by IFN- γ production (pg/mL). Negative control hIgG4 or humanized antibody hu13C5-hIgG1, hu13C5-hIgG4, hu5G11-hIgG1, or hu5G11-hIgG4 were tested at the following concentrations: 20 μ g/mL, 2 μ g/mL, 0.2 μ g/mL, 0.02 μ g/mL, and 0.002 μ g/mL.

DETAILED DESCRIPTION

[0064] PD1/PDL1 interactions inhibit T cell receptor signaling by recruiting the SHP1 and SHP2 phosphatases, which interfere with TCR signaling (Chemnitz et al. (2004) *J. Immunol.* 17:945–954). PD-L1 can not only promote tumor progression through inhibition of PD1-expressing immune effectors, but also modulate cell-mediated immunity in some infectious diseases (Mueller et al. (2010) *J. Clin. Invest.* 120:2508–2515). Furthermore, allogeneic effector T cell responses are susceptible to PD-1 pathway modulation in graft rejection (Lee et al. (2003) *J. Immunol.* 171:6929–6935). Therefore, the interaction of PD-1 with PD-L1 exerts a vital and diverse range of immunoregulatory roles in T cell activation, tolerance, and immune-mediated tissue damage. However, the interaction can be reversed by blocking the local binding of PD-1 with PD-L1 (Iwai et al. (2002) *Proc. Nat'l. Acad. Sci. USA* 99: 12293-7; Brown et al. (2003) *J. Immunol.* 170:1257-66).

[0065] 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); Winterle 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-1 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)).

[0066] 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 PDL1/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-1 or PD-L1 significantly inhibited tumor growth (Tsushima F. et al., Oral Oncol. 42:268-274 (2006)).

[0067] Furthermore, transfection of a murine mastocytoma line with PD-L1 led to decreased lysis of the tumor cells when co-cultured with a tumor-specific CTL clone. Lysis was restored when anti-PD-L1 mAb was added (Iwai Y. et al., Proc. Natl. Acad. Sci. USA. 99: 12293-12297 (2002)). In vivo, blocking the PD1/PD-L1 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-1 knockout mice. PD-L1 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-1 blockade using a fully human anti-PD-1 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-L1 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-L1 blockade augments tumor-specific T cell responses in vitro when used in combination with anti-CTLA-4 blockade. Overall, the PD-1/PD-L1 pathway is a target for the development of antibody therapeutics for cancer treatment. Anti-PD-L1 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-1 and PD-L1.

[0068] In one aspect, the present invention provides antibodies or antigen binding fragments thereof that bind to programmed death ligand 1 (PD-L1). In one embodiment, the antibodies or fragments thereof bind to human PD-L1. In another embodiment, the antibodies or fragments thereof bind to human and to cynomolgous PD-L1. In another embodiment, the antibodies or fragments thereof block the interaction of PD-L1 with its receptor PD-1 on T cells. In one aspect, the present invention provides methods of making and using the anti-PD-L1 antibodies or fragments thereof, and compositions comprising anti-PD-L1 antibodies or fragments thereof, including pharmaceutical compositions.

[0069] 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-L1 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-L1 antibodies and fragments thereof disclosed herein are of an IgG1 or an IgG4 isotype. The PD-L1 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-L1 antibodies and fragments thereof may be chimeric, humanized, or fully human antibodies. In one embodiment, the anti-PD-L1 antibodies are antibodies produced by a hybridoma cell line derived from a mouse. Thus, in one embodiment, the anti-PD-L1 antibodies are murine antibodies. In another embodiment, the anti-PD-L1 antibodies are chimeric antibodies. In a further embodiment, the chimeric antibodies are mouse-human chimeric antibodies. In another embodiment, the antibodies are humanized antibodies. In a further embodiment, the antibodies are derived from murine antibodies and are humanized.

[0070] 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.

[0071] 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-L1 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-L1 as the murine antibody from which the antibody’s CDRs are derived. Exemplary humanized antibodies are provided herein. Additional anti-PD-L1 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. In some embodiments, such 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 humanized 5G11 and humanized 13C5, several sites of framework amino acid of the aforementioned template human antibody were back mutated to the corresponding amino acid sequences in mouse 5G11 and 13C5 antibodies. In one embodiment, the amino acid at positions 53 and/or 60 and/or 67 of the light chain variable region is back mutated to the corresponding amino acid found at that position in the mouse 5G11 or 13C5 light chain variable region. In another embodiment, the amino acid at positions 24 and/or 28 and/or 30 and/or 49 and/or 73 and/or 83 and/or 94 of the heavy chain variable region is back mutated to the corresponding amino acid found at that position in the mouse 5G11 or 13C5 heavy chain variable region. In one embodiment, the humanized 5G11 antibody comprises a light chain variable region wherein the amino acid at position 60 is mutated from Ser (S) to Asp (D) and the amino acid at position 67 is mutated from Ser (S) to Tyr (Y); and a heavy chain variable region wherein the amino acid at position 24 is mutated from Phe (F) to Val (V), the amino acid at position 49 is mutated from Ala (A) to Gly (G), the amino acid at position 73 is mutated from Thr (T) to Asn (N), and the amino acid at position 83 is mutated from Thr (T) to Asn (N). In one embodiment, the humanized 13C5 antibody comprises a light chain variable region wherein the amino acid at position 53 is mutated from Tyr (Y) to Lys (K); and a heavy chain variable region wherein the amino acid at position 28 is mutated from Thr (T) to Ile (I), the amino acid at position 30 is mutated from Ser (S) to Arg (R), the amino acid at position 49 is mutated from Ser (S) to Ala (A), and the amino acid at position 94 is mutated from Tyr (Y) to Asp (D). 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. The present invention also encompasses humanized antibodies that bind to PD-L1 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.

[0072] 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.

[0073] The antibodies and antigen-binding fragments thereof disclosed herein are specific for PD-L1. In one embodiment, the antibodies and fragments thereof are specific for human PD-L1. In one embodiment, the antibodies and fragments provided herein bind to human or primate PD-L1 but not to PD-L1 from any other mammal. In a further embodiment, the antibodies and fragments thereof do not bind to mouse PD-L1. The terms “human PD-L1,” “hPD-L1”, and “huPD-L1” and the like are used interchangeably herein and refer to human PD-L1 and variants or isoforms of human PD-L1. By “specific for” is meant that the antibodies and fragments thereof bind PD-L1 with greater affinity than any other target. 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.

[0074] In one embodiment, the anti-PD1 antibodies and fragments or variants thereof have a binding affinity (KD) for PD-L1 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 a binding affinity (KD) for PD-L1 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 5 nM or less, about 4 nM or less, about 3 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 a binding affinity (KD) for PD-L1 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 nM.

[0075] 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 heavy chain CDR sequences (HCDR1, HCDR2, and HCDR3) for PD-L1 antibodies of the invention are provided below in **Table 1**. Exemplary light chain CDR sequences (LCDR1, LCDR2, and LCDR3) for PD-L1 antibodies of the invention are provided below in **Table 2**. Exemplary variable regions and full length heavy and light chain sequences for PD-L1 antibodies of the invention are provided below in **Table 3**.

Table 1. Heavy Chain CDR Sequences

Name	HCDR	SEQ ID NO	Sequence
13C5	1	81	SYGMS
	2	82	SISSGGSTYYPDVSKG
	3	83	GYDSGFAY
5G9	1	87	SYGMS
	2	88	SISSGGTTYYPDSVKG
	3	89	GYDSGFAY
5G11	1	93	TYGVH
	2	94	VIWRGVTTDYNAAFMS
	3	95	LGFYAMDY
8C6	1	99	SYGVH
	2	100	VIWSGGVTDYNAAFIS
	3	101	LGFYAMDY
7B4	1	105	TYWMH
	2	106	QINPDSTTINYAPSLKD
	3	107	PGDYGDFDC

4D1	1	111	SGYWN
	2	112	YISYSGSTYYNPSLKS
	3	113	SLLWFSTGFAY
4A8	1	117	SYGVH
	2	118	VIWSGGITDYNAAFKS
	3	119	LGFYAMDY
8H4	1	123	SYGMS
	2	124	SISSGGTTYLGSVQG
	3	125	GYDAGFAY
8H3	1	129	SGYWT
	2	130	YISYTGSTYYNPSLKS
	3	131	QRDWLGFAY
15F1	1	135	SYGMS
	2	136	SISSGGSIYYPDSVKG
	3	137	GYDAGFAF

Table 2. Light chain CDR Sequences

Name	LCDR	SEQ ID NO	Sequence
13C5	1	84	ASQSVSTSSSSFMH
	2	85	YASNLES
	3	86	QHSWEIPYT
5G9	1	90	RASQSVSTSSSSYM
	2	91	YASNLES
	3	92	QHSWEIPYT

5G11	1	96	KASQSVSNDVA
	2	97	YAANRYT
	3	98	QQDYTSPYT
8C6	1	102	KASQSVSNDVG
	2	103	YASNRYT
	3	104	QQDYTSPYT
7B4	1	108	RSSQIIVHSNANTYLE
	2	109	KVSNRFS
	3	110	FQGSHVPYT
4D1	1	114	SASSSVSSSYLY
	2	115	NTSNLAS
	3	116	HQWRSYPPT
4A8	1	120	SANSSVSYMH
	2	121	DTSKLAS
	3	122	QQWSSNPWT
8H4	1	126	RASQSVSTSSYSYMH
	2	127	YASNLES
	3	128	QNSWEIPYT
8H3	1	132	KSSQSLLYSSNQKNSLA
	2	133	WASNRES
	3	134	QQYYSYPLT
15F1	1	138	RASQSVSTSSYSYVH
	2	139	YASNLES
	3	140	QHSWEIPYT

Table 3. Heavy chain and light chain variable region and full length heavy and light chain amino acid sequences

Name	Region	SEQ ID NO	Sequence
13C5 murine	Heavy chain variable	2	EVKLVESGGGLVKPGGSLKLSCAASGFIFRSYGMSWVRQTPE KRLEWVASISSGGSTYYPD SVKGRFTISRDNAR NILYLQMSSLRSED TAMYDCARGYDSGFAYWGQGT LVT VSE
13C5 murine	Light chain variable	4	DIVLTQSPASLAVSLGQRATISCRASQSVSTSSSSFMHWYQQK PGQPPKLLIKYASNLESGV PARFSGSGSGTDFT LNIHPVEEEDTATYYCQHSWEIPYTFGGG TKLEIKR
5G9 murine	Heavy chain variable	6	EVKLVESGGGLVKPGGSLKLSCAASGFIFRSYGMSWVRQTP EKRLWVASISSGGSTYYPD SVKGRFIISRDNARNILYLQMSS LRSED TAMYYCAKGYDSGFAYWGQGT LVI VSA
5G9 murine	Light chain variable	8	DIVLTQSPPSLAVSLGQRATISCRASQSVSTSSSSYMHWYQQK PGQPPKLLIKYASNLESGV PARFSGSGSGTDFTLNIHPVEEEDT ATYYCQHSWEIPYTFGGG TKLEIK
5G11 murine	Heavy chain variable	10	QVQLKQSGPGLVQPSQSL SITCTVSGFSLTTYGVHWVRQSPG KGLEWLGVIWRGVTTDYNAAFMSRLTITKDNSKSQVFFKMN SLQANDTAIYYCARLGFYAMDYWGQGT SVTVSS
5G11 murine	Light chain variable	12	SIVMTQTPKFLLVSAGDRV TITCKASQSVSNDVAWYQQKPG QSPKLLIYYAANRYTGV PDRFTGSGYGTDFTFITISIVQAEDLA VYFCQQDYTSPYTFGGG TKLEIK
8C6 murine	Heavy chain variable	14	QVQLKQSGPGLVQPSQSL SITCTVSGFSLTSYGVHWVRQSPG KGLEWLGVIWWSGGVTDYNAAFISRLSISKDNSKSQVFFKMNS LQANDTAIYYCARLGFYAMDYWGQGT SVTVSS
8C6 murine	Light chain variable	16	SIVMTQTPKFLLVSAGDRV TITCKASQSVSNDVGWYQQKPG QSPKLLIYYASNRYSGV PDRFTGSGYGTDFTFITISTVQAEDLA VYFCQQDYTSPYTFGGG TKLEIK
7B4 murine	Heavy chain variable	18	EVKLFESGGGLVQPGGSLKLSCVASGFD FSTYWMHWVRQAP GQGLEWIGQINPDSTTINYAPSLKDRFIISRDN AKNTLFLQMS KVRSED TALYYCAKPGDYG YDFDCWQG GTTLTVSS
7B4 murine	Light chain variable	20	DVLMTQTPLYLPVSLGDQASISCRSSQIIVHSNANTYLEWFLQ KPGQSPKLLIYKVS NRFSGV PDRFSGSGSGTDFTLKISRVEAE DLGVYYCFQGSHVPYTFGGG TKLEIK
4D1 murine	Heavy	22	EVQLQESGPSLVKPSQTL SLTCSVTGDSITSGYWNWIRKFPGN KLEYMGYISYSGSTYYNPSLKSRI SITRDT SKNQYYLQLNSVT

	chain variable		TEDTATYYCARSLWFSTGFAYWGQGLTVTVSA
4D1 murine	Light chain variable	24	QIVLTQSPAIMASAPGEKVTLTCSASSSVSSSYLYWNQQKPGS SPKVWIYNTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAAS YFCHQWRSYPPTLGAGTKLELK
4A8 murine	Heavy chain variable	26	QVQLKQSGPGLVQPSQSLITCTVSGFSLTSYGVHWVRQSPG KGLEWLGVIWSGGITDYNAAFKSRLSISKDNSKSQVFFKMNS LQANDTAIYFCARLGFYAMDYWGQGSTVTVSS
4A8 murine	Light chain variable	28	QIVLTQSPAIMASAPGEKVTMTCSANSSVSVMHWYQQKSGTS PKRWIYDTSKLASGVPARFSGSGSGTSYSLTISSMGAEDAAT YYCQQWSSNPWTFGGGKLEIK
8H4 murine	Heavy chain variable	30	EVKLVESGGGLVKPGGSLKLSCAASGFTFRSYGMSWARQIPE KRLEWVASISSGGTTYLYLGSVQGRFTISRDNARNILYLQMSSL RSEDAMYYCARGYDAGFAYWGQGLTVSVSE
8H4 murine	Light chain variable	32	DIVLTQSPASLAVSLGQRATISCRASQSVSTSSYSVMHWYQQ KPGQPPKLLIKYASNLESGVPARFSGSGSGTDFTLNHPVEEE DTATYYCQNSWEIPYTFGGGKLEIK
8H3 murine	Heavy chain variable	34	EVQLQESGPSLVKPSQTLSTCTSGYWTWIRKFPNG KLEYMGYISYTGSTYYNPSLKSRLISRDTSKSQYYLQLNSVT TEDTATYYCARQRDWLGFAFWGQGLTVTVSA
8H3 murine	Light chain variable	36	DIVMTQTPSSLAVSLGEKVTMSCKSSQSLLYSSNQKNSLAWY QQKPGQSPKLLIYWASNRESGVDPDRFTGSSSGTDFTLTISSVK AEDLAVYYCQYYSYPLTFGAGTKLELK
15F1 murine	Heavy chain variable	38	EEKLVESGGGLVKPGGSLKLSCAASGFSFSSYGMSWVRQTPE KRLEWVASISSGGSIYYPDSVKGRFTISRDNARNILYLQMSSL RSEDAMYYCARGYDAGFAFWGQGLTVTASA
15F1 murine	Light chain variable	40	DIVLTQSPASLAVSLGQRATISCRASQSVSTSSYSYVHWYQQ KPGQPPKLLIKYASNLESGVPARFSGSGSGTDFTLNHPVEEE DTATYYCQHSWEIPYTFGGGKLEIK
5G11 humanized	Heavy chain variable	42	QITLKESGPTLVKPTQTLTLTCTVSGFSLSTYGVHWIRQPPGK ALEWLGVIWRGVTTDYNAAFMSRLTITKDNSKNQVVLTMN NMDPVDATATYYCARLGFYAMDYWGQGLTVTVSS
5G11 humanized	Light chain variable	44	DIQMTQSPSSLSASVGDRVITITCKASQSVSNDAWYQQKPGK APKLLIYYAANRYTGVPDRFSGSGYGTDFTFITISLQPEDAT YFCQQDYTSPYTFGQGTKLEIK

13C5 humanized	Heavy chain variable	46	EVQLVESGGGLVKPGGSLRLSCAASGFIERSYGMWVRQAP GKGLEWVASISSGGSTYYPD SVKGRFTISRDN AKNSLYLQMN SLRAEDTAVYDCARGYDSGFAYWGQGLTVTVSS
13C5 humanized	Light chain variable	48	DIVLTQSPASLAVSPGQRATITCRASQSVSTSSSSFMHWYQQK PGQPPKLLIKYASNLESGV PARFSGSGSGTDFTLTINPVEAND TANYYCQHSWEIPYTFGQGGTKLEIK
Chimeric 8C6-IgG4 (F234A/L235A)	Full length heavy chain (IgG4)	50	QVQLKQSGPGLVQPSQSL SITCTVSGFSLTSYGVHWVRQSPG KGLEWLGVIWWSGGVTDYNAAFISRLSISKDNSKSQVFFKMNS LQANDTAIYYCARLGFYAMDYWGQGSTVTVSSASTKGPSVF PLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLG TKTYTCNV DHKPSNTKV DKRVESKYGPCCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTP EVTCTVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKA KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDS DGSFFLYSRLTVDKSRWQEGNVF SCSVMHEALHNHYTQKSLSLSLG
Chimeric 8C6	Full length light chain	52	SIVMTQTPKFLLSAGDRV TITCKASQSVSNDVGWYQQKPG QSPKLLIYYASNRYSGVPDRFTGSGYGTDFTFITISTVQAEDLA VYFCQQDYTSPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGECE
Chimeric 8H4-IgG4 (F234A/L235A)	Full length heavy chain (IgG4)	54	EVKLVESGGGLVKPGGSLKLSCAASGFTFRSYGMSWARQIPE KRLEWVASISSGGTTYLGSVQGRFTISRDNARNILYLQMSSL RSED TAMYYCARGYDAGFAYWGQGLTVSVSSASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVTVPSSSLG TKTYTCNV DHKPSNTKV DKRVESKYGPCCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTP EVTCTVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKA KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDS DGSFFLYSRLTVDKSRWQEGNVF SCSVMHEALHNHYTQKSLSLSLG
Chimeric 8H4	Full length light chain	56	DIVLTQSPASLAVSLGQRATISCRASQSVSTSSYSYMHWYQQ KPGQPPKLLIKYASNLESGV PARFSGSGSGTDFTLNHPVEEE DTATYYCQNSWEIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGECE
Chimeric 5G11-IgG1 (D265A)	Full length heavy chain	58	QVQLKQSGPGLVQPSQSL SITCTVSGFSLTTYGVHWVRQSPG KGLEWLGVIWRGVTTDYNAAFMSRLTITKDNSKSQVFFKMN SLQANDTAIYYCARLGFYAMDYWGQGSTVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV

	(IgG1)		HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHNKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
Chimeric 5G11-IgG4 (F234A/ L235A)	Full length heavy chain (IgG4)	60	QVQLKQSGPGLVQPSQSLITCTVSGFSLTTYGVHWVRQSPG KGLEWLGVIWRGVTTDYNAAFMSRLTITKDNSKSQVFFKMN SLQANDTAIYYCARLGFYAMDYWGQGTSTVTVSSASTKGPSV FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHNKPSNTK VDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMHEALHNHYTQKSLSLSLG
Chimeric 5G11	Full length light chain	62	SIVMTQTPKFLVLSAGDRVTTITCKASQSVSNDVAWYQQKPG QSPKLLIYYAANRYTGVPDRFTGSGYGTDFTFITISIVQAEDLA VYFCQQDYTSPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEK
Chimeric 13C5-IgG1 (D265A)	Full length heavy chain (IgG1)	64	EVKLVESGGGLVKPGGSLKLSCAASGFIFRSYGMSWVRQTPE KRLEWVASISSGGSTYYPD SVKGRFTISRDNARNILYLQMSSL RSED TAMYDCARGYDSGFAYWGQGT LTVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHNKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
Chimeric 13C5-IgG4 (F234A/ L235A)	Full length heavy chain (IgG4)	66	EVKLVESGGGLVKPGGSLKLSCAASGFIFRSYGMSWVRQTPE KRLEWVASISSGGSTYYPD SVKGRFTISRDNARNILYLQMSSL RSED TAMYDCARGYDSGFAYWGQGT LTVTVSSASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHNKPSNTKV DKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVF SCSVMHEALHNHYTQKSLSLSLG

Chimeric 13C5	Full length light chain	68	DIVLTQSPASLAVSLGQRATISCRASQSVSTSSSSFMHWYQQK PGQPPKLLIKYASNLESGVPARFSGSGSGTDFTLNHPVEEEDT ATYYCQHSWEIPYTFGGGTKLEIKRTRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
Humanized 5G11-IgG1 (D265A)	Full length heavy chain (IgG1)	70	QITLKESGPTLVKPTQTLTLTCTVSGFSLSTYGVHWIRQPPGK ALEWLGVIWRGVTTDYNAAFMSRLTITKDNSKNQVVLTMN NMDPVDATATYYCARLGFYAMDYWGQGTLLTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
Humanized 5G11-IgG4 (F234A/ L235A)	Full length heavy chain (IgG4)	72	QITLKESGPTLVKPTQTLTLTCTVSGFSLSTYGVHWIRQPPGK ALEWLGVIWRGVTTDYNAAFMSRLTITKDNSKNQVVLTMN NMDPVDATATYYCARLGFYAMDYWGQGTLLTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVESKYGPCCPPCPAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGN VFSCSVMHEALHNHYTQKSLSLSLG
Humanized 5G11	Full length light chain	74	DIQMTQSPSSLSASVGDRVITICKASQSVSNDVAWYQQKPGK APKLLIYYAANRYTGVPDRFSGSGYGTDFFTISSLQPEDIAT YFCQQDYTSPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC
Humanized 13C5-IgG1 (D265A)	Full length heavy chain (IgG1)	76	EVQLVESGGGLVKPGGSLRLSCAASGFIFRSYGMSWVRQAP GKGLEWVASISSGGSTYYPD SVKGRFTISRDN AKNSLYLQMN SLRAEDTAVYDCARGYDSGFAYWGQGTLLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK

Humanized 13C5-IgG4 (F234A/L235A)	Full length heavy chain (IgG4)	78	EVQLVESGGGLVKPGGSLRLSCAASGFIERSYGMSWVRQAP GKGLEWVASISSGGSTYYPD SVKGRFTISRDN AKNSLYLQMN SLRAEDTAVYDCARGYDSGFAYWGQGLTVTVSSASTKGPSV FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGTKTYTCNVDHKPSNTK VDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMEALHNHYTQKSLSLGLG
Humanized 13C5	Full length light chain	80	DIVLTQSPASLAVSPGQRATITCRASQSVSTSSSSFMHWYQQK PGQPPKLLIKYASNLESGV PARFSGSGSTDFLTINPVEAND TANYYCQHSWEIPYTFGQG TKLEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS FNRGEC

[0076] In one embodiment, the invention provides anti-PD-L1 antibodies that comprise the light chain CDRs and heavy chain CDRs of antibodies 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and/or 15F1. 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 heavy chain CDR1, CDR2, and CDR3; and any light chain CDR1, CDR2, and CDR3 from the antibodies provided herein. Thus, the invention provides anti-PD-L1 antibodies that comprise a heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 81, 87, 93, 99, 105, 111, 117, 123, 129, and 135; a heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 82, 88, 94, 100, 106, 112, 118, 124, 130, and 136; a heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 83, 89, 95, 101, 107, 113, 119, 125, 131, and 137; a light chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 84, 90, 96, 102, 108, 114, 120, 126, 132, and 138; a light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 85, 91, 97, 103, 109, 115, 121, 127, 133, and 139; and a light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 86, 92, 98, 104, 110, 116, 122, 128, 134, and 140. In one embodiment, the present invention provides anti-PD-L1 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 provides anti-PD-L1 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.

[0077] In one embodiment, the invention provides anti-PD-L1 antibodies that comprise a variable heavy chain of an antibody selected from the group consisting of 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and/or 15F1 and a variable light chain of an antibody selected from the group consisting of 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and/or 15F1. 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 heavy chain variable region selected from the group consisting of SEQ ID NOs: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, and 46. 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: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 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.

[0078] 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 selected from the group consisting of SEQ ID NOs: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, or 48. 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: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 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.

[0079] The anti-PD-L1 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-L1 antibody that does not have an amino acid substitution, insertion, or deletion. Thus, the variant anti-PD-L1 antibodies provided herein retain binding to PD-L1. 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.

[0080] In some embodiments, the anti-PD-L1 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 Benjamin/Cummings Publication Company, 4th Ed. (1987).

[0081] 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-L1 antibodies comprising a heavy chain variable region having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, and 46; and a light chain variable region having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48.

[0082] 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-L1 with the exemplary antibodies provided herein.

[0083] The anti-PD-L1 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 cytotoxicity, 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 patterns 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)).

[0084] In one embodiment, the antibodies or fragments thereof provided herein are immunoconjugates comprising an anti-PD-L1 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: ³H, ¹⁴C, ³⁵S, ⁶²Cu, ⁶⁴Cu, ⁸⁹Zr, ⁹⁰Y, ⁹⁹Tc, ¹¹¹In, ¹²⁵I, ¹³¹I, ¹⁷⁷Lu, ¹⁶⁶Ho, and ¹⁵³Sm. 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.

[0085] In one embodiment, the present invention provides bispecific or multispecific antibodies specific for PD-L1 and at least one other antigen or epitope. The anti-PD-L1 antibodies and fragments thereof provided herein may be tested for binding to PD-L1 using the binding assays provided herein, or any other binding assay known in the art.

[0086] 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. Coligan et 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).

[0087] 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.

[0088] 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.

[0089] 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, schwannoma, 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 histiocytosis, 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, Schwannoma, and other carcinomas, as well as head and neck cancer.

[0090] 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, vibronaceae, campylobacter, pasteuraceae, bordetella, francisella, brucella, legionellaceae, bacteroidaceae, gram-negative bacilli, 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 syncytial 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.

[0091] 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.

[0092] 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-L1 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-L1 antibody and an antigen, wherein the vaccine elicits an antigen-specific immune response.

[0093] In one embodiment, the anti-PD-L1 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-L1 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-L1 antibodies provided herein restore the capacity for IFN γ production to effector T cells in contact with regulatory T cells.

[0094] 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.

[0095] 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-L1 antibody and the additional therapeutic agent exhibit therapeutic synergy when administered together, whether concurrently or sequentially. In one embodiment, the anti-PD-L1 antibody and the additional therapeutic agent are administered in separate formulations. In another embodiment, the anti-PD-L1 antibody and the additional therapeutic agent are administered in the same formulation. In one embodiment, the anti-PD-L1 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-L1 antibody or fragment thereof.

[0096] 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.

[0097] 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.

[0098] 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: Generation of hPD-L1 monoclonal antibody

Immunization of mice with hPD-L1-HisTag and hPD-L1-mFc

[0099] To generate antibodies against the human PD-L1, cDNAs encoding the open reading frame of the extracellular domain of hPD-L1 fused with a histidine tag (hPD-L1-HisTag, SEQ ID NOs:143 and 144), mouse Fc (hPD-L1-mFc, SEQ ID NOs:145 and 146), and human Fc tag (hPD-L1-hFc, SEQ ID NO:147 and 148) were obtained by PCR and subcloned into expression vector pcDNA3.1 (Invitrogen CAT#:V-790), respectively. After transient expression in freestyle 293 cells, hPD-L1-HisTag was purified with NTA column (GE healthcare), hPD-L1-mFc and hPD-L1-hFc were purified with Protein G column (GE healthcare).

[00100] BALB/cJ mice were immunized subcutaneously every 2 weeks for 6 weeks with recombinant hPD-L1-HisTag protein (100µg/mouse) or hPD-L1-mFc emulsified with an equal volume of Freund's complete/incomplete adjuvant. Three days before fusion, mice were boosted by intravenous injection of the antigen without adjuvant. Spleen cells (1×10^8) from immunized mouse were fused with SP2/0 myeloma cells (1.5×10^7) with PEG Hybri-Max (Sigma Inc., CAT#:7181). After fusion, the cells were distributed into 96-well plates at 0.1 ml per well and incubated at 37°C, 5% CO₂ incubator. On day 1, cells were fed by adding an additional 0.1 ml per well with media containing serum and HAT plus 2×methotrexate. On day 3 and day 7, 0.1 ml of media from each well was replaced with 0.1 ml of fresh HT media. The screening typically occurred between days 9-14, and culture supernatant was tested for antibody reacting with hPD-L1-hFc by ELISA.

[00101] To clone the selected hybridoma cell, limiting dilution was carried out four times. 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 NaHCO₃ 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 2: Anti-PD-L1 antibodies cDNA sequences cloning and humanization

Cloning of immunoglobulin cDNAs

[00102] Total RNA isolated from the hybridoma cell line producing hPD-L1 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.

[00103] 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 450 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.

[00104] The heavy and light variable region sequences of antibodies m4A8 (SEQ ID NOs: 25-28), m4D1 (SEQ ID NOs: 21-24), m5G9 (SEQ ID NOs: 5-8), m5G11 (SEQ ID NOs: 9-12), m8C6 (SEQ ID NOs: 13-16), m8H3 (SEQ ID NOs: 33-36), m8H4 (SEQ ID NOs: 29-32), m7B4 (SEQ ID NOs: 17-20), m13C5 (SEQ ID NOs: 1-4) and m15F1 (SEQ ID NOs: 37-40) were amplified from the corresponding hybridoma clones. These antibodies showed desired functions, such as blocking PD-L1 binding to PD-1, and enhanced T cell activation and cytokine release.

Construction and expression of chimeric 5G11 and 13C5 antibody

[00105] 8C6, 8H4, 5G11 and 13C5 chimeric light chains (SEQ ID NOs: 52, 56, 62, and 68, respectively) were constructed by linking the PCR-cloned cDNAs of mouse VL regions to human kappa chain constant region, respectively. 8C6, 8H4, 5G11 and 13C5 chimeric heavy chains (SEQ ID NOs: 50 (8C6-IgG4), 54 (8H4-IgG4), 58 (5G11-IgG1), 60 (5G11-IgG4), 64 (13C5-IgG1), and 66 (13C5-IgG4)) were constructed by linking the PCR-cloned cDNAs of mouse VH regions to human IgG1 and IgG4 constant regions. 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.

[00106] Freestyle 293 cells (200 mL at 10⁶/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 antibody with PD-L1 was measured by ELISA and Biacore, and was shown to bind to PD-L1 with comparable affinity to that of the murine parent antibody.

Antibody humanization design

[00107] 5G11 and 13C5 antibodies were humanized using CDR grafting approach (see, for example, U.S. Pat. No.5,225,539). The light chain and heavy chain variable chain sequences of the murine antibody 5G11 and 13C5 were compared to those available in the Research Collaboratory for Structural Bioinformatics (RCSB) protein databank (<http://www.ncbi.nlm.nih.gov/igblast/igblast.cgi>). The model of 5G11 and 13C5 were generated respectively based on the VH and VL structure with the highest sequence homology.

[00108] The template human antibodies to be grafted with the complementary determining regions (CDRs) in the VH and VL of mouse 5G11 and 13C5 antibody were selected from human antibody germlines having high sequence homology with mouse 5G11 and 13C5 antibody by searching the international immunogenetics information system website (<http://www.imgt.org/3Dstructure-DB/cgi/DomainGapAlign.cgi>). For 5G11, 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 13C5, 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.

[00109] CDR amino acid sequences of the aforementioned template human antibodies were substituted by the CDRs of hybridoma (mouse) 5G11 (SEQ ID NOs 93-98) and 13C5 (SEQ ID NOs 81-86) antibodies. 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 5G11 and 13C5 antibody to give a functional humanized antibody. As for VH and VL of 5G11 and 13C5, several sites of framework amino acid of the aforementioned template human antibody were backmutated to the corresponding amino acid sequences in mouse 5G11 and 13C5 antibody. For the light chain variable region of humanized 5G11 antibody, the amino acid at position 60 is mutated from Ser (S) to Asp (D), and the amino acid at position 67 is mutated from Ser (S) to Tyr (Y); and for the heavy chain variable region of humanized 5G11 antibody, the amino acid at position 24 is mutated from Phe (F) to Val (V), the amino acid at position 49 is mutated from Ala (A) to Gly (G), the amino acid at position 73 is mutated from Thr (T) to Asn (N), and the amino acid at position 83 is mutated from Thr (T) to Asn (N). For the light chain variable region of humanized 13C5, the amino acid at position 53 is mutated from Tyr (Y) to Lys

(K); and for the heavy chain variable region of humanized 13C5, the amino acid at position 28 is mutated from Thr (T) to Ile (I), the amino acid at position 30 is mutated from Ser (S) to Arg (R), the amino acid at position 49 is mutated from Ser (S) to Ala (A), and the amino acid at position 94 is mutated from Tyr (Y) to Asp (D). The amino acid sequences of VH and VL of humanized 5G11 are provided as SEQ ID NOs:42 and 44, respectively; DNA sequences encoding the VH and VL of humanized 5G11 are provided as SEQ ID NOs: 41 and 43, respectively. The amino acid sequences of VH and VL of humanized 13C5 are provided as SEQ ID NOs: 46 and 48, respectively); DNA sequences encoding the VH and VL of humanized 13C5 are provided as SEQ ID NOs: 45 and 47, respectively.

[00110] The amino acid sequences of the full light chain for humanized antibodies 5G11 and 13C5 are provided as SEQ ID NOs: 74 and 80, respectively. The DNA sequences encoding the full length humanized 5G11 and 13C5 are provided as SEQ ID NOs: 73 and 79, respectively. IgG1 and IgG4 versions of the humanized 5G11 and 13C5 antibodies were produced. 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 DNA and amino acid sequences for the full length IgG1 heavy chain of humanized antibody 5G11-hIgG1 are provided as SEQ ID NOs: 69 and 70, respectively. The DNA and amino acid sequences for the full length IgG4 heavy chain of humanized antibody 5G11-hIgG4 are provided as SEQ ID NOs: 71 and 72, respectively. The DNA and amino acid sequences for the full length IgG1 heavy chain of humanized antibody 13C5-hIgG1 are provided as SEQ ID NOs: 75 and 76, respectively. The DNA and amino acid sequences for the full length IgG4 heavy chain of humanized antibody 13C5-hIgG4 are provided as SEQ ID NOs: 77 and 78, respectively.

Construction and expression of humanized 5G11 and 13C5 antibody

[00111] DNA encoding humanized 5G11 and 13C5 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 100 μ g 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).

[00112] The binding kinetics between PD-L1 and PD-L1 antibodies were measured by Biacore analysis, which was performed at 25°C on a Biacore3000 instrument and recorded with a data collection rate of 1 Hz. 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-L1 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. 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 (k_a and k_d) 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, K_D , was calculated from the determined rate constants by the relation $K_D = k_d/k_a$. The binding affinity of anti-PD-L1 antibodies with human PD-L1 and cynomolgus PD-L1 (cyno-PD-L1) are summarized in **Table 4**.

Table 4. PD-L1 binding affinity of anti-PD-L1 antibodies

Selected Antibody	Antigen	K_D (M)
m4A8	Human PD-L1	2.33E-9
m4D1	Human PD-L1	4.39E-9
m5G9	Human PD-L1	4.78E-9
m5G11	Human PD-L1	1.90E-10
m7B4	Human PD-L1	6.01E-9
m8H3	Human PD-L1	6.60E-9
m8H4	Human PD-L1	4.56E-9
m8C6	Human PD-L1	1.53E-9

m13C5	Human PD-L1	1.35E-9
m15F1	Human PD-L1	3.59E-9
ch5G11	Human PD-L1	2.86E-10
ch13C5	Human PD-L1	2.28E-09
hu5G11	Human PD-L1	2.25E-10
hu13C5	Human PD-L1	1.74E-09
hu5G11	Cyno- PD-L1	2.75E-10
hu13C5	Cyno- PD-L1	2.43E-09

Example 3: ELISA based binding analysis of anti-PD-L1 antibodies

[00113] ELISA binding analyses were conducted based on human PD-L1-mFc (for chimeric and humanized antibody detection) and PD-L1-hFc protein (for hybridoma antibody detection). 96-well plates (Costar, Cat No: 9018) were coated with 100 μ L of 2 μ g/ml PD-L1-mFc (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 μ L of blocking buffer (PBS with 1% (w/v) of bovine serum albumin (BSA, Roche, Cat No:738328)) and incubating for 1 hour at 37° C. After the plates were washed three times with wash buffer (PBS with 0.05% (v/v) Tween20 (Sigma, Cat No:P1379)), 100 μ L/well of 1:10 serial dilutions of hybridoma (**Figure 1**), chimeric (**Figure 2**), or humanized (**Figure 3**) anti-PD-L1 antibodies in blocking buffer (starting from 20 μ g/mL) were added and incubated at room temperature for 1 hour. The plates were washed and incubated with 100 μ L/well of Goat anti-Mouse IgG (H+L) (Thermo, Cat No: 31432) in blocking buffer for 60 min. After the plates were washed, 100 μ L/well of substrate solution TMB (eBioscience, Cat No: 00-4201-56) were added and the plates were incubated for 2min at room temperature. 100 μ L/well of stop solution (2N H₂SO₄) was added to stop the reaction. The colorimetric signals were developed and read at 450 nm using an Auto Plate SpectraMax Plus (Supplier: Molecular Devices; Model: MNR0643; Software: SoftMax Pro v5.4). Data were analyzed using GraphPad Prism 5 and EC50 was calculated (**Figures 1-3; Tables 5-7**). These data demonstrated that anti-PD-L1 antibodies (hybridoma, chimeric, and humanized) bind PD-L1, as measured by ELISA.

Table 5. ELISA based binding EC50 of anti-PD-L1 hybridoma monoclonal antibody with PD-L1

hybridoma Ab	m5G11	m7B4	m4D1	m8H4	m13C5
EC50 ng/ml	45.9	31.42	7.14	29.04	65.1
hybridoma Ab	m8C6	m5G9	m4A8	m8H3	m15F1
EC50 ng/ml	18.2	31.2	57.6	48.7	48.7

Table 6. ELISA based binding EC50 of anti-PD-L1 chimeric antibody with PD-L1

Chimeric Ab	ch5G11 hIgG1	ch5G11 hIgG4	ch8C6 hIgG4	ch8H4- hIgG4	ch13C5 hIgG1	ch13C5 hIgG4
EC50 ng/ml	82.1	90	76	133.6	72.1	118

Table 7. ELISA based binding EC50 of humanized anti-PD-L1 antibody with PD-L1

Humanized Ab	hu13C5- hIgG1	hu13C5- hIgG4	hu5G11- hIgG1	hu5G11- hIgG4
EC50 (ng/ml)	85.6	126.82	49.5	69.9

[00114] ELISA based ligand blockage analyses were conducted via blocking biotinylated human PD-L1-mFc's binding to human PD-1-hFc. PD-1-hFc antigen (Crownbio) was suspended in PBS buffer (2ug/ml, 100ul/well) and coated on the 96 well plate (Costar, Cat No: 9018) 4°C overnight. The wells were aspirated and non-specific binding sites were blocked by adding 200 µL of blocking buffer (PBS with 1% (w/v) of bovine serum albumin (BSA, Roche, Cat No:738328)) and incubating for 1 hour at 37° C. After the plate was washed three times with wash buffer (PBS with 0.05% (v/v) Tween20 (Sigma, Cat No:P1379)), 100 µL/well of 1:3 serial dilutions of hybridoma (**Figure 4**), chimeric (**Figure 5**), or humanized (**Figure 6**) anti-PD-L1 antibodies in blocking buffer (starting from 20 µg/mL) were added and incubated at 37°C for 1 hour. 100 µl PDL-1-mFc-biotin (0.1µg/ml) was then added to each well and incubated at 37°C for 2h. After the plate was washed 3 times, secondary antibody (Avidin HRP eBioscience cat No.:E07418-1632, 1:500, 100 µl/well) was added and incubated at 37 °C for 0.5 hour. After the plate was washed, 100 µL/well of substrate solution TMB (eBioscience, Cat No: 00-4201-56) was added and the plate was incubated for 3min 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 an Auto Plate SpectraMax Plus (Supplier: Molecular Devices; Model:

MNR0643; Software: SoftMax Pro v5.4). Data were analyzed using GraphPad Prism 5 and IC50 was calculated (**Figure 4-6; Tables 8-10**). These data demonstrated that anti-PD-L1 antibodies (hybridoma, chimeric, and humanized) can block PD-1's binding with PD-L1 on the cell surface, as measured by ELISA.

Table 8. IC50 of anti-PD-L1 hybridoma monoclonal antibody inhibiting PD-1 binding with PD-L1 on solid surface

Hybridoma Ab	m5G11	m7B4	m4D1	m8H4	m13C5	m8C6	m5G9	m4A8	m8H3	m15F1
IC50 (ng/ml)	710.2	892.0	332.2	787.8	871.7	343.7	613.2	867.8	647.4	655.3

Table 9. IC50 of anti-PD-L1 chimeric antibody inhibiting PD-1 binding with PD-L1 on solid surface

Chimeric Ab	ch5G11-hIgG1	ch5G11-hIgG4	ch8C6-hIgG4	ch8H4-hIgG4	ch13C5-hIgG1	ch13C5-hIgG4
IC50 (ng/mL)	1006	926.1	476.6	848.1	805.2	375.3

Table 10. IC50 of humanized anti-PD-L1 antibody inhibiting PD-1 binding with PD-L1 on solid surface

Humanized Ab	hu5G11-hIgG1	hu5G11-hIgG4	hu13C5-hIgG1	hu13C5-hIgG4
IC50 (ng/ml)	793.6	822.5	1202.6	1192.4

Example 4: Cell-based binding analysis of anti-PD-L1 antibodies

[00115] Cell binding analyses of anti-PD-L1 antibodies were performed based on binding to a 293T cell line stably expressing PD-L1 (PD-L1-293T). 2×10^5 293T-PD-L1 cells were added into each well of 96-well culture plates and incubated with the indicated antibody (20 µg/ml with the dilution of 1:5) at 4°C for 1 h. After the cells were washed three times with FACS buffer, the secondary antibody (PE Goat anti-mouse: 1:200; PE mouse anti-human: 1:10) was added to the cells at 100 µl/well, and incubated at 4°C for 40min. Cells were washed three times with FACS buffer and analyzed by FACS Array. Binding of hybridoma antibodies is shown in **Figure 7a** and **7b**. Binding of chimeric antibodies is shown in **Figure 8**. Binding of humanized antibodies is shown in **Figure 9**. The calculated EC50 for hybridoma, chimeric, and humanized antibodies are shown below in **Tables 11, 12, and 13**, respectively. These data demonstrated that anti-PD-

L1 antibodies (hybridoma, chimeric, and humanized) bind PD-L1, as measured by FACS analysis.

Table 11. EC50 of anti-PD-L1 hybridoma monoclonal antibody with the PD-L1 on cell surface

Hybridoma Ab	m4D1	m4A8	m5G11	m8H4	m8H3
EC50 ng/ml	36.07	67.83	35.94	43.49	50.81
Hybridoma Ab	m8C6	m9G9	m7B4	m13C5	m15F1
EC50 ng/ml	40.97	33.7	47.41	45.29	47.8

Table 12. EC50 of anti-PD-L1 chimeric antibody with the PD-L1 on cell surface

Chimeric Ab	ch13C5 hIgG1	ch5G11 hIgG1	ch5G11 hIgG4
EC50 ng/ml	75.75	58.26	89.68

Table 13. EC50 of humanized anti-PD-L1 antibody with the PD-L1 on cell surface

Humanized Ab	hu5G11-hIgG1	hu5G11-hIgG4	hu13C5-hIgG1	hu13C5-hIgG4
EC50 ng/ml	47.93	54.33	80.01	80.39

[00116] The effect of anti-PD-L1 antibody on PD-1 binding to PD-L1 on the cell surface was also investigated. Briefly, PD-L1-293T cells were suspended in FACS buffer (PBS with 3% fetal calf serum). Various concentrations of the hybridoma (**Figure 10**), chimeric (**Figure 11**), or humanized (**Figure 12**) anti-PD-L1 antibodies were added to the cell suspension and incubated at 4°C for 60 minutes in 96 well plates. Biotin-labeled PD-L1 protein was then added to the wells and incubated at 4°C for 60 minutes. The cells were washed 3 times with PBS and incubated with mouse anti-biotin PE (Biolegend, cat# 409004). The cell-associated fluorescence was then detected by flow cytometry analysis using FACS array. The effects of anti-PD-L1 antibodies on PD-1 binding with PD-L1-293T were measured by the mean fluorescent intensity (MFI) of staining. Inhibition of PD-1 binding by anti-PD-L1 hybridoma antibodies is shown in **Figures 10a** and **10b**. Inhibition of PD-1 binding by anti-PD-L1 chimeric antibodies is shown in **Figure 11**. Inhibition of PD-1 binding by anti-PD-L1 humanized antibodies is shown in **Figure 12**. The calculated IC50 for the hybridoma (**Table 14**), chimeric (**Table 15**), and humanized (**Table 16**)

antibodies are shown in the tables below. These data demonstrated that anti-PD-L1 antibodies (hybridoma, chimeric, and humanized) can block PD-1's binding with PD-L1 on the cell surface, as measured by FACS analysis.

Table 14. IC50 of anti-PD-L1 hybridoma monoclonal antibody inhibiting PD-1 binding with PD-L1 on cell surface

Hybridoma Ab	mIgG1	m4D1	m5G11	m13C5	m7B4	m8H4
IC50 ng/ml	NA	27.3	16.3	28.9	38.1	30.6
Hybridoma Ab	m4A8	m5G9	m8C6	m8H3	m15F1	
IC50 ng/ml	29.1	49.1	8.2	33.6	21.1	

Table 15. IC50 of anti-PD-L1 chimeric antibody inhibiting PD-1 binding with PD-L1 on cell surface

Chimeric Ab	ch5G11-hIgG1	ch5G11-hIgG4	ch8C6-hIgG4	ch8H4-hIgG4	ch13C5-hIgG1	ch13C5-hIgG4
IC50 ng/ml	40.36	33.18	34.91	42.02	42.71	35.78

Table 16. IC50 of humanized anti-PD-L1 antibody inhibiting PD-1 binding with PD-L1 on cell surface

Humanized Ab	hIgG4	hu13C5-hIgG1	hu13C5-hIgG4	hu5G11-hIgG1	hu5G11-hIgG4
IC50 ng/ml	NA	18.5	49.9	16.5	9.6

Example 5: Effect of anti-PD-L1 antibodies on T cell activation in a mixed lymphocyte reaction

[00117] A mixed lymphocyte reaction was employed to demonstrate the effect of murine (**Figure 13a, 13b**), chimeric (**Figure 14a, 14b**), or humanized (**Figure 15a, 15b**) anti-PD-L1 antibodies in blocking the PD-L1/PD-1 pathway in lymphocyte effector cells. T cells in the assay were tested for IFN- γ and IL-2 secretion in the presence or absence of humanized anti-PD-L1 antibody.

[00118] 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, for each reaction, 10^5 purified T-cells and 10^4 allogeneic mature DC cells were added in a total volume of 200 μ l. The testing antibody was assayed at different concentrations as shown in **Figures 13a, 13b, 14a, 14b, 15a, and 15b** (i.e., 20 μ g/mL, 2 μ g/mL, 0.2 μ g/mL, 0.02 μ g/mL, and 0.002 μ g/mL). Either no antibody or an isotype control antibody was used as a negative control. The cells were cultured for 5 days at 37 $^{\circ}$ C. On day 6th, 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). The results are shown in **Figures 13a, 14a, and 15a** for IL-2 secretion, and **Figures 13b, 14b, and 15b** for IFN- γ secretion. The results of the study showed that hybridoma, chimeric, and humanized anti-PD-L1 antibodies promoted T-cell IFN- γ and IL-2 secretion in a concentration dependent manner. In contrast, cultures containing the isotype control antibody did not show increase in IFN- γ and IL-2 secretion.

Example 6: Effect of anti-PD-L1 antibody on the function of T regulatory cells

[00119] T regulatory cells (CD4⁺, CD25⁺) are lymphocytes that suppress the immune response. The effect of T regulatory cells on cytokine secretion of T effector cells in MLR was tested in the presence or absence of chimeric or humanized anti-PD-L1 antibodies. T regulatory cells (CD4⁺ CD25⁺) were purified from PBMC using a regulatory T cell isolation kit (Miltenyi Biotec, cat#130-091-301). 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. T regulatory cells were added into a mixed lymphocyte reaction containing purified CD4⁺ CD25⁻ T cells and allogeneic dendritic cells in a 4:1 ratio of CD4⁺ CD25⁻ to T regulatory cells. For example: the reaction was added with 1×10^5 cells/well of CD4⁺CD25⁻ cells, 1×10^4 cells/well of mDC, and 0.25×10^5 cells/well of CD4⁺CD25⁺ cells. Antibody was added to each reaction at a concentration of 10 μ g/ml. Either no antibody or an isotype control antibody was used as a negative control. The cells were cultured for 5 days at 37 $^{\circ}$ C. On the 5th day, 50 μ l medium was taken to detect IL-2 and IFN- γ concentration. After supplementing each well with 50 μ l culture medium, the cells were cultured for another 2 days before analyzed for cell proliferation by CTG (Promega, G7573). The

levels of IFN- γ and IL-2 in the culture medium were measured using a hIFN- γ ELISA kit (R&D, cat#DY285) and IL-2 ELISA kit (eBioscience). As shown in **Figure 16**, chimeric and humanized anti-PD-L1 antibodies, ch-13C5-hIgG1, ch-13C5-hIgG4, hu-13C5-IgG1, hu-13C5-IgG4, ch-5G11-IgG1, ch-5G11-IgG4, hu-5G11-IgG1, and hu-5G11-IgG4, can reduce the inhibitory effect of Treg cells on the secretion of IFN- γ by CD4⁺CD25⁻ T effector cells, suggesting that anti-PD-L1 antibodies can modulate the immune suppression function of T regulatory cells.

Example 7: Effect of humanized anti-PD-L1 antibody on autologous T cell activation

[00120] In this example, the effect of blocking PD-1/PD-L1 pathway by anti-PD-L1 antibody on T cell activation was examined. Purified human CD4⁺ T cells (Mitenyi Biotech, cat# 130-091-155) were activated with 1 μ g/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-L1 antibody, culture medium was harvested and the concentration of IFN γ was measured with ELISA. The results are shown in **Figure 17** and suggest that PD-L1 blockage by humanized anti-PD-L1 antibodies enhanced IFN- γ secretion by T cells.

Example 8: Human recall T cell response to tetanus toxoid challenge is enhanced by humanized anti-PD-L1 antibody

[00121] To investigate whether the antigen-specific T cell receptor triggering was modulated by blocking PD-1/PD-L1 pathway with anti-PD-L1 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 from recently [<1 year] TT immunized donors were plated into 96-well round bottom plates (costar, cat#3799) at 4×10^5 cells/well using RPMI1640 (Invitrogen, cat# A10491-01) supplemented with 80 U/ml penicillin, 80 g/ml streptomycin and 30% autologous serum, added with humanized 5G11 or 13C5 at various concentrations, and stimulated with 0.1 μ g/ml SEB and 1 μ g/ml TT (Astarte Biologies). After co-culture for 7 days at 37°C, 5% CO₂, the supernatant was harvested and the concentration of IFN- γ was measured. **Figures 18a** and **18b** provide the results of the assay using PBMC from two separate donors. The results of the study demonstrate that, compared to TT antigen alone, PD-L1 blockage with anti-PD-L1 antibody resulted in enhanced IFN- γ secretion by memory T cells.

[00122] In summary, the humanized 5G11 and 13C5 antibody retained the functional activity of their parental antibodies during the humanization process.

CLAIMS

1. An isolated antibody or fragment thereof that binds to PD-L1, wherein the antibody or fragment thereof comprises a heavy chain CDR1 sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 81, 87, 93, 99, 105, 111, 117, 123, 129, and 135; a heavy chain CDR2 sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 82, 88, 94, 100, 106, 112, 118, 124, 130, and 136; a heavy chain CDR3 sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 83, 89, 95, 101, 107, 113, 119, 125, 131, and 137; a light chain CDR1 sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 84, 90, 96, 102, 108, 114, 120, 126, 132, and 138; a light chain CDR2 sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 85, 91, 97, 103, 109, 115, 121, 127, 133, and 139; and a light chain CDR3 sequence having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 86, 92, 98, 104, 110, 116, 122, 128, 134, and 140.
2. The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof comprises a heavy chain CDR1 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 81, 87, 93, 99, 105, 111, 117, 123, 129, and 135; a heavy chain CDR2 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 82, 88, 94, 100, 106, 112, 118, 124, 130, and 136; a heavy chain CDR3 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 83, 89, 95, 101, 107, 113, 119, 125, 131, and 137; a light chain CDR1 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 84, 90, 96, 102, 108, 114, 120, 126, 132, and 138; a light chain CDR2 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 85, 91, 97, 103, 109, 115, 121, 127, 133, and 139; and a light chain CDR3 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 86, 92, 98, 104, 110, 116, 122, 128, 134, and 140.

3. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises 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: 81, 82, and 83, respectively; and 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: 84, 85, and 86, respectively.
4. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises 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: 87, 88, and 89, respectively; and 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: 90, 91, and 92, respectively.
5. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises 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: 93, 94, and 95, respectively; and 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: 96, 97, and 98, respectively.
6. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises 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: 99, 100, and 101, respectively; and 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: 102, 103, and 104, respectively.
7. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises 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:105, 106, and 107, respectively; and 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: 108, 109, 110, respectively.

8. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises 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: 111, 112, and 113, respectively; and 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: 114, 115, and 116, respectively.
9. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises 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: 117, 118, and 119, respectively; and 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: 120, 121, and 122, respectively.
10. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises 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: 123, 124, and 125, respectively; and 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: 126, 127, and 128, respectively.
11. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises 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: 129, 130, and 131, respectively; and 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: 132, 133, and 134, respectively.

12. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises 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: 135, 136, and 137, respectively; and 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: 138, 139, and 140, 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-L1, wherein the antibody or fragment thereof comprises 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: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, and 46; and 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: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48.
15. The isolated antibody or fragment thereof of claim 14, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, and 46; and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48.
16. An isolated antibody or fragment thereof that binds to PD-L1, wherein the antibody or fragment thereof comprises a heavy chain variable region according to SEQ ID NO: 42 and a light chain variable region according to SEQ ID NO: 44.

17. An isolated antibody or fragment thereof that binds to PD-L1, wherein the antibody or fragment thereof comprises a heavy chain variable region according to SEQ ID NO: 46 and a light chain variable region according to SEQ ID NO: 48.
18. An isolated antibody or fragment thereof that binds to PD-L1, wherein the antibody or fragment thereof comprises a heavy chain according to SEQ ID NO: 70 and a light chain according to SEQ ID NO: 74.
19. An isolated antibody or fragment thereof that binds to PD-L1, wherein the antibody or fragment thereof comprises a heavy chain according to SEQ ID NO: 72 and a light chain according to SEQ ID NO: 74.
20. An isolated antibody or fragment thereof that binds to PD-L1, wherein the antibody or fragment thereof comprises a heavy chain according to SEQ ID NO: 76 and a light chain according to SEQ ID NO: 80.
21. An isolated antibody or fragment thereof that binds to PD-L1, wherein the antibody or fragment thereof comprises a heavy chain according to SEQ ID NO: 78 and a light chain according to SEQ ID NO: 80.
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-L1 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-L1, wherein the antibody or fragment thereof has an affinity for PD-L1 of about 10 nM to about 0.01 nM.
28. The isolated antibody or fragment thereof according to claim 27, wherein the antibody or fragment thereof has an affinity for PD-L1 of about 10 nM or less.
29. The isolated antibody or fragment thereof according to claim 27, wherein the antibody or fragment thereof has an affinity for PD-L1 of about 1.0 nM or less.
30. An isolated antibody or fragment thereof that binds to PD-L1, 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-L1, wherein the antibody blocks binding of PD-L1 to PD-1.
32. An isolated antibody or fragment thereof of claim 31, wherein the antibody or fragment thereof blocks the binding of PD-L1 to PD-1 at an IC₅₀ of about 5 ng/mL to about 1000 ng/mL.
33. An isolated antibody or fragment thereof that binds to PD-L1, 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 IFN γ .
35. An isolated antibody or fragment thereof that binds to PD-L1, wherein the antibody or fragment thereof is produced by a hybridoma selected from the group consisting of 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and/or 15F1.
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 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and 15F1.
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.