

ANTI-PD-L1/ANTI-4-1BB BISPECIFIC ANTIBODIES AND USES THEREOF

BACKGROUND

Programmed death-ligand 1 (PD-L1), also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1), is a 40 kDa type 1 transmembrane protein believed to play a major role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states such as hepatitis. The binding of PD-L1 to PD-1 or B7.1 transmits an inhibitory signal which reduces the proliferation of CD8+ T cells at the lymph nodes and supplementary to that PD-1 is also able to control the accumulation of foreign antigen specific T cells in the lymph nodes through apoptosis which is further mediated by a lower regulation of the gene Bcl-2.

It has been shown that upregulation of PD-L1 may allow cancers to evade the host immune system. An analysis of tumor specimens from patients with renal cell carcinoma found that high tumor expression of PD-L1 was associated with increased tumor aggressiveness and an increased risk of death. Many PD-L1 inhibitors are in development as immuno-oncology therapies and are showing good results in clinical trials.

4-1BB is a member of TNF-receptor superfamily (TNFRSF) and is a costimulatory molecule which is expressed following the activation of immune cells, both innate and adaptive immune cells. 4-1BB plays important role in modulate the activity of various immune cells. 4-1BB agonists enhance immune cell proliferation, survival, secretion of cytokines and cytolytic activity CD8 T cells. Many other studies showed that activation of 4-1BB enhances immune response to eliminate tumors in mice. Therefore, it suggests that 4-1BB is a promising target molecule in cancer immunology. Despite of their anti-tumor efficacy, anti-4-1BB antibody induced severe liver toxicity in clinical application.

SUMMARY

The present disclosure provides an anti-PD-L1/anti-4-1BB bispecific antibody capable to effectively block the interactions between PD-L1 and its

receptor PD-1 and between 4-1BB and its ligand. The bispecific antibody may have high binding affinity to both of a PD-L1 protein (e.g., a human PD-L1 protein) and a 4-1BB protein (e.g., a human 4-1BB protein).

The anti-PD-L1/anti-4-1BB bispecific antibody may comprise an anti-PD-L1 antibody or an antigen-binding fragment thereof as a PD-L1 targeting moiety, which is capable of specifically recognizing and/or binding to a PD-L1 protein, and an anti-4-1BB antibody or an antigen-binding fragment thereof as a 4-1BB targeting moiety, which is capable of specifically recognizing and/or binding to a 4-1BB protein.

The anti-PD-L1/anti-4-1BB bispecific antibody may comprise an anti-PD-L1 antibody or an antigen-binding fragment thereof as a PD-L1 targeting moiety.

In an embodiment, the anti-PD-L1 antibody or fragment thereof comprised in the bispecific antibody can specifically bind to an immunoglobulin C (IgC) domain of PD-L1 (e.g., human PD-L1) protein. In some embodiments, the IgC domain consists of amino acid residues 133-225 of a human PD-L1 protein. In some embodiments, the anti-PD-L1 antibody or fragment thereof can bind to at least one of amino acid residues Y134, K162, and N183 of a human PD-L1 protein. In some embodiments, the anti-PD-L1 antibody or fragment thereof does not bind to an immunoglobulin V (IgV) domain of the PD-L1 protein, and for example, the IgV domain consists of amino acid residues 19-127 of a human PD-L1 protein. For example, the human PD-L1 protein may be selected from the group consisting of proteins represented by GenBank Accession No. NP_001254635.1, NP_001300958.1, NP_054862.1, etc., but may not be limited thereto. These anti-PD-L1 antibodies may be useful for therapeutic purposes such as treating various types of cancer, etc., and can also be used for diagnostic and prognostic purposes. In an embodiment, the anti-PD-L1 antibody or fragment thereof is capable of specificity to a human PD-L1 protein.

The anti-PD-L1/anti-4-1BB bispecific antibody comprise an anti-PD-L1 antibody or an antigen-binding fragment thereof and an anti-4-1BB antibody or an antigen-binding fragment thereof, wherein

the anti-PD-L1 antibody or antigen-binding fragment thereof is capable of specifically binding to an immunoglobulin C (Ig C) domain of a human Programmed death-ligand 1 (PD-L1) protein, wherein the Ig C domain consists of amino acid residues 133-225;

the anti-PD-L1 antibody or antigen-binding fragment thereof comprises a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 2 and 3; a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 5, 262, 263, 264, 265, 266, and 267; a VL CDR1 having an amino acid sequence of SEQ ID NOS: 6, 268, and 269; a VL CDR2 having an amino acid sequence of SEQ ID NO: 7; and a VL CDR3 having an amino acid sequence of SEQ ID NOS: 8, 270, 271, and 272; and

the anti-4-1BB antibody or antigen-binding fragment thereof comprises a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 10 and 11; a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 12 and 13; a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 14, 15, 16 and 17; a VL CDR1 having an amino acid sequence of SEQ ID NO: 18; a VL CDR2 having an amino acid sequence of SEQ ID NO: 19; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 20.

In an embodiment, the anti-PD-L1 antibody or antigen-binding fragment thereof comprises a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 2 and 3; a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 4 and 5; a VL CDR1 having an amino acid sequence of SEQ ID NO: 6; a VL CDR2 having an amino acid sequence of SEQ ID NO: 7; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 8.

In an embodiment, the anti-PD-L1 antibody or antigen-binding fragment thereof is capable of binding to at least one of amino acid residues Y134, K162, or N183 of the PD-L1 protein.

In an embodiment, the anti-PD-L1 antibody or antigen-binding fragment thereof is capable of binding to amino acid residues Y134, K162, and N183 of the PD-L1 protein.

In an embodiment, the anti-PD-L1 antibody or antigen-binding fragment thereof does not bind to an immunoglobulin V (Ig V) domain of the PD-L1 protein, wherein the Ig V domain consists of amino acid residues 19-127.

In an embodiment, the anti-PD-L1/anti-4-1BB bispecific antibody activates 4-1BB signaling, or immune response, depending on PD-L1 expressed on cell surfaces.

In an embodiment, each of the anti-PD-L1 antibody or antigen-binding fragment thereof and the anti-4-1BB antibody or antigen-binding fragment thereof is independently a chimeric antibody, a humanized antibody, or a fully human antibody.

The anti-PD-L1/anti-4-1BB bispecific antibody comprise an anti-PD-L1 antibody or an antigen-binding fragment thereof and an anti-4-1BB antibody or an antigen-binding fragment thereof, wherein

the anti-PD-L1 antibody or antigen-binding fragment thereof comprises:

- (1) a VH CDR1 having an amino acid sequence of SEQ ID NO: 1;
- (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 2 and 3;
- (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 5, 262, 263, 264, 265, 266 and 267;
- (4) a VL CDR1 having an amino acid sequence of SEQ ID NOS: 6, 268, and 269;
- (5) a VL CDR2 having an amino acid sequence of SEQ ID NO: 7; and
- (6) a VL CDR3 having an amino acid sequence of SEQ ID NOS: 8, 270, 271, and 272, and

the anti-4-1BB antibody or antigen-binding fragment thereof comprises:

- (i) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 10 and 11;

(ii) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 12 and 13;

(iii) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 14, 15, 16 and 17;

(iv) a VL CDR1 having an amino acid sequence of SEQ ID NO: 18;

(v) a VL CDR2 having an amino acid sequence of SEQ ID NO: 19; and

(vi) a VL CDR3 having an amino acid sequence of SEQ ID NO: 20.

The anti-PD-L1 antibody or fragment thereof may comprise (1) a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 2 and 3; (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 5, 262, 263, 264, 265, 266 and 267; (4) a VL CDR1 having an amino acid sequence of SEQ ID NOS: 6, 268 and 269; (5) a VL CDR2 having an amino acid sequence of SEQ ID NO: 7; and (6) a VL CDR3 having an amino acid sequence of SEQ ID NOS: 8, 270, 271 and 272. For example, the anti-PD-L1 antibody or fragment thereof may comprise a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; a VH CDR2 having an amino acid sequence of SEQ ID NO: 2 or 3; (3) a VH CDR3 having an amino acid sequence of SEQ ID NO: 4, 5, 262, 263, 264, 265, 266, or 267; a VL CDR1 having an amino acid sequence of SEQ ID NO: 6, 268 or 269; a VL CDR2 having an amino acid sequence of SEQ ID NO: 7; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 8, 270, 271 or 272.

In an embodiment, the anti-PD-L1 antibody or antigen-binding fragment thereof comprises a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 2 and 3; a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 4 and 5; a VL CDR1 having an amino acid sequence of SEQ ID NO: 6; a VL CDR2 having an amino acid sequence of SEQ ID NO: 7; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 8.

The anti-PD-L1/anti-4-1BB bispecific antibody may comprise an anti-4-1BB antibody or an antigen-binding fragment thereof as a 4-1BB targeting moiety. In an

embodiment, the anti-4-1BB antibody or fragment thereof can specifically bind to 4-1BB (e.g., human 4-1BB) protein.

The anti-4-1BB antibody or an antigen-binding fragment thereof is capable of enhancing immune response and/or treating tumor (cancer) in a mammal. The anti-4-1BB antibody or an antigen-binding fragment thereof is characterized by localizing and/or activating only in tumor microenvironment (TME) and/or considerably reducing liver toxicities compared to pre-existing anti-4-1BB antibodies, with maintaining the efficacies of enhancing immune response enhancement and/or tumor treatment.

For example, the human 4-1BB protein may be selected from the group consisting of proteins represented by NCBI Accession No. NP_001552, etc., but may not be limited thereto. These anti-4-1BB antibodies may be useful for therapeutic purposes such as treating various types of cancer, etc., and can also be used for diagnostic and prognostic purposes.

In an embodiment, the anti-4-1BB antibody or fragment thereof is capable of specificity to a human 4-1BB protein. The anti-4-1BB antibody or fragment thereof may comprise (i) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 10 and 11; (ii) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 12 and 13; (iii) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 14, 15, 16 and 17; (iv) a VL CDR1 having an amino acid sequence of SEQ ID NO: 18; (v) a VL CDR2 having an amino acid sequence of SEQ ID NO: 19; and (vi) a VL CDR3 having an amino acid sequence of SEQ ID NO: 20. For example, the anti-4-1BB antibody or fragment thereof may comprise a VH CDR1 having an amino acid sequence of SEQ ID NO: 10 or 11; a VH CDR2 having an amino acid sequence of SEQ ID NO: 12 or 13; a VH CDR3 having an amino acid sequence of SEQ ID NO: 14, 15, 16, or 17; a VL CDR1 having an amino acid sequence of SEQ ID NO: 18; a VL CDR2 having an amino acid sequence of SEQ ID NO: 19; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 20.

In an embodiment, each of the anti-PD-L1 antibody or antigen-binding fragment thereof and the anti-4-1BB antibody or antigen-binding fragment thereof is independently a chimeric antibody, a humanized antibody, or a fully human antibody.

In an embodiment, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof and the anti-4-1BB antibody or antigen-binding fragment thereof are humanized antibodies.

In an embodiment, the anti-PD-L1 antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 103 and 104, or a polypeptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 103 and 104.

In an embodiment, the anti-PD-L1 antibody or antigen-binding fragment thereof comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 105 and 106, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 105 and 106.

In an embodiment, the anti-4-1BB antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 21, 22, 23 and 24, or a polypeptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 21, 22, 23 and 24.

In an embodiment, the anti-4-1BB antibody or antigen-binding fragment thereof comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 25 and 26, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 25 and 26.

In an embodiment, the anti-PD-L1/anti-4-1BB bispecific antibody activates 4-1BB signaling depending on PD-L1 expressed on cell surfaces.

In an embodiment, the anti-PD-L1/anti-4-1BB bispecific antibody is in the form of IgG-scFv form.

The anti-PD-L1/anti-4-1BB bispecific antibody may comprise a heavy component comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 27, 29, 31, 33, 35, 37, 39, 41 and 43; and a light component an amino acid sequence selected from the group consisting of SEQ ID NOS: 28, 30, 32, 34, 36, 38, 40, 42 and 44.

Another embodiment provides a pharmaceutical composition comprising the bispecific antibody as described above. The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier. The pharmaceutical composition may be used for treating and/or preventing a cancer.

Another embodiment provides a method of treating and/or preventing a cancer in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of the bispecific antibody or the pharmaceutical composition. The method may further step of identifying the subject in need of treating and/or preventing a cancer, prior to the administering step.

Another embodiment provides a use of the bispecific antibody or the pharmaceutical composition in treating and/or preventing a cancer. Another embodiment provides a use of the bispecific antibody in preparing a pharmaceutical composition for treating and/or preventing a cancer.

In the pharmaceutical compositions, methods and/or uses provided herein, the cancer may be a solid cancer or blood cancer, preferably a solid cancer.

Another embodiment provides a composition for detection of PD-L1, 4-1BB, or both thereof simultaneously, in a biological sample, the composition comprising the bispecific antibody. Another embodiment provides a method of detection of PD-L1, 4-1BB, or both thereof simultaneously, in a biological sample, the method comprising contacting the biological sample with the bispecific antibody; and detecting (measuring) an antigen-antibody reaction (binding) between the bispecific antibody and PD-L1, 4-1BB, or both thereof.

The method of detection may further comprise, after the detecting step, determining that PD-L1, 4-1BB, or both thereof are present in the biological sample when an antigen-antibody reaction is detected, and/or that PD-L1, 4-1BB,

or both thereof are absent (not present) in the biological sample, when an antigen-antibody reaction is not detected.

Another embodiment provides a pharmaceutical composition for diagnosing a disease associated with PD-L1, 4-1BB, or both thereof, the composition comprising the bispecific antibody. In another embodiment, provided is a use of the bispecific antibody for diagnosing a disease associated with PD-L1, 4-1BB, or both thereof.

Another embodiment provides a method of diagnosing a disease associated with PD-L1, 4-1BB, or both thereof, the method comprising contacting a biological sample obtained from a patient with the bispecific antibody, and detecting antigen-antibody reaction or measuring a level of antigen-antibody reaction in the biological sample. In some embodiments, the method may further comprise contacting a normal sample with the bispecific antibody, and measuring a level of an antigen-antibody reaction in the normal sample. In addition, the method may further comprise comparing the level of the antigen-antibody reaction in the biological sample and in the normal sample, after the measuring step. In addition, after the detecting step or comparing step, the method may further comprise determining the patient as a patient with a disease associated with PD-L1, 4-1BB, or both thereof, when the antigen-antibody reaction is detected in the biological sample or the level of the antigen-antibody reaction in the biological sample is higher than that of the normal sample.

The disease associated with PD-L1, 4-1BB, or both thereof may be one associated with activation (e.g., abnormal activation or over-activation) and/or overproduction (overexpression) of PD-L1, 4-1BB, or both thereof. For example, the disease may be a cancer, as described above.

An embodiment provides a polynucleotide encoding the bispecific antibody. In particular, an embodiment provides a polynucleotide encoding a heavy chain of the bispecific antibody in an IgG-scFv form which comprises a full-length IgG and a scFv linked to a C-terminus and/or N-terminus of the full-length IgG. Other embodiment provides a polynucleotide encoding a light chain of the bispecific antibody in an IgG-scFv form. Another embodiment provides a recombinant vector

comprising the polynucleotide encoding a heavy chain of the bispecific antibody, the polynucleotide encoding a light chain of the bispecific antibody, or both thereof. Another embodiment provides a recombinant cell transfected with the recombinant vector.

Another embodiment provides a method of preparing the bispecific antibody, comprising expressing the polynucleotide encoding a heavy chain of the bispecific antibody, the polynucleotide encoding a light chain of the bispecific antibody in a cell. The step of expressing the polynucleotide may be conducted by culturing the cell comprising the polynucleotide (for example, in a recombinant vector) under a condition allowing the expression of the polynucleotide. The method may further comprise isolating and/or purifying the bispecific antibody from the cell culture, after the step of expressing or culturing.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B schematically illustrate an anti-PD-L1/anti-4-1BB bispecific antibody according to an embodiment.

FIG. 2 schematically illustrates the mechanism of action of an anti-PD-L1/anti-4-1BB bispecific antibody according to an embodiment.

FIG. 3 plots demonstrated selection criteria for PD-L1 variants in order to identify required residues for Hu1210-41 binding.

FIG. 4 illustrates the locations of Y134, K162, and N183, the residues (spheres) involved in binding to the anti-PD-L1 antibody according to an embodiment.

FIG. 5 show that the anti-4-1BB antibodies according to embodiments can bind to human 4-1BB with high affinity.

FIG. 6 shows that the anti-4-1BB antibodies according to embodiments can efficiently bind to 4-1BB expressed on mammalian cells.

FIGS. 7A-7B show graphs illustrating the binding of the anti-PD-L1/anti-4-1BB bispecific antibody according to an embodiment to human PD-L1 and human 4-1BB, measured by DACE (dual antigen capture ELISA).

FIG. 8 show the anti-PD-L1/anti-4-1BB bispecific antibodies according to an embodiment are stable in human serum.

FIGS. 9A-9F show that bispecific antibodies PD-L1x4-1BB according to embodiment activate 4-1BB signaling depending on PD-L1 expression on target cells.

FIGS. 10A and 10B show the PBMC assay results for the anti-PD-L1/anti-4-1BB bispecific antibodies according to embodiments. It also shows graphs illustrating the T-cell promoting activities of the anti-PD-L1/anti-4-1BB bispecific antibodies according to embodiments.

FIG. 11 shows a graph illustrating tumor growth inhibition effect of the anti-PD-L1/anti-4-1BB bispecific antibody according to an embodiment.

DETAILED DESCRIPTION

Definitions

It is to be noted that the term “a” or “an” entity refers to one or more of that entity for example, “an antibody,” is understood to represent one or more antibodies. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term “polypeptide” refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of “polypeptide,” and the term “polypeptide” may be used instead of, or interchangeably with any of these terms. The term “polypeptide” is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide

may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis.

The term “isolated” as used herein with respect to cells, nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs or RNAs, respectively, that are present in the natural source of the macromolecule. The term “isolated” as used herein also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an “isolated nucleic acid” is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term “isolated” is also used herein to refer to cells or polypeptides which are isolated from other cellular proteins or tissues. Isolated polypeptides are meant to encompass both purified and recombinant polypeptides.

As used herein, the term “recombinant” as it pertains to polypeptides or polynucleotides intends a form of the polypeptide or polynucleotide that does not exist naturally, a non-limiting example of which can be created by combining polynucleotides or polypeptides that would not normally occur together.

“Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An “unrelated” or “non-homologous” sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences of the present disclosure.

A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) of “sequence identity” to another sequence means that,

when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in Ausubel et al. eds. (2007) Current Protocols in Molecular Biology. Preferably, default parameters are used for alignment. One alignment program is BLAST, using default parameters. In particular, programs are BLASTN and BLASTP, using the following default parameters: Genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + SwissProtein + SPupdate + PIR. Biologically equivalent polynucleotides are those having the above-noted specified percent homology and encoding a polypeptide having the same or similar biological activity.

The term “an equivalent nucleic acid or polynucleotide” refers to a nucleic acid having a nucleotide sequence having a certain degree of homology, or sequence identity, with the nucleotide sequence of the nucleic acid or complement thereof. A homolog of a double stranded nucleic acid is intended to include nucleic acids having a nucleotide sequence which has a certain degree of homology with or with the complement thereof. In one aspect, homologs of nucleic acids are capable of hybridizing to the nucleic acid or complement thereof. Likewise, “an equivalent polypeptide” refers to a polypeptide having a certain degree of homology, or sequence identity, with the amino acid sequence of a reference polypeptide. In some aspects, the sequence identity is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%. In some aspects, the equivalent polypeptide or polynucleotide has one, two, three, four or five addition, deletion, substitution and their combinations thereof as compared to the reference polypeptide or polynucleotide. In some aspects, the equivalent sequence retains the activity (e.g., epitope-binding) or structure (e.g., salt-bridge) of the reference sequence.

Hybridization reactions can be performed under conditions of different “stringency.” In general, a low stringency hybridization reaction is carried out at

about 40°C in about 10xSSC or a solution of equivalent ionic strength/temperature. A moderate stringency hybridization is typically performed at about 50°C in about 6xSSC, and a high stringency hybridization reaction is generally performed at about 60°C in about 1xSSC. Hybridization reactions can also be performed under “physiological conditions” which is well known to one of skill in the art. A non-limiting example of a physiological condition is the temperature, ionic strength, pH and concentration of Mg^{2+} normally found in a cell.

A polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine when the polynucleotide is RNA. Thus, the term “polynucleotide sequence” is the alphabetical representation of a polynucleotide molecule. This alphabetical representation can be input into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching. The term “polymorphism” refers to the coexistence of more than one form of a gene or portion thereof. A portion of a gene of which there are at least two different forms, i.e., two different nucleotide sequences, is referred to as a “polymorphic region of a gene.” A polymorphic region can be a single nucleotide, the identity of which differs in different alleles.

The terms “polynucleotide” and “oligonucleotide” are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. Polynucleotides can have any three-dimensional structure and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, dsRNA, siRNA, miRNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be interrupted by non-

nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of this disclosure that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

The term “encode” as it is applied to polynucleotides refers to a polynucleotide which is said to “encode” a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for the polypeptide and/or a fragment thereof. The antisense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

As used herein, an “antibody” or “antigen-binding polypeptide” refers to a polypeptide or a polypeptide complex that specifically recognizes and binds to an antigen. An antibody can be a whole antibody and any antigen binding fragment or a single chain thereof. Thus the term “antibody” includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule having biological activity of binding to the antigen. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein.

The terms “antibody fragment” or “antigen-binding fragment”, as used herein, is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab' , Fab , Fv , $scFv$ and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term “antibody fragment” includes aptamers, spiegelmers, and diabodies. The term “antibody fragment” also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex.

A “single-chain variable fragment” or “ $scFv$ ” refers to a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins. In

some aspects, the regions are connected with a short linker peptide of ten to about 25 amino acids. The linker can be rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. ScFv molecules are known in the art and are described, e.g., in US patent 5,892,019.

The term antibody encompasses various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon (γ , μ , α , δ , ϵ) with some subclasses among them (e.g., γ 1- γ 4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) e.g., IgG1, IgG2, IgG3, IgG4, IgG5, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant disclosure. All immunoglobulin classes are clearly within the scope of the present disclosure, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

Antibodies, antigen-binding polypeptides, variants, or derivatives thereof of the disclosure include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, e.g., Fab, Fab' and F(ab')₂, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a V_L or V_H domain, fragments produced by a Fab expression library, and

anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to LIGHT antibodies disclosed herein). Immunoglobulin or antibody molecules of the disclosure can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

Light chains are classified as either kappa or lambda (K, λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CK) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen-binding site or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CK domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain, or subset of the complementarity determining regions (CDRs), of an antibody combine to form the variable region that defines a three dimensional antigen-binding site. This quaternary antibody structure forms the antigen-binding site present at the end of each arm of the Y. More specifically, the antigen-binding site is defined by three CDRs on each of the VH and VL chains (i.e. CDR-H1,

CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3). In some instances, e.g., certain immunoglobulin molecules derived from camelid species or engineered based on camelid immunoglobulins, a complete immunoglobulin molecule may consist of heavy chains only, with no light chains. See, e.g., Hamers-Casterman et al., *Nature* 363: 446-448 (1993).

In naturally occurring antibodies, the six “complementarity determining regions” or “CDRs” present in each antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen-binding domains, referred to as “framework” regions, show less inter-molecular variability. The framework regions largely adopt a β -sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β -sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (see www.bioinf.org.uk: Dr. Andrew C.R. Martin's Group; “Sequences of Proteins of Immunological Interest,” Kabat, E., et al., U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, 196: 901-917 (1987)).

In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term “complementarity determining region” (“CDR”) to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been

described by Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia et al., J. Mol. Biol. 196: 901-917 (1987), which are incorporated herein by reference in their entireties. The CDR definitions according to Kabat and Chothia include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth in the table below as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

[Table 1]

	Kabat	Chothia
CDR-H1	31-35	26-32
CDR-H2	50-65	52-58
CDR-H3	95-102	95-102
CDR-L1	24-34	26-32
CDR-L2	50-56	50-52
CDR-L3	89-97	91-96

Kabat et al. also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983).

In addition to table above, the Kabat number system describes the CDR regions as follows: CDR-H1 begins at approximately amino acid 31 (i.e., approximately 9 residues after the first cysteine residue), includes approximately 5-7 amino acids, and ends at the next tryptophan residue. CDR-H2 begins at the

fifteenth residue after the end of CDR-H1, includes approximately 16-19 amino acids, and ends at the next arginine or lysine residue. CDR-H3 begins at approximately the thirty third amino acid residue after the end of CDR-H2; includes 3-25 amino acids; and ends at the sequence W-G-X-G, where X is any amino acid. CDR-L1 begins at approximately residue 24 (i.e., following a cysteine residue); includes approximately 10-17 residues; and ends at the next tryptophan residue. CDR-L2 begins at approximately the sixteenth residue after the end of CDR-L1 and includes approximately 7 residues. CDR-L3 begins at approximately the thirty third residue after the end of CDR-L2 (i.e., following a cysteine residue); includes approximately 7-11 residues and ends at the sequence F or W-G-X-G, where X is any amino acid.

Antibodies disclosed herein may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region may be chondrichthoid in origin (e.g., from sharks).

As used herein, the term "heavy chain constant region" includes amino acid sequences derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy chain constant region comprises at least one of: a CH1 domain, a hinge (e.g., upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, an antigen-binding polypeptide for use in the disclosure may comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a polypeptide of the disclosure comprises a polypeptide chain comprising a CH3 domain. Further, an antibody for use in the disclosure may lack at least a portion of a CH2 domain (e.g., all or part of a CH2 domain). As set forth above, it will be understood by one of ordinary skill in the art that the heavy chain constant region may be modified such that they

vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

The heavy chain constant region of an antibody disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain constant region of a polypeptide may comprise a CH1 domain derived from an IgG1 molecule and a hinge region derived from an IgG3 molecule. In another example, a heavy chain constant region can comprise a hinge region derived, in part, from an IgG1 molecule and, in part, from an IgG3 molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG1 molecule and, in part, from an IgG4 molecule.

As used herein, the term “light chain constant region” includes amino acid sequences derived from antibody light chain. Preferably, the light chain constant region comprises at least one of a constant kappa domain or constant lambda domain.

A “light chain-heavy chain pair” refers to the collection of a light chain and heavy chain that can form a dimer through a disulfide bond between the CL domain of the light chain and the CH1 domain of the heavy chain.

As previously indicated, the subunit structures and three dimensional configuration of the constant regions of the various immunoglobulin classes are well known. As used herein, the term “VH domain” includes the amino terminal variable domain of an immunoglobulin heavy chain and the term “CH1 domain” includes the first (most amino terminal) constant region domain of an immunoglobulin heavy chain. The CH1 domain is adjacent to the VH domain and is amino terminal to the hinge region of an immunoglobulin heavy chain molecule.

As used herein the term “CH2 domain” includes the portion of a heavy chain molecule that extends, e.g., from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system; see Kabat et al., U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest” (1983). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains

are interposed between the two CH2 domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

As used herein, the term “hinge region” includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen-binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux et al., J. Immunol 161: 4083 (1998)).

As used herein the term “disulfide bond” includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CH1 and CH2 regions are linked by a disulfide bond and the two heavy chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

As used herein, the term “chimeric antibody” will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which may be intact, partial or modified in accordance with the instant disclosure) is obtained from a second species. In certain embodiments the target binding region or site will be from a non-human source (e.g. mouse or primate) and the constant region is human.

As used herein, “percent humanization” is calculated by determining the number of framework amino acid differences (i.e., non-CDR difference) between the humanized domain and the germline domain, subtracting that number from the total number of amino acids, and then dividing that by the total number of amino acids and multiplying by 100.

By “specifically binds” or “has specificity to,” it is generally meant that an antibody binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to “specifically bind” to an

epitope when it binds to that epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term “specificity” is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody “A” may be deemed to have a higher specificity for a given epitope than antibody “B,” or antibody “A” may be said to bind to epitope “C” with a higher specificity than it has for related epitope “D.” Preferably, the antibody binds to an antigen (or epitope) with “high affinity”, namely with a K_D of 1×10^{-7} M or less, more preferably 5×10^{-8} M or less, more preferably 3×10^{-8} M or less, more preferably 1×10^{-8} M or less, more preferably 25×10^{-9} M or less or even more preferably 1×10^{-9} M or less.

As used herein, the terms “treat” or “treatment” may refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

By “subject” or “individual” or “animal” or “patient” or “mammal,” may refer to any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sport, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, and so on.

As used herein, phrases such as “to a patient in need of treatment” or “a subject in need of treatment” includes subjects, such as mammalian subjects, that would benefit from administration of an antibody or composition of the present disclosure used, e.g., for detection, for a diagnostic procedure and/or for treatment.

The present disclosure provides an anti-PD-L1/anti-4-1BB bispecific antibody capable to effectively block the interactions between PD-L1 and its receptor PD-1 and between 4-1BB and its ligand. The bispecific antibody may have high binding affinity to both of a PD-L1 protein (e.g., a human PD-L1 protein) and a 4-1BB protein (e.g., a human 4-1BB protein).

The anti-PD-L1/anti-4-1BB bispecific antibody may comprise an anti-PD-L1 antibody or an antigen-binding fragment thereof as a PD-L1 targeting moiety, which is capable of specifically recognizing and/or binding to a PD-L1 protein, and an anti-4-1BB antibody or an antigen-binding fragment thereof as a 4-1BB targeting moiety, which is capable of specifically recognizing and/or binding to a 4-1BB protein.

Anti-PD-L1 antibody

The anti-PD-L1/anti-4-1BB bispecific antibody may comprise an anti-PD-L1 antibody or an antigen-binding fragment thereof as a PD-L1 targeting moiety. The anti-PD-L1 antibody or antigen-binding fragment thereof may exhibit potent binding and inhibitory activities to PD-L1, and be useful for therapeutic and diagnostics uses.

The PD-L1 protein is a 40 kDa type 1 transmembrane protein. The PD-L1 protein may be a human PD-L1 protein, and the human PD-L1 protein may be selected from the group consisting of proteins represented by GenBank Accession No. NP_001254635.1, NP_001300958.1, NP_054862.1, etc., but may not be limited thereto. The human PD-L1 protein includes an extracellular portion including an N-terminal immunoglobulin V (IgV) domain (amino acids 19-127) and a C-terminal immunoglobulin C (IgC) domain (amino acids 133-225). Unlike pre-existing anti-PD-L1 antibodies, which bind to the IgV domain of PD-L1, thereby disrupting the binding between PD-1 and PD-L1, the anti-PD-L1 antibody or fragment thereof comprised in the bispecific antibody may not bind to an immunoglobulin V (IgV) domain of the PD-L1 protein but bind to the IgC domain of PD-L1, to effectively inhibit PD-L1, thereby improving therapeutic effects.

In particular, the anti-PD-L1 antibody or fragment thereof comprised in the bispecific antibody can specifically bind to an immunoglobulin C (IgC) domain of PD-L1 protein. In the case of human PD-L1 protein, the Ig C domain comprises or consists essentially of amino acid residues 133-225 of full-length of the human PD-L1 protein. More specifically, the anti-PD-L1 antibody or fragment thereof can bind to at least one selected from the amino acid residues Y134, K162, and N183 of human PD-L1 protein. In some embodiments, the anti-PD-L1 antibody or fragment thereof can bind to at least two selected from the amino acid residues Y134, K162, and N183 of human PD-L1 protein. In some embodiments, the anti-PD-L1 antibody or fragment thereof does not bind to an immunoglobulin V (IgV) domain of the PD-L1 protein, wherein the IgV domain consists of amino acid residues 19-127 of human PD-L1 protein.

In an embodiment, the anti-PD-L1 antibody or fragment thereof is capable of specificity to a human PD-L1 protein.

The anti-PD-L1 antibody or fragment thereof may comprise (1) a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 3; (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 5, 262, 263, 264, 265, 266 and 267; (4) a VL CDR1 having an amino acid sequence of SEQ ID NO: 6, 268 and 269; (5) a VL CDR2 having an amino acid sequence of SEQ ID NO: 7; and (6) a VL CDR3 having an amino acid sequence of SEQ ID NO: 8, 270, 271 and 272.

[Table 2] CDRs of anti-PD-L1 antibodies

Name	Sequence	SEQ ID NO:
VH CDR1	<u>SYDMS</u>	1
VH CDR2	TISD <u>A</u> GGYIYYSDSVKG	2
	TISD <u>A</u> GGYIYYRDSVKG	3
VH CDR3	<u>EFGKRY</u> ALDY	4
	<u>ELPWRY</u> ALDY	5
	EFGKRYALDS	262
	<u>E</u> IFNRYALDY	263
	<u>ELH</u> FRYALDY	264

	ELYFRYALDY	265
	ELLHRYALDY	266
	ELRGRYALDY	267
VL CDR1	KASQDVTPAVA	6
	KAKQDVTPAVA	268
	KASQDVWPAVA	269
VL CDR2	STSSRYT	7
VL CDR3	QQHYTTPLT	8
	MQHYTTPLT	270
	QQHSTTPLT	271
	QQHSDAPLT	272

In some embodiments, an antibody or fragment thereof includes no more than one, no more than two, or no more than three of the above substitutions. In some embodiments, the antibody or fragment thereof includes a VH CDR1 of SEQ ID NO: 1, a VH CDR2 of SEQ ID NO: 2 or 3, a VH CDR3 of SEQ ID NO: 4, 5, 262, 263, 264, 265, 266 or 267, a VL CDR1 of SEQ ID NO: 6, 268 or 269, a VL CDR2 of SEQ ID NO: 7, and a VL CDR3 of SEQ ID NO: 8, 270, 271 or 272.

For example, the anti-PD-L1 antibody or fragment thereof may comprise a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; a VH CDR2 having an amino acid sequence of SEQ ID NO: 2 or 3; a VH CDR3 having an amino acid sequence of SEQ ID NO: 4 or 5; a VL CDR1 having an amino acid sequence of SEQ ID NO: 6; a VL CDR2 having an amino acid sequence of SEQ ID NO: 7; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 8.

The back-mutations may be useful for retaining certain characteristics of the anti-PD-L1 antibodies. In some embodiments, the anti-PD-L1 antibodies of the present disclosure, in particular the human or humanized ones, may include one or more of the back-mutations. In some embodiments, the back-mutation (i.e., included amino acid at the specified position) in a heavy chain variable region (VH) is one or more selected from (a) Ser at position 44, (b) Ala at position 49, (c) Ala at position 53, (d) Ile at position 91, (e) Glu at position 1, (f) Val at position 37, (g) Thr at position 40, (h) Val at position 53, (i) Glu at position 54, (j) Asn at position 77, (k)

Arg at position 94, and (l) Thr at position 108, of the heavy chain variable region, according to Kabat numbering, and combinations thereof. In some embodiments, the VH back-mutations are selected from (a) Ser at position 44, (b) Ala at position 49, (c) Ala at position 53, and/or (d) Ile at position 91, of the heavy chain variable region, according to Kabat numbering, and combinations thereof.

In some embodiments, the back-mutation in a light chain variable region (VL) is one or more selected from (a) Ser at position 22, (b) Gln at position 42, (c) Ser at position 43, (d) Asp at position 60, and (e) Thr at position 63, of the light chain variable region, according to Kabat numbering, and combinations thereof.

In some embodiments, the anti-PD-L1 antibody or fragment thereof further comprises a heavy chain constant region, a light chain constant region, an Fc region, or the combination thereof. In some embodiments, the light chain constant region may be a kappa or lambda chain constant region. In some embodiments, the antibody is of an isotype of IgG, IgM, IgA, IgE or IgD, for example, human IgG, human IgM, human IgA, human IgE, or human IgD. In some embodiments, the isotype may be IgG, for example human IgG, such as, IgG1, IgG2, IgG3, or IgG4. In some embodiments, the fragment (antigen-binding fragment of the anti-PD-L1 antibody) may be any fragment comprising heavy chain CDRs and/or light chain CDRs of the antibody, and for example, it may be selected from the group consisting of Fab, Fab', F(ab')₂, Fd (comprising a heavy chain variable region and a CH1 domain), Fv (a heavy chain variable region and/or a light chain variable region), single-chain Fv (scFv; comprising or consisting essentially of a heavy chain variable region and a light chain variable region, in any order, and a peptide linker between the heavy chain variable region and the light chain variable region), single-chain antibodies, disulfide-linked Fvs (sdFv), and the like.

Without limitation, the anti-PD-L1 antibody or fragment thereof is a chimeric antibody, a humanized antibody, or a fully human antibody. In one aspect, antibody or fragment thereof is not naturally occurring, or chemically or recombinantly synthesized.

The binding of an antibody of the disclosure to PD-L1 can be assessed using one or more techniques well established in the art. For example, in a

preferred embodiment, an antibody can be tested by a flow cytometry assay in which the antibody is reacted with a cell line that expresses human PD-L1, such as CHO cells that have been transfected to express PD-L1, e.g., human PD-L1, or monkey PD-L1, e.g., rhesus or cynomolgus monkey or mouse PD-L1 on their cell surface. Other suitable cells for use in flow cytometry assays include anti-CD3-stimulated CD4⁺ activated T cells, which express native PD-L1. Still other suitable binding assays include ELISA assays, for example using a recombinant PD-L1 protein. Additionally, or alternatively, the binding of the antibody, including the binding kinetics (e.g., K_D value) can be tested in Biacore analysis. Preferred binding affinities of an antibody of the disclosure include those with a dissociation constant or K_D of 4.25×10^{-9} M or less.

Given that each of these antibodies can bind to PD-L1 such as human PD-L1, the CDR sequences or V_H and V_L sequences can be “mixed and matched” to create other anti-PD-L1 binding molecules of the disclosure. Preferably, when the CDR sequences or V_H and V_L chains are mixed and matched, for example, a V_H sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_H sequence. Likewise, preferably a V_L sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_L sequence.

Anti-4-1BB antibody

The anti-PD-L1/anti-4-1BB bispecific antibody may comprise an anti-4-1BB antibody or an antigen-binding fragment thereof as a 4-1BB targeting moiety.

In an embodiment, the anti-4-1BB antibody or fragment thereof can specifically bind to 4-1BB (e.g., human 4-1BB) protein.

For example, the human 4-1BB protein may be selected from the group consisting of proteins represented by NCBI Accession No. NP_001552, etc., but may not be limited thereto. These anti-4-1BB antibodies or antigen-binding fragments thereof are capable of enhancing immune response and/or treating tumor (cancer) in a mammal. The anti-4-1BB antibody or an antigen-binding fragment thereof is characterized by localizing and/or activating only in tumor microenvironment (TME) and/or considerably reducing liver toxicities compared to

pre-existing anti-4-1BB antibodies, with maintaining the efficacies of enhancing immune response enhancement and/or tumor treatment.

The term “4-1BB” refers to CD137, or TNFRSF9 (TNF Receptor 25 Superfamily Member 9), is a member of TNF-receptor superfamily (TNFRSF) and is a co-stimulatory molecule which is expressed following the activation of immune cells, both innate and adaptive immune cells. As used herein, 4-1BB may be originated from a mammal, for example, Homo sapiens (human) (NCBI Accession No. NP_001552). For example, the human 4-1BB protein (NP_001552) may be represented by the amino acid sequence (SEQ ID NO: 9), as follows:

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1  mgnsncyniva tllvlInfer trslqdpesn cpagtfcdnn nqicspcpp nsfssaggqr
61  tcdicrqckg vfrtrkecss tsnaecdctp gfhclgagcs mceqdckqgg eltkkgckdc
121  cfgtfndqkr gicrpwtncs ldgksvlvng tkerdvvcgp spadlspgas svtpapare
181  pghspqiisf flaltstall flffltlrf svvkrgrkkl lyifkqpfmr pvqttqeedg
241  cscrpfeeee ggcel

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As described herein, the term “4-1BB” includes variants, isoforms, homologs, orthologs, and paralogs. For example, antibodies specific for a human 4-1BB protein may, in certain cases, cross-react with a 4-1BB protein from a species other than human. In other embodiments, the antibodies specific for a human 4-1BB protein may be completely specific for the human 4-1BB protein and may exhibit species or other types of cross-reactivity, or may cross-react with 4-1BB from certain other species but not all other species (e.g., cross-react with monkey 4-1BB, but not mouse 4-1BB). The term “human 4-1BB” refers to human sequence 4-1BB, such as the complete amino acid sequence of human 4-1BB having NCBI Accession No. NP_001552. The term “mouse 4-1BB” refers to mouse sequence 4-1BB, such as the complete amino acid sequence of mouse 4-1BB having NCBI Accession No. NP_033430.1. 4-1BB also can be known in the art as, for example, CD137. The human 4-1BB sequence in the disclosure may differ from human 4-1BB of NCBI Accession No. NP_001552 by having, e.g., conserved mutations or mutations in non-conserved regions and the 4-1BB in the

disclosure has substantially the same biological function as the human 4-1BB of NCBI Accession No. NP_001552.

As demonstrated in the experimental examples, the anti-4-1BB antibodies disclosure herein show 4-1BB binding abilities, binding abilities to 4-1BB which expressed on cell surface, and high 4-1BB binding affinities. In addition, as demonstrated in the experimental example, the anti-4-1BB antibody disclosure herein, particularly when combined with anti-PD-L1 antibody disclosure herein, is capable of activation T cell. Furthermore, as demonstrated in the experimental example, the anti-4-1BB antibody disclosure herein has increased in vivo antitumor effect.

These anti-4-1BB antibodies may be useful for therapeutic purposes such as treating various types of cancer, etc., and can also be used for diagnostic and prognostic purposes.

In an embodiment, the anti-4-1BB antibody or fragment thereof is capable of specificity to a human 4-1BB protein. The anti-4-1BB antibody or fragment thereof may comprise (i) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 10 and 11; (ii) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 12 and 13; (iii) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 14, 15, 16 and 17; (iv) a VL CDR1 having an amino acid sequence of SEQ ID NO: 18; (v) a VL CDR2 having an amino acid sequence of SEQ ID NO: 19; and (vi) a VL CDR3 having an amino acid sequence of SEQ ID NO: 20. For example, the anti-4-1BB antibody or fragment thereof may comprise a VH CDR1 having an amino acid sequence of SEQ ID NO: 10 or 11; a VH CDR2 having an amino acid sequence of SEQ ID NO: 12 or 13; a VH CDR3 having an amino acid sequence of SEQ ID NO: 14, 15, 16, or 17; a VL CDR1 having an amino acid sequence of SEQ ID NO: 18; a VL CDR2 having an amino acid sequence of SEQ ID NO: 19; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 20.

[Table 3] CDRs of anti-4-1BB antibodies

Name	Sequence	SEQ ID NO:
VH CDR1	SYDMS	10

	GYDMS	11
VH CDR2	WISYSGGSIYYADSVKG	12
	VIYPDDGNTYYADSVKG	13
VH CDR3	DGQRNSMREFDY	14
	HGGQKPTTKSSSAYGMDG	15
	DAQRNSMREFDY	16
	DAQRQSMREFDY	17
VL CDR1	SGSSSNIGNNYVT	18
VL CDR2	ADSHRPS	19
VL CDR3	ATWDYSLSGYV	20

In non-limiting examples of the anti-4-1BB antibody or fragment thereof,

(1) the heavy chain variable region may comprise or consist essentially of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 21, 22, 23 and 24, or a polypeptide having a sequence identity of at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% to the above described amino acid sequences; and/or

(2) the light chain variable region may comprise or consist essentially of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 25 and 26, or a polypeptide having a sequence identity of at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% to the above described amino acid sequences.

Non-limiting examples of the anti-4-1BB antibody or fragment thereof may comprise a heavy chain variable region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 21, 22, 23 or 24.

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKCLEW
VSWISYSGGSIYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDGQ
RNSMREFDYWGQGT LTVSS (SEQ ID NO: 21)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSGYDMSWVRQAPGKCLEW
VSVIYPDDGNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDAAVYYCAKHGG
QKPTTKSSSAYGMDGWGQGTLTVTVSS (SEQ ID NO: 22)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKCLEW
VSWISYSGGSIYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDAAQ
RNSMREFDYWGQGTLTVTVSS (SEQ ID NO: 23)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKCLEW
VSWISYSGGSIYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDAAQ
RQSMREFDYWGQGTLTVTVSS (SEQ ID NO: 24)

Non-limiting examples of the anti-4-1BB antibody or fragment thereof may comprise a light chain variable region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 25 or 26.

QSVLTQPPSASGTPGRRVTISCSGSSSNIGNNNYVTWYQQLPGTAPKLLIY
ADSHRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCATWDYSLSGYVFGC
GTKLTVL (SEQ ID NO: 25)

QSVLTQPPSASGTPGQRTISCSGSSSNIGNNNYVTWYQQLPGTAPKLLIY
ADSHRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCATWDYSLSGYVFGC
GTKLTVL (SEQ ID NO: 26)

The antibodies of the disclosure are characterized by particular functional features or properties of the antibodies. For example, the antibodies specifically bind to human 4-1BB and may bind to 4-1BB from certain other species, e.g., monkey 4-1BB, e.g., cynomolgus monkey, rhesus monkey, but may not substantially bind to 4-1BB from certain other species, e.g., mouse 4-1BB. Preferably, an antibody of the disclosure binds to human 4-1BB with high affinity.

Further, the antibody of the disclosure, particularly as bispecific antibody comprising an anti-PD-L1 antibody of the disclosure herein, has the ability to enhance immune cell proliferation, survival, secretion of cytokines and cytolytic activity CD8 T cells. In certain embodiments, an antibody of the disclosure, particularly as bispecific antibody comprising an anti-PD-L1 antibody, binds to human 4-1BB and exhibits an ability to activate T cells.

Other means by which to evaluate the ability of the antibody to stimulate an immune response include the ability of the antibody to inhibit tumor growth, such as in an *in vivo* tumor graft model.

The binding of an antibody of the disclosure to 4-1BB can be assessed using one or more techniques well established in the art. For example, in a preferred embodiment, an antibody can be tested by a flow cytometry assay in which the antibody is reacted with a cell line that expresses human 4-1BB, such as CHO cells that have been transfected to express 4-1BB, e.g., human 4-1BB, or monkey 4-1BB, e.g., rhesus or cynomolgus monkey or mouse 4-1BB on their cell surface. Other suitable cells for use in flow cytometry assays include anti-CD3-stimulated CD4⁺ activated T cells, which express native 4-1BB. Still other suitable binding assays include ELISA assays, for example using a recombinant 4-1BB protein. Additionally, or alternatively, the binding of the antibody, including the binding kinetics (e.g., KD value) can be tested in Octet analysis. Preferred binding affinities of an antibody of the disclosure include those with a dissociation constant or KD of 1.80×10^{-10} M or less.

In some embodiments, the anti-4-1BB antibody or fragment thereof further comprises a heavy chain constant region, a light chain constant region, an Fc region, or the combination thereof. In some embodiments, the light chain constant region may be a kappa or lambda chain constant region. In some embodiments, the antibody is of an isotype of IgG, IgM, IgA, IgE or IgD, for example, human IgG, human IgM, human IgA, human IgE, or human IgD. In some embodiments, the isotype may be IgG, for example human IgG, such as, IgG1, IgG2, IgG3, or IgG4. In some embodiments, the fragment (antigen-binding fragment of the anti-PD-L1 antibody) may be any fragment comprising heavy chain CDRs and/or light chain CDRs of the antibody, and for example, it may be selected from the group consisting of Fab, Fab', F(ab')₂, Fd (comprising a heavy chain variable region and a CH1 domain), Fv (a heavy chain variable region and/or a light chain variable region), single-chain Fv (scFv; comprising or consisting essentially of a heavy chain variable region and a light chain variable region, in any order, and a peptide

linker between the heavy chain variable region and the light chain variable region), single-chain antibodies, disulfide-linked Fvs (sdFv), and the like.

Without limitation, the anti-4-1BB antibody or fragment thereof is a chimeric antibody, a humanized antibody, or a fully human antibody. In one aspect, antibody or fragment thereof is not naturally occurring, or chemically or recombinantly synthesized.

Given that each of these antibodies can bind to 4-1BB such as human 4-1BB, the CDR sequences or the V_H and V_L sequences can be "mixed and matched" to create other anti-4-1BB binding molecules of the disclosure. Preferably, when the CDRs sequences or V_H and V_L chains are mixed and matched, for example, a V_H sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_H sequence. Likewise, preferably a V_L sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_L sequence.

Anti-PD-L1/anti-4-1BB bispecific antibody

In the bispecific antibody comprising the PD-L1 targeting moiety and the 4-1BB targeting moiety, one of the PD-L1 targeting moiety and the 4-1BB targeting moiety can be a full-length antibody, and the other can be an antigen-binding fragment (e.g., scFv) comprising heavy chain CDRs, light chain CDRs, or a combination thereof. The full-length antibody targeting one of PD-L1 and 4-1BB proteins, and the antigen-binding fragment targeting the other protein may be chemically linked (e.g., covalently linked) directly or via a peptide linker. The antigen-binding fragment (e.g., scFv) may be linked directly or via a peptide linker to N-terminus of the full-length antibody (e.g., N-terminus of a light chain or a heavy chain of the full-length antibody), C-terminus of the full-length antibody (e.g., C-terminus of a heavy chain (or Fc or CH3 domain) of the full-length antibody), or both thereof (see FIGS. 1A and 1B).

In an embodiment, the bispecific antibody may comprise a full-length anti-PD-L1 antibody, an antigen-binding fragment (e.g., scFv) of an anti-4-1BB antibody, and a peptide linker therebetween. In other embodiment, the bispecific

antibody may comprise a full-length anti-4-1BB antibody, an antigen-binding fragment (e.g., scFv) of an anti-PD-L1 antibody, and a peptide linker therebetween.

In an embodiment, the scFv contained in the bispecific antibody may comprise a heavy chain variable region and a light chain variable region in any order. For example, the scFv contained in the bispecific antibody may comprise a heavy chain variable region and a light chain variable, in a direction from N-terminus to C-terminus, and optionally a peptide linker therebetween, or alternatively, the scFv contained in the bispecific antibody may comprise a light chain variable region and a heavy chain variable, in a direction from N-terminus to C-terminus, and optionally a peptide linker therebetween.

In an embodiment, the anti-PD-L1/anti-4-1BB bispecific antibody activates 4-1BB signaling, and as a result immune response, depending on PD-L1 expressed on cell surfaces.

The use of a peptide linker for the bispecific antibody may lead to a high purity of the antibody.

As used herein, the term "peptide linker" may be those including any amino acids of 1 to 100, particularly 2 to 50, and any kinds of amino acids may be included without any restrictions. The peptide linker may include for example, Gly, Asn and/or Ser residues, and also include neutral amino acids such as Thr and/or Ala. Amino acid sequences suitable for the peptide linker may be those known in the relevant art. Meanwhile, a length of the peptide linker may be variously determined within such a limit that the functions of the fusion protein will not be affected. For instance, the peptide linker may be formed by including a total of about 1 to about 100, about 2 to about 50, or about 5 to about 25 of one or more selected from the group consisting of Gly, Asn, Ser, Thr, and Ala. In one embodiment, the peptide linker may be represented as $(G_mS_l)_n$ (m , l , and n , are independently an integer of about 1 to about 10, particularly an integer of about 2 to about 5). For example, the examples of the peptide liners are summarized as follows:

Linker Function	Examples			Ref.
	Fusion Protein	Linker		
		Type	Sequence ^a	
Increase Stability/Folding	scFv	flexible	(GGGGGS) ₃	[46]
	G-CSF-Tf	flexible	(GGGGGS) ₃	[20]
	HBsAg preS1	flexible	(GGGGGS) ₃	[85]
	Myc- Est2p	flexible	(Gly) ₆	[30]
	albumin-ANF	flexible	(Gly) ₆	[31]
	virus coat protein	rigid	(EAAAK) ₃	[50]
	beta-glucanase-xylanase	rigid	(EAAAK) _n (n=1-3)	[52]
Increase expression	hGH-Tf and Tf-hGH	rigid	A(EAAAK) ₆ ALEA(EAAAK) ₆ A	[18]
	G-CSF-Tf and Tf-G-CSF	rigid	A(EAAAK) ₆ ALEA(EAAAK) ₆ A	[18]
Improve biological activity	G-CSF-Tf	flexible	(GGGGGS) ₃	[20]
	G-CSF-Tf	rigid	A(EAAAK) ₆ ALEA(EAAAK) ₆ A	[20]
	hGH-Tf	rigid	A(EAAAK) ₆ ALEA(EAAAK) ₆ A	[40]
	HSA-IFN- α 2b	flexible	GGGGGS	[17]
	HSA-IFN- α 2b	rigid	PAPAP	[17]
	HSA-IFN- α 2b	rigid	AEEAAAKEAAAKA	[17]
	PGA-rTHS	flexible	(GGGGGS) _n (n=1, 2, 4)	[55]
	interferon- γ gp120	rigid	(Ala-Pro) _n (10 – 34 aa)	[54]
	GSE-S-S-Tf	cleavable	disulfide	[39]
IFN- α 2b-HSA	cleavable	disulfide	[42]	
Enable targeting	FIX-albumin	cleavable	VSQTSKLTR AETVFPDV ^b	[59]
	LAP-IFN-	cleavable	PLG LWA ^c	[64]
	MaxE-MaxF	cleavable	RVL AEA; EDVVCC SMSV; GGIEGR GS ^e	[68]
	Immunotoxins	cleavable	TRHKQPR GWE; AGNRVRR SVG; RRRRRRR R R ^d	[72]
	Immunotoxin	cleavable	GFLG ^e	[77]
Alter PK	G-CSF-Tf and hGH-Tf	dipeptide	LE	[79]
		rigid	A(EAAAK) ₆ ALEA(EAAAK) ₆ A	
		cleavable	Disulfide	

In another embodiment, both of the PD-L1 targeting moiety and the 4-1BB targeting moiety may be a full-length antibody or an antigen-binding fragment comprising heavy chain CDRs, light chain CDRs, or a combination thereof.

In another embodiment, the bispecific antibody may be in a heterodimeric form, which comprises a first arm including a pair of a first heavy chain and a first light chain targeting one of PD-L1 and 4-1BB, and a second arm including a pair of a second heavy chain and a second light chain targeting the other one.

In an embodiment, the full-length antibody may be in a full-length immunoglobulin form (e.g., IgG, IgM, IgA, IgE or IgD, such as, human IgG, human IgM, human IgA, human IgE, or human IgD), and the antigen-binding fragment may be selected from the group consisting of Fab, Fab', F(ab')₂, Fd, Fv, scFv, single-chain antibodies, sdFv, and the like, as described above. For example, the full-length antibody may be in a full-length human IgG (human IgG1, human IgG2, human IgG3, or human IgG4) form, and the antigen-binding fragment may be scFv.

For example, an antibody described herein may comprise a flexible linker sequence, or may be modified to add a functional moiety (e.g., PEG, a drug, a toxin, or a label).

Antibodies or variants described herein may comprise derivatives that are modified, e.g., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to the antigen (e.g., an epitope). For example, but not by way of limitation, the antibodies can be modified, e.g., by at least one selected from the group consisting of glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, and the like. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the antibodies may contain one or more non-classical amino acids.

The antibodies or fragments thereof can be detectably labeled by tagging (coupling) with a conventional labeling material selected from chemiluminescent

compounds, fluorescent compounds (e.g., fluorescence emitting metals), radioisotopes, dyes, etc. The presence of the tagged antibodies or fragments thereof can be detected by measuring a signal arising during a chemical reaction between the antibody (or fragment thereof) and the labeling material. Examples of particularly useful labeling material may be at least one selected from the group consisting of luminol, isoluminol, thermotropic acridinium ester, imidazole, acridinium salt, oxalate ester, fluorescence emitting metals, and the like. For example, the fluorescence emitting metals may be ^{152}Eu , or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

In certain embodiments, the prepared bispecific antibodies will not elicit a deleterious immune response in the animal to be treated, e.g., in a human. In one embodiment, the bispecific antibody may be modified to reduce their immunogenicity using any conventional techniques. For example, the bispecific antibody may be a humanized, primatized, deimmunized, or chimeric antibody. These types of antibodies are derived from a non-human antibody, typically a murine or primate antibody, that retains or substantially retains the antigen-binding properties of the parent antibody, but which is less immunogenic in humans. This may be achieved by various methods, including (a) grafting the entire non-human variable domains onto human constant regions to generate chimeric antibodies; (b) grafting at least a part of one or more of the non-human complementarity determining regions (CDRs) into a human framework and constant regions with or without retention of critical framework residues; or (c) transplanting the entire non-human variable domains, but “cloaking” them with a human-like section by replacement of surface residues.

De-immunization can also be used to decrease the immunogenicity of an antibody. As used herein, the term “de-immunization” may include alteration of an antibody to modify T-cell epitopes (see, e.g., International Application Publication Nos. : WO/9852976 A1 and WO/0034317 A2). For example, variable heavy chain and variable light chain sequences from the starting antibody are analyzed and a

human T-cell epitope “map” from each V (variable) region showing the location of epitopes in relation to complementarity-determining regions (CDRs) and other key residues within the sequence is created. Individual T-cell epitopes from the T-cell epitope map are analyzed in order to identify alternative amino acid substitutions with a low risk of altering activity of the final antibody. A range of alternative variable heavy and variable light sequences are designed comprising combinations of amino acid substitutions and these sequences are subsequently incorporated into a range of binding polypeptides. Typically, between 12 and 24 variant antibodies are generated and tested for binding and/or function. Complete heavy and light chain genes comprising modified variable and human constant regions are then cloned into expression vectors and the subsequent plasmids introduced into cell lines for the production of whole antibody. The antibodies are then compared in appropriate biochemical and biological assays, and the optimal variant is identified.

The binding specificity and/or affinity of the bispecific antibody to each target protein can be determined by any conventional assay, for example, in vitro assays such as immunoprecipitation, radioimmunoassay (RIA), or enzyme-linked immunoabsorbent assay (ELISA), but not be limited thereto.

Alternatively, techniques described for the production of single-chain units (U.S. Pat. No. 4,694,778, etc.) can be adapted to produce single-chain units of the present disclosure. Single-chain units are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge (peptide linker), resulting in a single-chain fusion peptide (scFv). Techniques for the assembly of functional Fv fragments in *E. coli* may also be used.

Examples of techniques which can be used to produce single-chain Fvs (scFvs) and antibodies include those described in U.S. Pat. Nos. 4,946,778, 5,258,498, etc.). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human

immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See, e.g., U.S. Pat. Nos. 5,807,715, 4,816,567, and 4,816,397, which are incorporated herein by reference in their entireties.

Humanized antibodies are antibody molecules derived from a non-human species antibody that bind the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen-binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen-binding and sequence comparison to identify unusual framework residues at particular positions (See, e.g., Queen et al., U.S. Pat. No. 5,585,089, which are incorporated herein by reference in their entireties). Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (U.S. Pat. Nos. 5,225,539, 5,530,101, 5,585,089, etc., each of which is incorporated by reference in its entirety), veneering or resurfacing (EP 592,106; EP 519,596, each of which is incorporated by reference in its entirety), and chain shuffling (U.S. Pat. No. 5,565,332, which is incorporated by reference in its entirety).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887, 4,716,111, etc., each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the

human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring that express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a desired target polypeptide. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B-cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies.

Completely human antibodies which recognize a selected epitope can also be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope.

In another embodiment, DNA encoding desired monoclonal antibodies may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The isolated and subcloned hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into prokaryotic or eukaryotic host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells or myeloma cells that do not otherwise produce immunoglobulins. More particularly, the isolated DNA (which may be synthetic as described herein) may be used to clone constant and variable region sequences for the manufacture antibodies as described in Newman et al.,

U.S. Pat. No. 5,658,570, which is incorporated by reference herein. Essentially, this entails extraction of RNA from the selected cells, conversion to cDNA, and amplification by PCR using Ig specific primers. Suitable primers for this purpose are also described in U.S. Pat. No. 5,658,570. As will be discussed in more detail below, transformed cells expressing the desired antibody may be grown up in relatively large quantities to provide clinical and commercial supplies of the immunoglobulin.

Additionally, using routine recombinant DNA techniques, one or more of the CDRs of the bispecific antibody may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). For example, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds to at least one epitope of a desired polypeptide, e.g., LIGHT. Preferably, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen (or epitope). Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present disclosure and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" by splicing genes from a mouse antibody molecule, of appropriate antigen specificity, together with genes from a human antibody molecule of appropriate biological activity can be used. As used herein, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region.

Alternatively, antibody-producing cell lines may be selected and cultured using techniques well known to the skilled artisan. Such techniques are described in a variety of laboratory manuals and primary publications.

Additionally, standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding an antibody of the present disclosure, including, but not limited to, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference variable heavy chain region, CDR-H1, CDR-H2, CDR-H3, variable light chain region, CDR-L1, CDR-L2, or CDR-L3. Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity.

Non-limiting examples of Anti-PD-L1/anti-4-1BB bispecific antibody are provided in Table 4 below.

As used herein, "Heavy Component" means a component of anti-PD-L1/anti-4-1BB bispecific antibody of the present disclosure, which comprises (1) heavy chain of anti-PD-L1 antibody and (2) heavy chain and light chain of anti-4-1BB antibody.

As used herein, "Light Component" means a component of anti-PD-L1/anti-4-1BB bispecific antibody of the present disclosure, which comprises light chain of anti-PD-L1 antibody.

[Table 4] Examples of Anti-PD-L1/anti-4-1BB bispecific antibody

ABLPNB.01	Heavy Component	EVQLVESGGGLVQPGGSLRLSCAASGFTFSS SYDMS WVRQAPGKSLEWVA TISDAGGYIYYSDSVKGRFTISR DNAKNSLYLQMNSLRDEDTAVYICARE FGKRYALDY WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG	SEQ ID NO: 27
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		LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKVV EPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGKGGGGSGGGGSGGGGSSQSVLTQ PPSASGTPGRRVTISCSGSSSNIGNNYYVTWYQQLP TAPKLLIYADSHRPSGVPDRFSGSKSGTSASLAISGLR SEDEADYYCATWDYSLSGYVFGCGTKLTVLGGGGS GGGGSGGGGSGGGGSEVQLLESGLLVQPGGSLR LSCAASGFTFSSYDMSWVRQAPGKCLEWVSWISYS GGSIYYADSVKGRFTISRDNKNTLYLQMNSLRAEDT AVYYCARDGQRNSMREFDYWGQGLTVTVSS	
	Light Compo nent	DIQMTQSPSSLSASVGDRVTITCKASQDVT PAVAWY QQKPGKAPKLLIYSTSSRYTGVP SRFSGSGSGTDFTF TISSLQPEDIATYYCQQHYTTPLT FGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSK DSTYLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTK SFNRGEC	SEQ ID NO: 28
ABLPNB.02	Heavy Compo nent	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMS WVRQAPGKSLEWVATISDAGGYIYSDSVKGRFTISR DNAKNSLYLQMNSLRDEDTAVYICAREFGKRYALDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKVV EPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGKGGGGSGGGGSGGGGSSQSVLTQ PPSASGTPGRRVTISCSGSSSNIGNNYYVTWYQQLP TAPKLLIYADSHRPSGVPDRFSGSKSGTSASLAISGLR SEDEADYYCATWDYSLSGYVFGCGTKLTVLGGGGS GGGGSGGGGSGGGGSEVQLLESGLLVQPGGSLR LSCAASGFTFSSYDMSWVRQAPGKCLEWVSWISYS GGSIYYADSVKGRFTISRDNKNTLYLQMNSLRAEDT AVYYCARDAGQRNSMREFDYWGQGLTVTVSS	SEQ ID NO: 29
	Light Compo nent	DIQMTQSPSSLSASVGDRVTITCKASQDVT PAVAWY QQKPGKAPKLLIYSTSSRYTGVP SRFSGSGSGTDFTF TISSLQPEDIATYYCQQHYTTPLT FGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSK DSTYLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTK SFNRGEC	SEQ ID NO: 30

ABLPNB.03	Heavy Component	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMS WVRQAPGKSLEWVATISDAGGYIYYSDSVKGRFTISR DNAKNSLYLQMNSLRDEDTAVYICAREFGKRYALDY WGQGTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKHTHTCPPCPAPPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGIFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGKGGGSGGGGSGGGGSSQSVLTQ PPSASGTPGRRVTISCSGSSSNIGNNYVTWYQQLP TAPKLLIYADSHRPSGVPDRFSGSKSGTSASLAISGLR SEDEADYYCATWDYSLSGYVFGCGTKLTVLGGGGS GGGGSGGGGSGGGGSEVQLLESGLVQPGGSLR LSCAASGFTFSSYDMSWVRQAPGKCLEWVSVIYDD GNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDA AVYYCAKHGGQKPTTKSSSAYGMDGWGQGT LVTVS S	SEQ ID NO: 31
	Light Component	DIQMTQSPSSLSASVGDRVTITCKASQDVT PAVAWY QQKPGKAPKLLIYSTSSRYTGVP SRFSGSGSGTDFTF TISSLQPEDATYYCQQHYTTPLTFGQGT KLEIKRTVA APSVFIFPPSDEQLKSGTASVCLLNNFY PREAKVQW KVDNALQSGNSQESVTEQDSKSTYLSSTLT LSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 32
ABLPNB.04	Heavy Component	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMS WVRQAPGKSLEWVATISDAGGYIYYRDSVKGRFTISR DNAKNSLYLQMNSLRDEDTAVYICARELPWRYALDY WGQGTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKHTHTCPPCPAPPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGIFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGKGGGSGGGGSGGGGSSQSVLTQ PPSASGTPGRRVTISCSGSSSNIGNNYVTWYQQLP TAPKLLIYADSHRPSGVPDRFSGSKSGTSASLAISGLR SEDEADYYCATWDYSLSGYVFGCGTKLTVLGGGGS GGGGSGGGGSGGGGSEVQLLESGLVQPGGSLR LSCAASGFTFSSYDMSWVRQAPGKCLEWVSWISYS GGSIYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDT AVYYCARDGQRNSMREFDYWGQGT LVTVSS	SEQ ID NO: 33
	Light Component	DIQMTQSPSSLSASVGDRVTITCKASQDVT PAVAWY QQKPGKAPKLLIYSTSSRYTGVP SRFSGSGSGTDFTF TISSLQPEDATYYCQQHYTTPLTFGQGT KLEIKRTVA APSVFIFPPSDEQLKSGTASVCLLNNFY PREAKVQW	SEQ ID NO: 34

		KVDNALQSGNSQESVTEQDSKDYSLSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC	
ABLPNB.05	Heavy Compo nent	EVQLVESGGGLVQPGGSLRLSCAASGFTFSS SYDMS WVRQAPGKSLEWVA TISDAGGYIYYRDSVKGR FTISR DNAKNSLYLQMNSLRDEDTAVYICARELP PWRYALDY WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LSDGGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGKGGGGSGGGSGGGGSQSVLTQ PPSASGTPGRRVTISCS SGSSSNIGNNYVTWYQQ LP TAPKLLIY ADSHRPS GVDPDRFSGSKSGTSASLAISGLR SEDEADYY CATWDYSLSGYV FGCGTKLTVLGGGG GGGGSGGGSGGGGSEVQLLESGLLVQPGGSLR LSCAASGFTFSS SYDMS WVRQAPGKCLEWV SWISYS GGSIYYADSVKGR FTISRDNKNTLYLQMNSLRAEDT AVYYCARD DAQRNSMREFDY WGQGLVTVSS	SEQ ID NO: 35
	Light Compo nent	DIQMTQSPSSLSASVGDRVTITCK KASQDVT PAVAWY QQKPGKAPKLLIY STSSRYT GVPSRFSGSGSGTDFTF TISSLQPEDIAITYYC QQHYTTPLT FGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVCLLNNFYPRKAKVQW KVDNALQSGNSQESVTEQDSKDYSLSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 36
ABLPNB.06	Heavy Compo nent	EVQLVESGGGLVQPGGSLRLSCAASGFTFSS SYDMS WVRQAPGKSLEWVA TISDAGGYIYYRDSVKGR FTISR DNAKNSLYLQMNSLRDEDTAVYICARELP PWRYALDY WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LSDGGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGKGGGGSGGGSGGGGSQSVLTQ PPSASGTPGRRVTISCS SGSSSNIGNNYVTWYQQ LP TAPKLLIY ADSHRPS GVDPDRFSGSKSGTSASLAISGLR SEDEADYY CATWDYSLSGYV FGCGTKLTVLGGGG GGGGSGGGSGGGGSEVQLLESGLLVQPGGSLR LSCAASGFTFSS SYDMS WVRQAPGKCLEWV SWISYS GGSIYYADSVKGR FTISRDNKNTLYLQMNSLRAEDT AVYYCARD DAQRQSMREFDY WGQGLVTVSS	SEQ ID NO: 37
	Light Compo nent	DIQMTQSPSSLSASVGDRVTITCK KASQDVT PAVAWY QQKPGKAPKLLIY STSSRYT GVPSRFSGSGSGTDFTF TISSLQPEDIAITYYC QQHYTTPLT FGQGTKLEIKRTVA	SEQ ID NO:

		APSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNREGC	38
ABLPNB.07	Heavy Compo nent	EVQLVESGGGLVQPGGSLRLSCAASGFTFSS SYDMS WVRQAPGKSLEWVA TISDAGGYIYYRDSVKGR FTISR DNAKNSLYLQMNSLRDEDTAVYICARELP PWRYALDY WGQGTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGKGGGGSGGGGSGGGGSQSVLTQ PPSASGTPGQRTVISC SGSSSNIGNNNYVTWYQQ LPG TAPKLLIY ADSHRPS GVDPDRFSGSKSGTSASLAISGLR SEDEADYY CATWDYSLSGYV FGCGTKLTVLGGGGG GGGGSGGGGSGGGGSEVQLLESGLLVQPGGSLR LSCAASGFTFSS SYDMS WVRQAPGKCLEWV SWISYS GGSIYYADSVKGR FTISRDN SKNTLYLQMNSLRAEDT AVYYCARD DAQRNSMREFDY WGQGLTVTVSS	SEQ ID NO: 39
	Light Compo nent	DIQMTQSPSSLSASVGDRVTITCK KASQDVT PAVAWY QQKPGKAPKLLIY STSSRYT GVPSRFSGSGSGTDFTF TISSLQPEDVATYYC QQHYTTPLT FGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNREGC	SEQ ID NO: 40
ABLPNB.08	Heavy Compo nent	EVQLVESGGGLVQPGGSLRLSCAASGFTFSS SYDMS WVRQAPGKSLEWVA TISDAGGYIYYRDSVKGR FTISR DNAKNSLYLQMNSLRDEDTAVYICARELP PWRYALDY WGQGTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGKGGGGSGGGGSGGGGSQSVLTQ PPSASGTPGQRTVISC SGSSSNIGNNNYVTWYQQ LPG TAPKLLIY ADSHRPS GVDPDRFSGSKSGTSASLAISGLR SEDEADYY CATWDYSLSGYV FGCGTKLTVLGGGGG GGGGSGGGGSGGGGSEVQLLESGLLVQPGGSLR LSCAASGFTFSS SYDMS WVRQAPGKCLEWV SWISYS GGSIYYADSVKGR FTISRDN SKNTLYLQMNSLRAEDT AVYYCARD DAQRQSMREFDY WGQGLTVTVSS	SEQ ID NO: 41
	Light Compo	DIQMTQSPSSLSASVGDRVTITCK KASQDVT PAVAWY QQKPGKAPKLLIY STSSRYT GVPSRFSGSGSGTDFTF	SEQ ID

	nent	TISSLQPEDIATYYC QQHYTTPL TFGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC	NO: 42
ABLPNB.09	Heavy Compo nent	EVQLVESGGGLVQPGGSLRLSCAASGFTF SSYDMS WVRQAPGKSLEWVA TISDAGGYIYYRDSVKGR FTISR DNAKNSLYLQMNSLRDEDTAVYICARE LPWRYALDY WGQGTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFPYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGKGGGSGGGGSGGGGSGSVLTQ PPSASGTPGRRVTIS CSGSSSNIGNNYVTWY QQLPG TAPKLLI YADSHRPS GVDPDRFSGSKSGTSASLAISGLR SEDEADYY CATWDYSLSGYV FGCGTKLTVLGGGGG GGGGSGGGGSGGGGSEVQLLESGLVQPGGSLR LSCAASGFTF SGYDMS WVRQAPGKCLEWWS VIYPDD GNTYYADSVKGR FTISRDN SKNTLYLQMNSLRAEDA AVYYCAKH GGQKPTTKSSSAYGMDG WGQGT LTVTS S	SEQ ID NO: 43
	Light Compo nent	DIQMTQSPSSLSASVGDRVTIT CKASQDVT PAVAWY QQKPGKAPKLLI YSTSSRYT GVPSRFSGSGSGTDFTF TISSLQPEDIATYYC QQHYTTPL TFGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 44

Therapeutic Use of the bispecific antibody

The bispecific antibody provided herein is capable of simultaneously blocking the activities of PD-L1 and 4-1BB, thereby exhibiting improved effects in immunotherapies and/or cancer therapies, for example, by activating immune response (see FIG. 2). Given the ability of the bispecific antibodies of the disclosure to inhibit the binding of PD-L1 to PD-1 molecules and to stimulate antigen-specific T cell responses, the disclosure also provides a composition or in vitro and in vivo methods of using the antibodies of the disclosure to stimulate, enhance or upregulate antigen-specific T cell responses.

An embodiment provides a pharmaceutical composition comprising the bispecific antibody as described above. The pharmaceutical composition may

further comprise a pharmaceutically acceptable carrier. The pharmaceutical composition may be used for stimulating an immune response (e.g., an antigen-specific T cell response), and/or treating and/or preventing a disease associated with PD-L1, 4-1BB, or both thereof.

Another embodiment provides a method of stimulating an immune response (e.g., an antigen-specific T cell response), and/or treating and/or preventing a disease associated with PD-L1, 4-1BB, or both thereof, in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of the bispecific antibody or the pharmaceutical composition. The method may further step of identifying the subject in need of treating and/or preventing a disease associated with PD-L1, 4-1BB, or both thereof, prior to the administering step.

The disease associated with PD-L1, 4-1BB, or both thereof may be selected from cancers (or tumors), infectious diseases, autoimmune reactions, nervous system disorders, and the like.

In an embodiment, the subject may be selected from mammals including humans, for example, a mammal (e.g., a human) suffering from a cancer mammalian cells. In other embodiment, the subject may be a cell separated (isolated) from a mammal, for example, a mammal suffering from the disease selected from cancers infectious diseases, autoimmune reactions, nervous system disorders, and the like (e.g., a cancer cell or a cell separated (isolated) from an infectious region in the mammal, or a T cell, such as a tumor-infiltrating T lymphocyte, a CD4+ T cell, a CD8+ T cell, or the combination thereof).

Another embodiment provides a use of the bispecific antibody or the pharmaceutical composition in treating and/or preventing a cancer. Another embodiment provides a use of the bispecific antibody in preparing a pharmaceutical composition for treating and/or preventing a cancer.

In the pharmaceutical compositions, methods and/or uses provided herein, the disease associated with PD-L1, 4-1BB, or both thereof may be one associated with activation (e.g., abnormal activation or over-activation) and/or overproduction

(overexpression) of PD-L1, 4-1BB, or both thereof. For example, the disease may be a cancer.

The cancer may be a solid cancer or blood cancer, preferably a solid cancer.

The administration of the bispecific antibody may be conducted by one or more techniques well established in the art.

A “therapeutically effective dosage” of the antibody of the disclosure preferably results in a decrease in severity of disease symptoms, an increase infrequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. For example, for the treatment of tumor bearing subjects, a “therapeutically effective dosage” preferably inhibits tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject, which is typically a human or can be another mammal.

The pharmaceutical compositions may comprise an effective amount of the bispecific antibody, and an acceptable carrier. In some embodiments, the composition further includes a second anticancer agent (e.g., an immune checkpoint inhibitor).

In a specific embodiment, the term “pharmaceutically acceptable” may refer to approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. Further, a “pharmaceutically acceptable carrier” will generally be a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

The composition comprising the antibody or the antigen-binding fragment thereof of the present disclosure may further comprise a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier is the one conventionally used in preparing a formulation.

Diagnostic Use of the bispecific antibody

Over-expression and/or over-activation of PD-L1 and/or 4-1BB is observed in a biological sample (e.g., cells, tissues, blood, serum, etc.) from a patient suffering from a certain cancer (for example, tumor cell), and/or patients having PD-L1- and/or 4-1BB-over-expressing cells are likely responsive to treatments with the bispecific antibody. Accordingly, the bispecific antibody of the present disclosure can also be used for diagnostic and prognostic purposes.

An embodiment provides a pharmaceutical composition for diagnosing a disease associated with PD-L1, 4-1BB, or both thereof, the composition comprising the bispecific antibody. In another embodiment, provided is a use of the bispecific antibody for diagnosing a disease associated with PD-L1, 4-1BB, or both thereof.

Another embodiment provides a method of diagnosing a disease associated with PD-L1, 4-1BB, or both thereof, the method comprising contacting a biological sample obtained from a patient with the bispecific antibody, and detecting antigen-antibody reaction or measuring a level of antigen-antibody reaction in the biological sample. In this method, when the antigen-antibody reaction is detected in the biological sample or the level of the antigen-antibody reaction in the biological sample is higher than that of a normal sample, the patient from whom the biological sample is obtained may be determined as a patient with a disease associated with PD-L1, 4-1BB, or both thereof. Therefore, in some embodiments, the method may further comprise contacting a normal sample with the bispecific antibody, and measuring a level of an antigen-antibody reaction in the normal sample. In addition, the method may further comprise comparing the level of the antigen-antibody reaction in the biological sample and in the normal sample, after the measuring step. In addition, after the detecting step or comparing step, the method may further comprise determining the patient as a patient with a disease associated with PD-L1, 4-1BB, or both thereof, when the antigen-antibody reaction is detected in the biological sample or the level of the antigen-antibody reaction in the biological sample is higher than that of the normal sample.

The disease associated with PD-L1, 4-1BB, or both thereof may be one associated with activation (e.g., abnormal activation or over-activation) and/or overproduction (overexpression) of PD-L1, 4-1BB, or both thereof. For example, the disease may be a cancer, as described above.

In the diagnosing composition and method, the biological sample may be at least one selected from the group consisting of a cell, a tissue, body fluid (e.g., blood, serum, lymph, etc.) and the like, obtained (separated) from a patient to be diagnosed. The normal sample may be at least one selected from the group consisting of a cell, a tissue, body fluid (e.g., blood, serum, lymph, urine, etc.) and the like, obtained (separated) from a patient having no disease associated with PD-L1, 4-1BB, or both thereof. The patient may be selected from a mammal, such as a human. Upon optional pre-treatment of the sample, the sample can be incubated with the bispecific antibody of the present disclosure under conditions allowing the antibody to interact with a PD-L1 and/or 4-1BB protein potentially present in the sample.

Presence and/or level (concentration) of the PD-L1 and/or 4-1BB protein in the sample can be used for identifying a patient who is suitable for a treatment with the bispecific antibody, or a patient who is responsive or susceptible to the treatment with the bispecific antibody.

An embodiment provides a pharmaceutical composition identifying a patient who is suitable for a treatment with the bispecific antibody, or a patient who is responsive or susceptible to the treatment with the bispecific antibody, the composition comprising the bispecific antibody. In another embodiment, provided is a use of the bispecific antibody for identifying a patient who is suitable for a treatment with the bispecific antibody, or a patient who is responsive or susceptible to the treatment with the bispecific antibody. Another embodiment provides a method of identifying a patient who is suitable for a treatment with the bispecific antibody, or a patient who is responsive or susceptible to the treatment with the bispecific antibody, the method comprising contacting a biological sample obtained from a patient with the bispecific antibody, and detecting antigen-

antibody reaction or measuring a level of antigen-antibody reaction in the biological sample.

An embodiment provides a composition for detection of PD-L1, 4-1BB, or both thereof simultaneously, in a biological sample, the composition comprising the bispecific antibody. Another embodiment provides a method of detection of PD-L1, 4-1BB, or both thereof simultaneously, in a biological sample, the method comprising contacting the biological sample with the bispecific antibody; and detecting (measuring) an antigen-antibody reaction (binding) between the bispecific antibody and PD-L1, 4-1BB, or both thereof.

In the detecting composition and the detecting method, the term “detection of PD-L1, 4-1BB, or both thereof” may refer to, but not be limited to, detection of presence (and/or absence) and/or level of PD-L1, 4-1BB, or both thereof in the biological sample.

In the method of detection, when an antigen-antibody reaction is detected, it can be determined that PD-L1, 4-1BB, or both thereof are present in the biological sample, and when an antigen-antibody reaction is not detected, it can be determined that PD-L1, 4-1BB, or both thereof are absent (not present) in the biological sample. Therefore, the method of detection may further comprise, after the detecting step, determining that PD-L1, 4-1BB, or both thereof are present in the biological sample when an antigen-antibody reaction is detected, and/or that PD-L1, 4-1BB, or both thereof are absent (not present) in the biological sample, when an antigen-antibody reaction is not detected.

In the method of detection, the level of PD-L1, 4-1BB, or both thereof may be determined according to the degree of the antigen-antibody reaction (e.g., the amount of antigen-antibody complex formed by the antigen-antibody reaction, the intensity of any signal obtained by the antigen-antibody reaction, and the like, which can be measured by any conventional means).

The biological sample may comprise at least one selected from the group consisting of a cell (e.g., a tumor cell), a tissue (e.g., a tumor tissue), body fluid (e.g., blood, serum, etc.), and the like, obtained or isolated from a mammal such as a human. The steps of the method of detection may be conducted *in vitro*.

In the diagnosing method and/or detecting method, the step of detecting the antigen-antibody reaction or measuring a level of the antigen-antibody reaction may be performed by any general method known to the relevant art, such as general enzymatic reactions, fluorescent reactions, luminescent reactions, and/or detection of radiation. For example, the step may be performed by a method selected from, but not limited to, the group consisting of immunochromatography, immunohistochemistry (IHC), enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), enzyme immunoassay (EIA), fluorescence immunoassay (FIA), luminescence immunoassay (LIA), western blotting, microarray, flow cytometry, surface plasmon resonance (SPR), and the like, but not be limited thereto.

Polynucleotides Encoding the Antibodies and Methods of Preparing the Antibodies

An embodiment provides a polynucleotide encoding the bispecific antibody. In particular, an embodiment provides a polynucleotide encoding a heavy chain of the bispecific antibody in an IgG-scFv form. Other embodiment provides a polynucleotide encoding a light chain of the bispecific antibody in the IgG-scFv form. The IgG-scFv form may refer to a kind of a bispecific antibody comprising a full-length IgG antibody targeting (binding to) one of PD-L1 and 4-1BB proteins and a scFv fragment targeting (binding to) the other one, wherein the scFv is linked to a C-terminus and/or N-terminus of the full-length IgG antibody directly (without a peptide linker) or via a peptide linker.

In an embodiment, when the bispecific antibody in an IgG-scFv form comprises a full-length IgG antibody against PD-L1 and a scFv fragment against 4-1BB, the polynucleotide encoding a heavy chain of the bispecific antibody may encode a heavy chain of the full-length IgG antibody against PD-L1 and a scFv fragment against 4-1BB that is linked to a C-terminus and/or N-terminus of the full-length IgG antibody directly or via a peptide linker; and the polynucleotide encoding a light chain of the bispecific antibody may encode a light chain of the full-length IgG antibody against PD-L1.

In another embodiment, when the bispecific antibody in an IgG-scFv form comprises a full-length IgG antibody against 4-1BB and a scFv fragment against PD-L1, the polynucleotide encoding a heavy chain of the bispecific antibody may encode a heavy chain of the full-length IgG antibody against 4-1BB and a scFv fragment against PD-L1 that is linked to a C-terminus and/or N-terminus of the full-length IgG antibody directly or via a peptide linker; and the polynucleotide encoding a light chain of the bispecific antibody may encode a light chain of the full-length IgG antibody against 4-1BB.

Another embodiment provides a recombinant vector comprising the polynucleotide encoding a heavy chain of the bispecific antibody, the polynucleotide encoding a light chain of the bispecific antibody, or both thereof. Another embodiment provides a recombinant cell transfected with the recombinant vector.

Another embodiment provides a method of preparing the bispecific antibody, comprising expressing the polynucleotide encoding a heavy chain of the bispecific antibody, the polynucleotide encoding a light chain of the bispecific antibody in a cell. The step of expressing the polynucleotide may be conducted by culturing the cell comprising the polynucleotide (for example, in a recombinant vector) under a condition allowing the expression of the polynucleotide. The method may further comprise isolating and/or purifying the bispecific antibody from the cell culture, after the step of expressing or culturing.

[EXAMPLES]

Hereafter, the present invention will be described in detail by examples.

The following examples are intended merely to illustrate the invention and are not construed to restrict the invention.

Example 1: Preparation of anti-PD-L1 monoclonal antibodies

1.1. Preparation of Anti-human-PD-L1 mouse monoclonal antibodies and analysis thereof

Anti-human-PD-L1 mouse monoclonal antibodies were generated using the hybridoma technology, as disclosed in International Application Publication WO2017-215590.

The amino acid and polynucleotide sequences of the variable regions of the hybridoma supernatants, named Hybridoma HL1210-3, are provided in Table 5 below.

[Table 5] HL1210-3 variable sequences

Name	Sequence	SEQ ID NO:
HL1210-3 VH	GAAGTGAAACTGGTGGAGTCTGGGGGAGACTTAGTG AAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAGCC TCTGGATTCACTTTCAGTAGCTATGACATGTCTTGGG TTCGCCAGACTCCGGAGAAGAGTCTGGAGTGGGTCTG CAACCATTAGTGATGGTGGTGGTTACATCTACTATTC AGACAGTGTGAAGGGGCGATTTACCATCTCCAGAGA CAATGCCAAGAACAACCTGTACCTGCAAATGAGCAGT CTGAGGTCTGAGGACACGGCCTTGTATATTTGTGCAA GAGAATTTGGTAAGCGCTATGCTTTGGACTACTGGG GTCAAGGAACCTCAGTCACCGTCTCCTCA	45
HL1210-3 VH	EVKLVESGGDLVKPGGSLKLSCAASGFTFSSYDMSWV RQTPEKSLEWWATISDGGGYIYYSDSVKGRFTISRDNK NNLYLQMSSLRSEDALYICAREFGKRYALDYWGQGTST VT	46
HL1210-3 VL	GACATTGTGATGACCCAGTCTCACAAATTCATGTCCA CATCGGTAGGAGACAGGGTCAGCATCTCCTGCAAGG CCAGTCAGGATGTGACTCCTGCTGTCGCCTGGTATC AACAGAAGCCAGGACAATCTCCTAACTACTGATTTA CTCCACATCCTCCCGGTACACTGGAGTCCCTGATCG CTTCACTGGCAGTGGATCTGGGACGGATTTCACTTTC ACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTT TATTACTGTCAGCAACATTATACTACTCCGCTCACGTT CGGTGCTGGGACCAAGCTGGAGCTGAAA	47
HL1210-3 VL	DIVMTQSHKFMSTSVGDRVSISCKASQDVTPAVAWYQQ KPGQSPKLLIYSTSSRYTGVPDRFTGSGSGTDFTFTISS VQAEDLAVYYCQQHYTTPITFGAGTKLELK	48

1.2. Humanization of the HL1210-3 mouse mAb

The mAb HL1210-3 variable region genes were employed to create a humanized Mab, per the methods commonly employed in the art and as disclosed in International Application Publication WO 2017-215590.

The amino acid and nucleotide sequences of some of the resultant humanized antibody are listed in Table 6 below.

[Table 6] Humanized antibody sequences (bold indicates CDR)

Name	Amino Acid Sequence	SEQ ID NO:
HL1210-VH	EVKLVESGGDLVKPGGSLKLSCAASGFTFSS YDMSWVRQ TPEKSLEWVATISDGGGGYIYYSDSVKGRFTISRDNKNNL YLQMSSLRSEDALYICAREFGKRYALDYWGQGTSVTVS S	49
Hu1210 VH.1	EVQLVESGGGLVKPGGSLRLSCAASGFTFSS YDMSWVR QAPGKGLEWVSTISDGGGGYIYYSDSVKGRFTISRDNKNS LYLQMNSLRAEDTAVYYCAREFGKRYALDYWGQGTTVT VSS	50
Hu1210 VH.1a	EVQLVESGGGLVKPGGSLRLSCAASGFTFSS YDMSWVR QAPGKGLEWVATISDGGGGYIYYSDSVKGRFTISRDNKNS LYLQMNSLRAEDTAVYYCAREFGKRYALDYWGQGTTVT VSS	51
Hu1210 VH.1b	EVQLVESGGGLVKPGGSLRLSCAASGFTFSS YDMSWVR QAPGKSLEWVATISDGGGGYIYYSDSVKGRFTISRDNKNS LYLQMNSLRAEDTAVYYCAREFGKRYALDYWGQGTTVT SS	52
Hu1210 VH.2	EVQLVESGGGLVKPGGSLRLSCAASGFTFSS YDMSWIRQ APGKGLEWVSTISDGGGGYIYYSDSVKGRFTISRDNKNSL YLQMNSLRAEDTAVYYCAREFGKRYALDYWGQGTTVT SS	53
Hu1210 VH.2a	EVQLVESGGGLVKPGGSLRLSCAASGFTFSS YDMSWIRQ APGKGLEWVATISDGGGGYIYYSDSVKGRFTISRDNKNSL YLQMNSLRAEDTAVYYCAREFGKRYALDYWGQGTTVT SS	54
Hu1210 VH.2b	EVQLVESGGGLVKPGGSLRLSCAASGFTFSS YDMSWVR QAPGKSLEWVATISDGGGGYIYYSDSVKGRFTISRDNKNS LYLQMNSLRAEDTAVYYCAREFGKRYALDYWGQGTTVT SS	55
Hu1210 VH.3	EVQLLESGGGLVQPGGSLRLSCAASGFTFSS YDMSWVR QAPGKGLEWVSTISDGGGGYIYYSDSVKGRFTISRDNKNT LYLQMNSLRAEDTAVYYCAREFGKRYALDYWGQGTTVT VSS	56
Hu1210 VH.3a	EVQLLESGGGLVQPGGSLRLSCAASGFTFSS YDMSWVR QAPGKSLEWVATISDGGGGYIYYSDSVKGRFTISRDNKNT LYLQMNSLRAEDTAVYYCAREFGKRYALDYWGQGTTVT SS	57
Hu1210 VH.4	EVQLVESGGGLVQPGGSLRLSCAASGFTFSS YDMSWVR QAPGKGLEWVSTISDGGGGYIYYSDSVKGRFTISRDNKNS LYLQMNSLRDEDTAVYYCAREFGKRYALDYWGQGTTVT VSS	58
Hu1210 VH.4a	EVQLVESGGGLVQPGGSLRLSCAASGFTFSS YDMSWVR QAPGKGLEWVATISDGGGGYIYYSDSVKGRFTISRDNKNS LYLQMNSLRDEDTAVYYCAREFGKRYALDYWGQGTTVT VSS	59
Hu1210 VH.4b	EVQLVESGGGLVQPGGSLRLSCAASGFTFSS YDMSWVR	60

	QAPGKSLEWWATISDGGGGYIYYSDSVKGRFTISRDNAKNS LYLQMNSLRDEDTAVYICAREFGKRYALDYWGQGTTVTV SS	
Hu1210 VH.4c	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYDMSWVR QAPGKSLEWWATISEGGGGYIYYSDSVKGRFTISRDNAKNS LYLQMNSLRDEDTAVYICAREFGKRYALDYWGQGTTVTV SS	61
Hu1210 VH.4d (H12 VH)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYDMSWVR QAPGKSLEWWATISDAGGGYIYYSDSVKGRFTISRDNAKNS LYLQMNSLRDEDTAVYICAREFGKRYALDYWGQGTTVTV SS	62
Hu1210 VH.4e	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYDMSWVR QAPGKSLEWWATISDVGGYIYYSDSVKGRFTISRDNAKNS LYLQMNSLRDEDTAVYICAREFGKRYALDYWGQGTTVTV SS	63
Hu1210 VH.5	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYDMSWVR QAPGKGLEWWATISDGGGGYIYYSDSVKGRFTISRDNAKNS LYLQMNSLRAEDTAVYYCAREFGKRYALDYWGQGTTLTV SS	64
HU1210 VH.5a	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYDMSWVR QAPGKGLEWWATISDGGGGYIYYSDSVKGRFTISRDNAKNS LYLQMNSLRAEDTAVYICAREFGKRYALDYWGQGTTLTV SS	65
HU1210 VH.5b	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYDMSWVR QAPGKGLEWWATISDGGGGYIYYSDSVKGRFTISRDNAKNS LYLQMNSLRAEDTAVYICAREFGKRYALDYWGQGTTVTV SS	66
HU1210 VH.5c	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYDMSWVR QAPGKGLEWWATISDGGGGYIYYSDSVKGRFTISRDNAKNN LYLQMNSLRAEDTAVYICAREFGKRYALDYWGQGTTLTV SS	67
HU1210 VH.5d	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYDMSWVR QTPEKSLEWWATISDGGGGYIYYSDSVKGRFTISRDNAKNN LYLQMNSLRAEDTAVYICAREFGKRYALDYWGQGTTLTV SS	68
HL1210-VK	DIVMTQSHKFMSTSVGDRVSISCKASQDVTPAVAWYQQK PGQSPKLLIYSTSSRYTGVDPDRFTGSGSGTDFTFTISSVQ AEDLAVYYCQQHYTTPLTFGAGTKLEIK	69
Hu1210 VK.1 (H12 VL)	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQK PGKAPKLLIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQP EDIATYYCQQHYTTPLTFGQGGTKLEIK	70
Hu1210 VL.1a	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQK PGKSPKLLIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQP EDIATYYCQQHYTTPLTFGQGGTKLEIK	71
Hu1210 VK.2	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQK PGKAPKLLIYSTSSRYTGVPSRFSGSGSGTDFTLTSSSLQP EDFATYYCQQHYTTPLTFGQGGTKLEIKR	72

Hu1210 VK.2a	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKLLIY STSSRYT GVDPDRFTGSGSGTDFTLTISSLQPEDFATYYC QQHYTTP LTFGQGGTKLEIKR	73
Hu1210 VK.2b	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGQSPKLLIY STSSRYT GVDPDRFTGSGSGTDFTLTISSLQPEDFATYYC QQHYTTP LTFGQGGTKLEIKR	74
Hu1210 VK.2c	DIQMTQSPSSLSASVGDRVTISCKASQDVTPAVAWYQQKPGQSPKLLIY STSSRYT GVDPDRFTGSGSGTDFTLTISSLQPEDFATYYC QQHYTTP LTFGQGGTKLEIKR	75
Name	Nucleic Acid Sequence	SEQ ID NO:
HL1210 VH	GAGGTGAAGCTGGTGGAGAGCGGCGGAGATCTGGTGAAGCCTGGCGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGTGAGGAGACCCCGAGAAGAGCCTGGAGTGGGTGGCCACATCAGCGATGGCGGCGGCTACATCTACTACAGCGACAACGCGTGAAGGGCAGGTTACCATCAGCAGGGACAACGCAAGAACAACCTGTACCTGCAGATGAGCAGCCTGAGGAGCGAGGACACCGCCCTGTACATCTGCGCCAGGGAGTTCGCAAGAGGTACGCCCTGGACTACTGGGGACAGGGCACAGCGTGACCGTGAGCAGC	76
Hu1210 VH.1	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTG AAGCCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGT GAGACAGGCCCTGGCAAAGGCCTGGAGTGGGTGAGC ACCATCTCCGATGGCGGCGGCTACATCTATTACTCCGA CAGCGTGAAGGGCAGGTTACCATCAGCAGGGACAAC GCCAAGAAGCAGCCTGTACCTGCAGATGAACAGCCTGAG GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGAG TTCGGCAAAAGGTACGCCCTGGACTACTGGGGCCAGG GCACAACCGTGACCGTGAGCAGC	77
Hu1210 VH.1a	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTG AAGCCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGT GAGACAGGCCCTGGCAAAGGCCTGGAGTGGGTGGCC ACCATCTCCGATGGCGGCGGCTACATCTATTACTCCGA CAGCGTGAAGGGCAGGTTACCATCAGCAGGGACAAC GCCAAGAAGCAGCCTGTACCTGCAGATGAACAGCCTGAG GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGAG TTCGGCAAAAGGTACGCCCTGGACTACTGGGGCCAGG GCACAACCGTGACCGTGAGCAGC	78
Hu1210 VH.1b	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTG AAGCCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGT GAGACAGGCCCTGGCAAAGGCCTGGAGTGGGTGGCC ACCATCTCCGATGGCGGCGGCTACATCTATTACTCCGA CAGCGTGAAGGGCAGGTTACCATCAGCAGGGACAAC GCCAAGAAGCAGCCTGTACCTGCAGATGAACAGCCTGAG GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGAG TTCGGCAAAAGGTACGCCCTGGACTACTGGGGCCAGG	79

	GCACAACCGTGACCGTGAGCAGC	
Hu1210 VH.2	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTG AAGCCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGATC AGACAGGCCCCCTGGCAAAGGCCTGGAGTGGGTGAGCA CCATCTCCGATGGCGGCGGCTACATCTATTACTCCGAC AGCGTGAAGGGCAGGTTACCATCAGCAGGGACAACG CCAAGAACAGCCTGTACCTGCAGATGAACAGCCTGAGG GCCGAGGACACCGCCGTGTACTACTGCGCCAGGGAGT TCGGCAAAAGGTACGCCCTGGACTACTGGGGCCAGGG CACAACCGTGACCGTGAGCAGC	80
Hu1210 VH.2a	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTG AAGCCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGATC AGACAGGCCCCCTGGCAAAGGCCTGGAGTGGGTGGCCA CCATCTCCGATGGCGGCGGCTACATCTATTACTCCGAC AGCGTGAAGGGCAGGTTACCATCAGCAGGGACAACG CCAAGAACAGCCTGTACCTGCAGATGAACAGCCTGAGG GCCGAGGACACCGCCGTGTACTACTGCGCCAGGGAGT TCGGCAAAAGGTACGCCCTGGACTACTGGGGCCAGGG CACAACCGTGACCGTGAGCAGC	81
Hu1210 VH.2b	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTG AAGCCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGT GAGACAGGCCCCCTGGCAAAGGCCTGGAGTGGGTGGCC ACCATCTCCGATGGCGGCGGCTACATCTATTACTCCGA CAGCGTGAAGGGCAGGTTACCATCAGCAGGGACAAC GCCAAGAACAGCCTGTACCTGCAGATGAACAGCCTGAG GGCCGAGGACACCGCCGTGTACATCTGCGCCAGGGAG TTCGGCAAAAGGTACGCCCTGGACTACTGGGGCCAGG GCACAACCGTGACCGTGAGCAGC	82
Hu1210 VH.3	GAGGTGCAGCTGCTGGAGAGCGGAGGAGGACTGGTG CAACCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGT GAGACAGGCCCCCTGGCAAAGGCCTGGAGTGGGTGAGC ACCATCTCCGATGGCGGCGGCTACATCTATTACTCCGA CAGCGTGAAGGGCAGGTTACCATCAGCAGGGACAAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAG GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGAG TTCGGCAAAAGGTACGCCCTGGACTACTGGGGCCAGG GCACAACCGTGACCGTGAGCAGC	83
Hu1210 VH.3a	GAGGTGCAGCTGCTGGAGAGCGGAGGAGGACTGGTG CAACCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGT GAGACAGGCCCCCTGGCAAAGGCCTGGAGTGGGTGGCC ACCATCTCCGATGGCGGCGGCTACATCTATTACTCCGA CAGCGTGAAGGGCAGGTTACCATCAGCAGGGACAAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAG GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGAG TTCGGCAAAAGGTACGCCCTGGACTACTGGGGCCAGG	84

	GCACAACCGTGACCGTGAGCAGC	
Hu1210 VH.4	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTG CAACCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGT GAGACAGGCCCCCTGGCAAAGGCCTGGAGTGGGTGAGC ACCATCTCCGATGGCGGCGGCTACATCTATTACTCCGA CAGCGTGAAGGGCAGGTTCAACATCAGCAGGGACAAC GCCAAGAACAGCCTGTACCTGCAGATGAACAGCCTGAG GGATGAGGACACCGCCGTGTACTACTGCGCCAGGGAG TTCGGCAAAGGTACGCCCTGGACTACTGGGGCCAGG GCACAACCGTGACCGTGAGCAGC	85
Hu1210 VH.4a	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTG CAACCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGT GAGACAGGCCCCCTGGCAAAGGCCTGGAGTGGGTGGCC ACCATCTCCGATGGCGGCGGCTACATCTATTACTCCGA CAGCGTGAAGGGCAGGTTCAACATCAGCAGGGACAAC GCCAAGAACAGCCTGTACCTGCAGATGAACAGCCTGAG GGATGAGGACACCGCCGTGTACTACTGCGCCAGGGAG TTCGGCAAAGGTACGCCCTGGACTACTGGGGCCAGG GCACAACCGTGACCGTGAGCAGC	86
Hu1210 VH.4b	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTG CAACCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGT GAGACAGGCCCCCTGGCAAAGGCCTGGAGTGGGTGGCC ACCATCTCCGATGGCGGCGGCTACATCTATTACTCCGA CAGCGTGAAGGGCAGGTTCAACATCAGCAGGGACAAC GCCAAGAACAGCCTGTACCTGCAGATGAACAGCCTGAG GGATGAGGACACCGCCGTGTACATCTGCGCCAGGGAG TTCGGCAAAGGTACGCCCTGGACTACTGGGGCCAGG GCACAACCGTGACCGTGAGCAGC	87
Hu1210 VH.4c	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTG CAACCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGT GAGACAGGCCCCCTGGCAAAGGCCTGGAGTGGGTGGCC ACCATCTCCGAAGGCGGCGGCTACATCTATTACTCCGA CAGCGTGAAGGGCAGGTTCAACATCAGCAGGGACAAC GCCAAGAACAGCCTGTACCTGCAGATGAACAGCCTGAG GGATGAGGACACCGCCGTGTACATCTGCGCCAGGGAG TTCGGCAAAGGTACGCCCTGGACTACTGGGGCCAGG GCACAACCGTGACCGTGAGCAGC	88
Hu1210 VH.4d	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTG CAACCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGT GAGACAGGCCCCCTGGCAAAGGCCTGGAGTGGGTGGCC ACCATCTCCGATGCGGGCGGCTACATCTATTACTCCGA CAGCGTGAAGGGCAGGTTCAACATCAGCAGGGACAAC GCCAAGAACAGCCTGTACCTGCAGATGAACAGCCTGAG GGATGAGGACACCGCCGTGTACATCTGCGCCAGGGAG TTCGGCAAAGGTACGCCCTGGACTACTGGGGCCAGG	89

	GCACAACCGTGACCGTGAGCAGC	
Hu1210 VH.4e	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTG CAACCCGGAGGCAGCCTGAGACTGAGCTGCGCTGCCA CGGGCTTCACCTTCAGCAGCTACGACATGAGCTGGGT GAGACAGGCCCTGGCAAAAGCCTGGAGTGGGTGGCC ACCATCTCCGATGTTGGCGGCTACATCTATTACTCCGA CAGCGTGAAGGGCAGGTTACCATCAGCAGGGACAAC GCCAAGAACAGCCTGTACCTGCAGATGAACAGCCTGAG GGATGAGGACACCGCCGTGTACATCTGCGCCAGGGAG TTCGGCAAAAGGTACGCCCTGGACTACTGGGGCCAGG GCACAACCGTGACCGTGAGCAGC	90
Hu1210 VH.5	GAGGTGCAGCTGGTGGAGTCCGGAGGAGGCCTGGTG CAACCTGGAGGCTCCCTGAGGCTGTCCTGTGCCGCTT CCGGCTTCACCTTCAGCTCCTACGATATGAGCTGGGTG AGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGGCCA CCATCTCCGACGGAGGCGGCTACATCTACTACTCCGAC TCCGTGAAGGGCAGGTTACCATCTCCCGGGACAACG CCAAGAACTCCCTGTACCTGCAGATGAACTCTCTCAGG GCTGAGGACACCGCCGTGTATTACTGCGCCAGGGAGT TTGGCAAGAGGTACGCCCTGGATTACTGGGGCCAGGG CAACTGGTGACAGTGAGCTCC	91
Hu1210 VH.5a	GAGGTGCAGCTGGTGGAGTCCGGAGGAGGCCTGGTG CAACCTGGAGGCTCCCTGAGGCTGTCCTGTGCCGCTT CCGGCTTCACCTTCAGCTCCTACGATATGAGCTGGGTG AGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGGCCA CCATCTCCGACGGAGGCGGCTACATCTACTACTCCGAC TCCGTGAAGGGCAGGTTACCATCTCCCGGGACAACG CCAAGAACTCCCTGTACCTGCAGATGAACTCTCTCAGG GCTGAGGACACCGCCGTGTATATCTGCGCCAGGGAGT TTGGCAAGAGGTACGCCCTGGATTACTGGGGCCAGGG CAACTGGTGACAGTGAGCTCC	92
Hu1210 VH.5b	GAGGTGCAGCTGGTGGAGTCCGGAGGAGGCCTGGTG CAACCTGGAGGCTCCCTGAGGCTGTCCTGTGCCGCTT CCGGCTTCACCTTCAGCTCCTACGATATGAGCTGGGTG AGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGGCCA CCATCTCCGACGGAGGCGGCTACATCTACTACTCCGAC TCCGTGAAGGGCAGGTTACCATCTCCCGGGACAACG CCAAGAAACACCTGTACCTGCAGATGAACTCTCTCAGG GCTGAGGACACCGCCGTGTATATCTGCGCCAGGGAGT TTGGCAAGAGGTACGCCCTGGATTACTGGGGCCAGGG CAACTGGTGACAGTGAGCTCC	93
Hu1210 VH.5c	GAGGTGCAGCTGGTGGAGTCCGGAGGAGGCCTGGTG CAACCTGGAGGCTCCCTGAGGCTGTCCTGTGCCGCTT CCGGCTTCACCTTCAGCTCCTACGATATGAGCTGGGTG AGGCAGACCCCTGAGAAGAGCCTGGAGTGGGTGGCCA CCATCTCCGACGGAGGCGGCTACATCTACTACTCCGAC TCCGTGAAGGGCAGGTTACCATCTCCCGGGACAACG CCAAGAAACACCTGTACCTGCAGATGAACTCTCTCAGG GCTGAGGACACCGCCGTGTATATCTGCGCCAGGGAGT TTGGCAAGAGGTACGCCCTGGATTACTGGGGCCAGGG	94

	CACACTGGTGACAGTGAGCTCC	
Hu1210 VH.5d	GAGGTGCAGCTGGTGGAGTCCGGAGGAGGCCTGGTG CAACCTGGAGGCTCCCTGAGGCTGTCCTGTGCCGCTT CCGGCTTCACCTTCAGCTCCTACGATATGAGCTGGGTG AGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGGCCA CCATCTCCGACGGAGGCGGCTACATCTACTACTCCGAC TCCGTGAAGGGCAGGTTACCATCTCCCGGGACAACG CCAAGAACTCCCTGTACCTGCAGATGAACTCTCTCAGG GCTGAGGACACCGCCGTGTATATCTGCGCCAGGGAGT TTGGCAAGAGGTACGCCCTGGATTACTGGGGCCAGGG CACAACCGTGACAGTGAGCTCC	95
HL1210 VK	GACATCGTGATGACCCAGAGCCACAAGTTCATGAGCAC CAGCGTGGGCGATAGGGTGAGCATCAGCTGCAAGGCC AGCCAGGATGTGACCCCTGCCGTGGCCTGGTACCAGC AGAAGCCCGGCCAGAGCCCCAAGCTGCTGATCTACAG CACCAGCAGCAGGTACACCGGCGTGCCCGACAGGTTT ACAGGAAGCGGCAGCGGCACCGACTTCACCTTCACCA TCAGCAGCGTGACAGGCCGAGGACCTGGCCGTGTACTA CTGCCAGCAGCACTACACCACCCCTCTGACCTTCGGCG CCGGCACCAAGCTGGAGCTGAAG	96
Hu1210 VK.1	GACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCG CTAGCGTGGGCGACAGGGTGACCATCACCTGCAAGGC CAGCCAGGATGTGACCCCTGCCGTGGCCTGGTACCAG CAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACA GCACCAGCAGCAGGTACACCGGCGTGCCAGCAGGTT TAGCGGAAGCGGCAGCGGCACCGACTTCACCTTCACC ATCAGCAGCCTGCAGCCCGAGGACATCGCCACCTACTA CTGCCAGCAGCACTACACCACCCCTCTGACCTTCGGCC AGGGCACCAAGCTGGAGATCAAG	97
Hu1210 VK.1a	GACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCG CTAGCGTGGGCGACAGGGTGACCATCACCTGCAAGGC CAGCCAGGATGTGACCCCTGCCGTGGCCTGGTACCAG CAGAAGCCCGGCAAGTCCCCCAAGCTGCTGATCTACA GCACCAGCAGCAGGTACACCGGCGTGCCAGCAGGTT TAGCGGAAGCGGCAGCGGCACCGACTTCACCTTCACC ATCAGCAGCCTGCAGCCCGAGGACATCGCCACCTACTA CTGCCAGCAGCACTACACCACCCCTCTGACCTTCGGCC AGGGCACCAAGCTGGAGATCAAG	98
Hu1210 VK.2	GACATTAGATGACCCAGTCCCCTAGCAGCCTGTCCGC TTCCGTGGGCGACAGGGTGACCATCACCTGCAAGGCC AGCCAGGACGTGACACCTGCTGTGGCCTGGTATCAACA GAAGCCTGGCAAGGCTCCTAAGCTCCTGATCTACAGCA CATCCTCCCGGTACACCGGAGTGCCCTCCAGGTTTAGC GGCAGCGGCTCCGGCACCGATTTACCCCTGACCATTTT CTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCC AGCAGCACTACACCACACCCCTGACCTTCGGCCAGGG CACCAAGCTGGAGATCAAGCGG	99
Hu1210 VK.2a	GACATTAGATGACCCAGTCCCCTAGCAGCCTGTCCGC TTCCGTGGGCGACAGGGTGACCATCACCTGCAAGGCC	100

	AGCCAGGACGTGACACCTGCTGTGGCCTGGTATCAACA GAAGCCTGGCAAGGCTCCTAAGCTCCTGATCTACAGCA CATCCTCCCGGTACACCGGAGTGCCCGACAGGTTTACC GGCAGCGGCTCCGGCACCGATTTACCCCTGACCATTTC CTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCC AGCAGCACTACACCACACCCCTGACCTTCGGCCAGGG CACCAAGCTGGAGATCAAGCGG	
Hu1210 VK.2b	GACATTCAGATGACCCAGTCCCCTAGCAGCCTGTCCGC TTCCGTGGGCGACAGGGTGACCATCACCTGCAAGGCC AGCCAGGACGTGACACCTGCTGTGGCCTGGTATCAACA GAAGCCTGGCCAGAGCCCTAAGCTCCTGATCTACAGCA CATCCTCCCGGTACACCGGAGTGCCCGACAGGTTTACC GGCAGCGGCTCCGGCACCGATTTACCCCTGACCATTTC CTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCC AGCAGCACTACACCACACCCCTGACCTTCGGCCAGGG CACCAAGCTGGAGATCAAGCGG	101
Hu1210 VK.2c	GACATTCAGATGACCCAGTCCCCTAGCAGCCTGTCCGC TTCCGTGGGCGACAGGGTGACCATCAGCTGCAAGGCC AGCCAGGACGTGACACCTGCTGTGGCCTGGTATCAACA GAAGCCTGGCCAGAGCCCTAAGCTCCTGATCTACAGCA CATCCTCCCGGTACACCGGAGTGCCCGACAGGTTTACC GGCAGCGGCTCCGGCACCGATTTACCCCTGACCATTTC CTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCC AGCAGCACTACACCACACCCCTGACCTTCGGCCAGGG CACCAAGCTGGAGATCAAGCGG	102

The humanized VH and VK genes were produced synthetically and then respectively cloned into vectors containing the human gamma 1 and human kappa constant domains. The pairing of the human VH and the human VK created the 40 humanized antibodies (see Table 7).

[Table 7] Humanized antibodies with their VH and VL regions

VH VK	Hu1210 VH.1	Hu1210 VH.1a	Hu1210 VH.1b	Hu1210 VH.2	Hu1210 VH.2a	Hu1210 VH 2.b	Hu1210 VH
Hu1210 VK.1	Hu1210-1	Hu1210-2	Hu1210-3	Hu1210-4	Hu1210-5		
Hu1210 VK.1a	Hu1210-7	Hu1210-8	Hu1210-9	Hu1210-10	Hu1210-11		
Hu1210 VK							H1210 chimera

VH VK	Hu1210 VH.3	Hu1210 VH.3a	Hu1210 VH.4	Hu1210 VH.4a	Hu1210 VH.4b
Hu1210 VK.1	Hu1210-13	Hu1210-14	Hu1210-15	Hu1210-16	Hu1210-17
Hu1210 VK.1a	Hu1210-18	Hu1210-19	Hu1210-20	Hu1210-21	Hu1210-22

VK	VH	Hu1210 VH.5	HU1210 VH.5a	HU1210 VH.5b	HU1210 VH.5c	HU1210 VH.5d
Hu1210 VK.2		Hu1210-23	Hu1210-27	Hu1210-31	Hu1210-32	Hu1210-36
Hu1210 VK.2a		Hu1210-24	Hu1210-28		Hu1210-33	Hu1210-37
Hu1210 VK.2b		Hu1210-25	Hu1210-29		Hu1210-34	Hu1210-38
Hu1210 VK.2c		Hu1210-26	Hu1210-30		Hu1210-35	Hu1210-39

VK	VH	Hu1210 VH.4c	Hu1210 VH.4d	Hu1210 VH.4e
Hu1210 VK.1		Hu1210-40	Hu1210-41	Hu1210-42

1.3. Identification of PD-L1 Epitope

This study was conducted to identify amino acid residues involved in the binding of PD-L1 to the antibodies of the present disclosure.

An alanine-scan library of PD-L1 was constructed. Briefly, 217 mutant clones of PD-L1 were generated on Integral Molecular's protein engineering platform. Binding of Hu1210-41 Fab to each variant in the PD-L1 mutation library was determined, in duplicate, by high-throughput flow cytometry. Each raw data point had background fluorescence subtracted and was normalized to reactivity with PD-L1 wild-type (WT). For each PD-L1 variant, the mean binding value was plotted as a function of expression. To identify preliminary critical clones (circles with crosses), thresholds (dashed lines) of >70% WT binding to control MAb (MIH1 Mab, in house prepared) and <30%WT reactivity to Hu1210-41 Fab were applied (FIG. 3). Y134, K162, and N183 of PD-L1 were identified as required residues for Hu1210-41 binding. The low reactivity of N183A clone with Hu1210-41 Fab suggests that it is the major energetic contributor to Hu1210-41 binding, with lesser contributions by Y134 and K162.

The critical residues (spheres) were identified on a 3D PD-L1 structure, as illustrated in FIG. 4. These residues, Y134, K162, and N183, therefore, constitute epitopes of PD-L1 responsible for binding to antibodies of various embodiments of the present disclosure.

It is interesting to note that Y134, K162, and N183 are all located within the IgC domain of the PD-L1 protein. Both PD-1 and PD-L1's extracellular portions have an IgV domain and an IgC domain. It is commonly known that PD-L1 binds

to PD-1 through bindings between their IgV domains. Unlike such conventional antibodies, however, Hu1210-41 binds to the IgC domain, which would have been expected to be ineffective in inhibiting PD-1/PD-L1 binding. This different epitope of Hu1210-41, surprisingly, likely contributes to the excellent activities of Hu1210-41.

1.4. Antibody Engineering of anti-PD-L1 antibody

Examples 1.4 attempted to identify further improved antibodies based on Hu1210-41 using mutagenesis.

Four sub-libraries were constructed for antibody engineering of anti-PD-L1 monoclonal antibody, using either of the following strategies. In strategy 1, mutagenesis of heavy chain variable domain VH CDR3 or VL-CDR3 was performed by highly random mutation. In strategy 2, two CDR combination libraries composed of (VH-CDR3, VL-CDR3 and VL-CDR1) or (VH-CDR1, VH-CDR2 and VL-CDR2) were generated by CDR walking with controlled mutation rates.

Bio-Panning: the phage panning methods were adapted by shortening the incubation/binding time prior to the harsh washing condition. Briefly, 100 μ l magnetic streptavidin beads (Invitrogen, USA) were blocked with 1 ml of MPBS for 1 hr at room temperature. In another tube, library phage was pre-incubated ($5 \times 10^{11} \sim 12$ for each round) with 100 μ l magnetic streptavidin beads in 1 ml of MPBS to remove unwanted binders. Magnet particle concentrator was used to separate the phage and beads. The biotinylated PD-L1 protein was added to the phage and incubated 2h at room temperature, and gently mixed using an overhead shaker. Beads carrying phage from the solution were separated in the magnetic particle concentrator and the supernatant was discarded. The beads were washed with fresh wash buffer, ten times with PBST and ten times with PBS (pH7.4). 0.8ml, 0.25% Trypsin in PBS (Sigma, USA) was added and incubated for 20 min at 37°C to elute the phage. The output phage was titrated and rescued for next round panning, decreasing antigen concentration round by round.

ELISA screening and On/off rate ranking

Clones were picked and induced from the desired panning output; phage ELISA was conducted for primary screening; positive clones were analyzed by sequencing; unique hotspots were found.

Table 8 shows the mutations identified. As shown below, the hotspot mutation residues and/or their substitutes are underlined.

[Table 8] Mutations in the CDRs

	CDR-H1 (SEQ ID No.)	CDR-H2 (SEQ ID No.)	CDR-H3 (SEQ ID No.)
WT (H12)	SYDMS (107)	TISDAGGYIYYSDSVKG (117)	EFGKRYALDY (127)
B3	SYDMS (108)	TISDAGGYIYYRDSVKG (118)	EFGKRYALDY (128)
C4	SYDMS (109)	TISDAGGYIYYRDSVKG (119)	EFGKRYALD <u>S</u> (129)
B1	SYDMS (110)	TISDAGGYIYYRDSVKG (120)	<u>E</u> IFNRYALDY (130)
B6	SYDMS (111)	TISDAGGYIYYRDSVKG (121)	<u>EL</u> PWRYALDY (131)
C3	SYDMS (112)	TISDAGGYIYYRDSVKG (122)	<u>EL</u> HFRYALDY (132)
C6	SYDMS (113)	TISDAGGYIYYRDSVKG (123)	<u>EL</u> YFRYALDY (133)
A1	SYDMS (114)	TISDAGGYIYYRDSVKG (124)	<u>EL</u> LHRYALDY (134)
A2	SYDMS (115)	TISDAGGYIYYRDSVKG (125)	<u>EL</u> RGRYALDY (135)
A3	SYDMS (116)	TISDAGGYIYYRDSVKG (126)	EFGKRYALDY (136)
	CDR-L1 (SEQ ID No.)	CDR-L2 (SEQ ID No.)	CDR-L3 (SEQ ID No.)
WT	KASQDVTPAVA (137)	STSSRYT (147)	QQHYTTPLT (157)
B3	K <u>A</u> KQDVTPAVA (138)	STSSRYT (148)	<u>M</u> QHYTTPLT (158)
C4	KASQDV <u>W</u> PAVA (139)	STSSRYT (149)	QQH <u>S</u> TTPLT (159)
B1	KASQDVTPAVA (140)	STSSRYT (150)	QQHYTTPLT (160)
B6	KASQDVTPAVA (141)	STSSRYT (151)	QQHYTTPLT (161)
C3	KASQDVTPAVA (142)	STSSRYT (152)	QQHYTTPLT (162)
C6	KASQDVTPAVA (143)	STSSRYT (153)	QQHYTTPLT (163)
A1	KASQDVTPAVA (144)	STSSRYT (154)	QQHYTTPLT (164)
A2	KASQDVTPAVA (145)	STSSRYT (155)	QQHYTTPLT (165)
A3	KASQDVTPAVA (146)	STSSRYT (156)	QQH <u>S</u> DAPLT (166)

The amino acid sequences of the variable regions of these antibodies are shown in Table 9 below.

[Table 9] Antibody sequences

Name	Sequence	SEQ ID NO:
WT-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQ APGKSLEWVATISDAGGYIYYRDSVKGRFTISRDNKNSLY LQMNSLRDEDTAVYICAREFGKRYALDYWGQGTTVTVSS	167
WT-VK	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKP GKAPKLLIYSTSSRYTGVPSPRFSGSGSGTDFTFTISLQPE DIATYYCQQHYTTPLTFGQGTKLEIK	168
B3-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQ APGKSLEWVATISDAGGYIYYRDSVKGRFTISRDNKNSLY LQMNSLRDEDTAVYICAREFGKRYALDYWGQGTTVTVSS	169

B3-VK	DIQMTQSPSSLSASVGDRVITITCKAKQDVTPAVAWYQQKP GKAPKLLIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQPE DIATYYCMQHYTTPLTFGQGTKLEIK	170
C4-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQ APGKSLEWWATISDAGGYIYYRDSVKGRFTISRDNKNSLY LQMNSLRDEDTAVYICAREFGKRYALDSWGQGTTVTVSS	171
C4-VK	DIQMTQSPSSLSASVGDRVITITCKASQDVTPAVAWYQQK PGKAPKLLIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQP EDIATYYCQQHSTTPLTFGQGTKLEIK	172
B1-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVR QAPGKSLEWWATISDAGGYIYYRDSVKGRFTISRDNKNS LYLQMNSLRDEDTAVYICAREIFNRYALDYWGQGTTVTVS S	173
B1-VK	DIQMTQSPSSLSASVGDRVITITCKASQDVTPAVAWYQQK PGKAPKLLIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQP EDIATYYCQQHYTTPLTFGQGTKLEIK	174
B6-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVR QAPGKSLEWWATISDAGGYIYYRDSVKGRFTISRDNKNS LYLQMNSLRDEDTAVYICARELPWRYALDYWGQGTTVTV SS	175
B6-VK	DIQMTQSPSSLSASVGDRVITITCKASQDVTPAVAWYQQK PGKAPKLLIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQP EDIATYYCQQHYTTPLTFGQGTKLEIK	176
C3-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVR QAPGKSLEWWATISDAGGYIYYRDSVKGRFTISRDNKNS LYLQMNSLRDEDTAVYICARELHFRYALDYWGQGTTVTV SS	177
C3-VK	DIQMTQSPSSLSASVGDRVITITCKASQDVTPAVAWYQQK PGKAPKLLIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQP EDIATYYCQQHYTTPLTFGQGTKLEIK	178
C6-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVR QAPGKSLEWWATISDAGGYIYYRDSVKGRFTISRDNKNS LYLQMNSLRDEDTAVYICARELYFRYALDYWGQGTTVTV SS	179
C6-VK	DIQMTQSPSSLSASVGDRVITITCKASQDVTPAVAWYQQK PGKAPKLLIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQP EDIATYYCQQHYTTPLTFGQGTKLEIK	180
A1-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVR QAPGKSLEWWATISDAGGYIYYRDSVKGRFTISRDNKNS LYLQMNSLRDEDTAVYICARELLHRYALDYWGQGTTVTV SS	181
A1-VK	DIQMTQSPSSLSASVGDRVITITCKASQDVTPAVAWYQQK PGKAPKLLIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQP EDIATYYCQQHYTTPLTFGQGTKLEIK	182
A2-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVR QAPGKSLEWWATISDAGGYIYYRDSVKGRFTISRDNKNS LYLQMNSLRDEDTAVYICARELRGRYALDYWGQGTTVTV SS	183
A2-VK	DIQMTQSPSSLSASVGDRVITITCKASQDVTPAVAWYQQK PGKAPKLLIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQP	184

	EDIATYYCQQHYTTPLTFGQGKLEIK	
A3-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSLEWVATISDAGGYIYSDSVKGRFTISRDNKNSLYLQMNSLRDEDTAVYICAREFGKRYALDYWGQGT TVT VSS	185
A3-VK	DIQMTQSPSSLSASVGDRVITITCKASQDVTPAVAWYQQKPGKAPKLLIYSTSSRYTGVP SRFSGSGSGTDFTFTISLQPED IATYYCQQHSDAPLTFGQGKLEIK	186

[Table 10] Heavy chain variable regions for H12 and B6 clones

Antibody No.	VH	SEQ ID NO:
H12	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSLEWVATISDAGGYIYSDSVKGRFTISRDNKNSLYLQMNSLRDEDTAVYICAREFGKRYALDYWGQGT TVT VSS	103
B6	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSLEWVATISDAGGYIYRDSVKGRFTISRDNKNSLYLQMNSLRDEDTAVYICARELPWRYALDYWGQGT TVT VSS	104

[Table 11] Light chain variable regions for H12 and B6 clones

Antibody No.	VL	SEQ ID NO:
H12	DIQMTQSPSSLSASVGDRVITITCKASQDVTPAVAWYQQKPGKAPKLLIYSTSSRYTGVP SRFSGSGSGTDFTFTISLQPED IATYYCQQHYTTPLTFGQGT KLEIK	105
B6	DIQMTQSPSSLSASVGDRVITITCKASQDVTPAVAWYQQKPGKAPKLLIYSTSSRYTGVP SRFSGSGSGTDFTFTISLQPED IATYYCQQHYTTPLTFGQGT KLEIK	106

1.5. Protein kinetic for PD-L1

To explore the binding kinetics of the humanized antibody, this example performed the affinity ranking by using Biacore. As shown in Table 12 below, H12 and B6.

[Table 12]

Antibody	KD (M)	kon(1/Ms)	kdis(1/s)	Chi
H12	6.122E-09	7.124E+04	4.361E-04	0.0415
B6	4.248E-09	9.827E+04	4.175E-04	0.0766

As shown in Table 12, the anti-PD-L1 antibodies tested show high PD-L1 binding affinities.

Example 2. Preparation of anti-4-1BB monoclonal antibodies

2.1. Screening of full human monoclonal antibodies against 4-1BB

Phage library immunotube panning against 4-1BB

For panning of the library against target molecules, four rounds of panning were carried out in total using 4-1BB coated immunotubes.

Bacterial colonies from the 3 rounds of panning output were grown in SB-Carbenicillin in 96 deepwell plate until turbid, at which point 1011 pfu of VCSM13 helper phage was added to each well. After 1 h infection at 37°C with gentle shaking (80 rpm), 70 µg/mL of kanamycin was added and the cells were cultured overnight at 30°C with shaking at 200 rpm.

Next day, the plates were centrifuged and the supernatants containing the phages were added to 4-1BB antigen-coated ELISA plates blocked with 3% BSA in PBST. After 1 h incubation at room temperature, the plates were washed three times with PBST and anti M13 antibody was added. The plates were incubated for 1 h, washed three times with PBST, and the binding activity was measured using tetramethylbenzidine (TMB).

The 4-1BB specific binders were amplified for plasmid DNA sequencing. Ig light chain V genes (VL) and VH sequences were analyzed to identify unique sequences and determine sequence diversity.

[Table 13] heavy chain variable regions

Antibody No.	VH	SEQ ID NO:
41B01	EVQLLES GGGLVQP GGSRLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVS WISYSGGSIYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDG QRNSMREFDYWGQGTLTVSS	187
41B01.01	EVQLLES GGGLVQP GGSRLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVS WISYSGGSIYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDQA RNSMREFDYWGQGTLTVSS	188
41B01.02	EVQLLES GGGLVQP GGSRLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVS WISYSGGSIYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDQA RQSMREFDYWGQGTLTVSS	189
41B01.03	EVQLLES GGGLVQP GGSRLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVS WISYSGGSIYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDQA RNSMREFDYWGQGTLTVSS	190
41B01.04	EVQLLES GGGLVQP GGSRLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVS WISYSGGSIYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDQA RQSMREFDYWGQGTLTVSS	191
41B02	EVQLLES GGGLVQP GGSRLRLSCAASGFTFSGYDMSWVRQAPGKGLEWVS VIYPDDGNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDAAVYYCAKHG GQKPTTKSSSAYGMDGWGQGTLTVSS	192

[Table 14] Heavy Chain CDRs

Antibody No.	CDR H1	SEQ ID NO:	CDR H2	SEQ ID NO:	CDR H3	SEQ ID NO:
41B01	SYDMS	10	WISYSGGSIYYADSVK G	12	DGQRNSMREFDY	14
41B01.01	SYDMS	10	WISYSGGSIYYADSVK G	12	DAQRNSMREFDY	16
41B01.02	SYDMS	10	WISYSGGSIYYADSVK G	12	DAQRQSMREFDY	17
41B01.03	SYDMS	10	WISYSGGSIYYADSVK G	12	DAQRNSMREFDY	16
41B01.04	SYDMS	10	WISYSGGSIYYADSVK G	12	DAQRQSMREFDY	17
41B02	GYDMS	11	VIYPDDGNTYYADSVK G	13	HGGQKPTTKSSSAY GMDG	15

[Table 15] Light chain variable regions

Antibody No.	VL	SEQ ID NO:
41B01	QSVLTQPPSASGTPGRRVTISCSGSSSNIGNNYYVTWYQQLPGTAPKLLIYADSHRP SGVPDRFSGSKSGTSASLAISGLRSEDEADYYCATWDYSLSGYVFGGGTKLTVL	193
41B01.01	QSVLTQPPSASGTPGRRVTISCSGSSSNIGNNYYVTWYQQLPGTAPKLLIYADSHRP SGVPDRFSGSKSGTSASLAISGLRSEDEADYYCATWDYSLSGYVFGGGTKLTVL	194
41B01.02	QSVLTQPPSASGTPGRRVTISCSGSSSNIGNNYYVTWYQQLPGTAPKLLIYA DSHRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCATWDYSLSGYVF GGGTKLTVL	195
41B01.03	QSVLTQPPSASGTPGRRVTISCSGSSSNIGNNYYVTWYQQLPGTAPKLLIYA DSHRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCATWDYSLSGYVF GGGTKLTVL	196
41B01.04	QSVLTQPPSASGTPGRRVTISCSGSSSNIGNNYYVTWYQQLPGTAPKLLIYA DSHRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCATWDYSLSGYVF GGGTKLTVL	197
41B02	QSVLTQPPSASGTPGRRVTISCSGSSSNIGNNYYVTWYQQLPGTAPKLLIYA DSHRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCATWDYSLSGYVF GGGTKLTVL	198

[Table 16] Light Chain CDRs

Antibody No.	CDR L1	SEQ ID NO:	CDR L2	SEQ ID NO:	CDR L3	SEQ ID NO:
41B01	SGSSSNIGNNYYVT	18	ADSHRPS	19	ATWDYSLSG YV	20
41B01.01	SGSSSNIGNNYYVT	18	ADSHRPS	19	ATWDYSLSG YV	20
41B01.02	SGSSSNIGNNYYVT	18	ADSHRPS	19	ATWDYSLSG YV	20

41B01.03	SGSSSNIGNNYVT	18	ADSHRPS	19	ATWDYSLSG YV	20
41B01.04	SGSSSNIGNNYVT	18	ADSHRPS	19	ATWDYSLSG YV	20
41B02	SGSSSNIGNNYVT	18	ADSHRPS	19	ATWDYSLSG YV	20

2.2. Antigen Binding Abilities of Anti-4-1BB Antibodies to human 4-1BB

(1) Antigen binding measured by ELISA

To evaluate the antigen binding activity, the antibody candidates were subjected to ELISA test. Briefly, microtiter plates were coated with human 4-1BB-Fc protein at 0.1 µg/ml in PBS, 100 µl/well at 4°C overnight, then blocked with 100 µl/well of 5% BSA. Five-fold dilutions of humanized antibodies{41B01 and 41B02} starting from 10 µg/ml were added to each well and incubated for 1-2 hours at RT. The plates were washed with PBS/Tween and then incubate with goat-anti-human IgG antibody conjugated with Horse Radish Peroxidase (HRP) for 1 hour at RT. After washing, the plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-630nm. As shown in FIG. 5, the anti-4-1BB antibodies tested show 4-1BB binding abilities.

(2) Cell binding measured by FACS

To evaluate the antigen binding property, the antibody candidates were analyzed for its binding to mammalian expressed 4-1BB by FACS. Briefly, 4-1BB-Jurkat cells were incubated with antibodies(41B01 and 41B02). After wash by FACS buffer (1%BSA in PBS), the FITC-anti-human IgG antibody was added to each well and incubated at 4°C for 1 hour. The MFI of FITC was evaluated by FACS Caliber. As shown in FIG. 6, the anti-4-1BB antibodies tested show binding abilities to 4-1BB which expressed on cell surface and can efficiently bind to 4-1BB expressed on mammalian cells.

(3) Protein kinetic for 4-1BB

To explore the binding kinetics of the humanized antibody, this example performed the affinity ranking by using Octet Red 96. As shown in Table 17 below, 41B01 and 41B02.

[Table 17]

Antibody	KD (M)	kon(1/Ms)	kdis(1/s)	Chi	R ²
41B01	1.80E-10	6.58E+05	1.19E-04	0.0392	0.9987
41B02	1.01E-09	5.95E+05	6.03E-04	0.0525	0.9973

As shown in Table 17, the anti-4-1BB antibodies tested show high 4-1BB binding affinities.

Example 3. Preparation of anti-PD-L1/anti- 4-1BB bispecific antibodies

Hu1210-41 (Hu1210 VH.4dxHu1210 VK.1, see Table 6; hereinafter, "H12") and B6 (see Table 12) clones among the anti-PD-L1 clones prepared in Example 1 and 41B01, 41B01.01, 41B01.02, 41B01.03, 41B01.04, and 41B02 clones among the anti-4-1BB clones prepared in Example 2 were exemplarily selected, to prepare anti-PD-L1/anti-4-1BB bispecific antibodies in a full-length IgG X scFv form. When PD-L1 is placed in full IgG part, IgG1 with ADCC reduced mutant backbone (N297A mutation; US Patent. No. 7332581, 8219149, etc.) was used, and when 4-1BB is placed in full IgG part, IgG4 was used.

A DNA segment 1 having a nucleotide sequence encoding a heavy chain of an IgG antibody of the anti-PD-L1/anti-4-1BB bispecific antibody was inserted into pcDNA 3.4 (Invitrogen, A14697; plasmid 1), and a DNA segment 2 having a nucleotide sequence encoding a light chain of an IgG antibody of the anti-PD-L1/anti-4-1BB bispecific antibody was inserted into pcDNA 3.4 (Invitrogen, A14697; plasmid 2). Thereafter, a DNA segment 3 encoding a scFv was fused at a part of the DNA segment 1 corresponding to the c-terminus of the Fc region of the IgG antibody inserted into the plasmid 1, using a DNA segment 4 encoding a linker peptide having 10 amino acid lengths consisting of (GGGGS)₂, to construct vectors for the expression of bispecific antibodies. Furthermore, in order to stabilize scFv, additional modification was applied to generate disulfide bridge

fusing VL103-VH44 to C-terminus of light chain and C-terminus of heavy chain, respectively.

The sequences of the heavy chain, light chain, scFv and DNA segments were summarized in Table 18:

[Table 18] Bispecific antibody comprising the anti-PD-L1 clone in IgG form and the anti-4-1BB clone in scFv form (PD-L1x4-1BB)

ABLPNB.01 (bispecific antibody comprising the anti-PD-L1 H12 clone in IgG form and the anti-4-1BB 41B01 clone in scFv form)			
		Amino acid sequence (N'→C') (Seq ID No.)	Nucleotide Sequence (5'→3') (Seq ID No.)
Heavy compo nent	Heavy chain of H12	EVQLVESGGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKSLEWV ATISDAGGYIYYS DSVKGRFTISRDN AKNSLYLQMNSL RDEDTAVYICARE FGKRYALDYWG QGTTVTVSSASTK GPSVFPLAPSSKS TSGGTAALGCLV KDYFPEPVTVSW NSGALTSGVHTF PAVLQSSGLYSLS SVVTVPSSSLGTQ TYICNVNHKPSNT KVDKKVEPKSCD KTHTCPPCPAPEL LGGPSVFLFPPKP KDTLMISRTP EVT CVVVDVSHEDPE VKFNWYVDGVEV HNAKTKPREEQY ASTYRVVSVLTVL HQDWLNGKEYKC KVSNAKALPAIEK TISKAKGQPREPQ VYTLPPSREEMTK NQVSLTCLVKGFY PSDIAVEWESNG QPENNYKTTTPVL DSDGSFFLYSKLT VDKSRWQQGNV	GAGGTGCAGCTGGTGGAGAGCGGA GGAGGACTGGTGCAACCCGGAGGC AGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGA CATGAGCTGGGTGAGACAGGCCCT GGCAAAGCCTGGAGTGGGTGGCCA CCATCTCCGATGCGGGCGGCTACAT CTATTACTCCGACAGCGTGAAGGGC AGGTTCAACCATCAGCAGGGACAACG CCAAGAACAGCCTGTACCTGCAGAT GAACAGCCTGAGGGATGAGGACACC GCCGTGTACATCTGCGCCAGGGAGT TCGGCAAAGGTACGCCCTGGACTA CTGGGGCCAGGGCACAACCGTGACC GTGAGCAGCgctAgcAccAAgGGCCCC TCTGTGTTCCCTCTGGCCCCCTCCTC TAAATCCACCTCTGGCGGAACCGCT GCTCTGGGCTGTCTGGTCAAGGACT ACTTCCCTGAGCCCGTGACCGTGTC TTGGAATTCTGGCGCTCTGACCAGC GGAGTGACACCTTTCCAGCTGTGC TGCAGTCCTCCGGCCTGTACTCTCT

			FSCSVMHEALHN HYTQKSLSLSPGK (199)	GTCTCTGTGTCGTGACAGTGCCTTCCA GCTCTCTGGGCACCCAGACCTACAT
	Linker		GGGSGGGGSG GGGS (200)	CTGCAACGTGAACCACAAGCCCTCC
	scFv of 41B0 1	VL	QSVLTQPPSASG TPGRRVTISCSGS SSNIGNNYVTWY QQLPGTAPKLLIY ADSHRPSGVPDR FSGSKSGTSASLA ISGLRSEDEADYY CATWDYSLSGYV FGCGTKLTVL (201)	AACACCAAGGTGGACAAGAAGGTGG AACCCAAGTCCTGCGACAAGACCCA CACCTGTCCTCCATGTCCTGCTCCAG AACTGCTGGGCGGACCCTCCGTGTT CCTGTTCCCTCCAAAGCCTAAGGACA CCCTGATGATCTCCCGGACCCCTGA AGTGACCTGCGTGGTGGTGGATGTG TCCCACGAGGATCCCGAAGTGAAGT
		Linker	GGGSGGGGSG GGGSGGGGS (202)	TCAATTGGTACGTGGACGGCGTGGA AGTGCACAACGCCAAGACCAAGCCT
		VH	EVQLLES GGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKCLEWV SWISYSGGSIYYA DSVKGRFTISRDN SKNTLYLQMNSL RAEDTAVYYCAR DGQRNSMREFDY WGQGTLLTVSS (203)	AGAGAGGAACAGTAC _{gcc} TCCACCTA CCGGGTGGTGTCCGTGCTGACCGTT CTGCACCAGGATTGGCTGAACGGCA AAGAGTACAAGTGCAAGGTGTCCAA CAAGGCCCTGCCTGCCCCTATCGAA AAGACCATCTCTAAGGCCAAGGGCC AGCCCCGGGAACCTCAAGTGACAC CTTGCTCCCAGCCGGGAAGAGATG ACCAAGAACCAGGTGTCCCTGACCT GCCTGGTTAAGGGCTTCTACCCCTC CGATATCGCCGTGGAATGGGAGTCT AACGGCCAGCCCGAGAACAACACTACA AGACCACCCCTCCTGTGCTGGACTC CGACGGCTCATTCTTCTGTACTCCA AGCTGACCGTGGACAAGTCTCGGTG GCAGCAGGGCAACGTGTTCTCCTGC TCTGTGATGCACGAGGCCCTGCACA ACCACTACACCCAGAAGTCCCTGTC CCTGTCTCCCGGCAAAGGTGGGGGG GGATCTGGTGGTGGTGGATCAGGGG GTGGGGGGTCTCAAAGCGTACTCAC

			CCAACCTCCATCTGCATCCGGTACAC CTGGTCGGCGAGTAACCATCTCCTG CTCTGGGAGCTCTTCTAATATTGGTA ACAACTATGTCACCTGGTATCAGCAG TTGCCTGGGACAGCACCCAACTTCT TATATATGCCGATAGCCATCGGCCTT CCGGCGTACCCGATCGCTTCTCCGG GTCAAAATCTGGAACATCTGCCTCAC TCGCAATTAGTGGATTGCGATCTGAG GATGAAGCAGATTATTATTGCGCTAC CTGGGATTATTCACCTTCTGGCTACG TCTTTGGTgtggaACAAAACCTTACCGT GTTGGGCGGCGGAGGAAGCGGAGG CGGCGGTTCTGGTGGTGGCGGTAGC GGAGGTGGTGGATCTGAAGTACAGC TTCTTGAGTCTGGCGGAGGATTGGT CCAGCCAGGCGGTTCCCTCCGCCTG TCATGTGCCGCATCCGGCTTTACTTT CTCTAGTTATGATATGAGCTGGGTTT GCCAAGCTCCTGGCAAAtgcCTGGAG TGGGTCTCCTGGATTTCACTCAGG TGGCAGCATCTATTATGCTGACAGTG TGAAAGGTGCCTTTACAATCTCCCGA GATAACAGCAAAAACACCTTGTACCT GCAAATGAACAGCCTTCGCGCAGAG GACACAGCCGTATATTATTGCGCTCG CGATGGACAACGTAATTCTATGCGTG AGTTTGACTACTGGGGACAGGGGAC ATTGGTCACTGTATCTTCCtga (204)
Light compo nent	Light chain of H12	DIQMTQSPSSLSA SVGDRVITITCKAS QDVTPAVAWYQ QKPGKAPKLLIYS TSSRYTGVPSRF SGSGSGTDFTFI SSLQPEDIATYYC	GACATCCAGATGACCCAGAGCCCTA GCAGCCTGAGCGCTAGCGTGGGCG ACAGGGTGACCATCACCTGCAAGGC CAGCCAGGATGTGACCCCTGCCGTG GCCTGGTACCAGCAGAAGCCCGGCA AGGCCCCCAAGCTGCTGATCTACAG CACCAGCAGCAGGTACACCGGCGTG

		QQHYTTPLTFGQ GTKLEIKRTVAAP SVFIFPPSDEQLK SGTASVVCLLNNF YPREAKVQWKVD NALQSGNSQESV TEQDSKDSTYSLS STLTLSKADYEKH KVVACEVTHQGL SSPVTKSFNRGE C (28)	CCCAGCAGGTTTAGCGGAAGCGGCA GCGGCACCGACTTCACCTTCACCAT CAGCAGCCTGCAGCCCGAGGACATC GCCACCTACTACTGCCAGCAGCACT ACACCACCCCTCTGACCTTCGGCCA GGGCACCAAGCTGGAGATCAAGAGA ACCGTGGCCGCTCCCTCCGTGTTCA TCTTCCCACCATCTGACGAGCAGCT GAAGTCCGGCACCCTTCTGTCTGTG TGCCTGCTGAACAATTCTACCCTCG GGAAGCCAAGGTGCAGTGGAAGGTG GACAATGCCCTGCAGTCCGGCAACT CCAAGAGTCTGTGACCGAGCAGGA CTCCAAGGACAGCACCTACTCCCTG TCCTCTACCCTGACCCTGTCCAAGG CCGACTACGAGAAGCACAAGGTGTA CGCCTGCGAAGTGACCCACCAGGGA CTGTCTAGCCCCGTGACCAAGTCCTT CAACAGAGGCGAGTGCTGA (205)
ABLPNB.02 (bispecific antibody comprising the anti-PD-L1 H12 clone in IgG form and the anti-4-1BB 41B01.03 clone in scFv form)			
		Amino acid sequence (N'→C') (Seq ID No.)	Nucleotide Sequence (5'→3') (Seq ID No.)
Heavy compo nent	Heavy chain of H12	EVQLVESGGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKSLEWV ATISDAGGYIYYS DSVKGRFTISRDN AKNSLYLQMNSL RDEDTAVYICARE FGKRYALDYWG QGTTTVTVSSASTK GPSVFPLAPSSKS TSGGTAALGCLV KDYFPEPVTVSW NSGALTSGVHTF PAVLQSSGLYSLS SVVTVPSSSLGTQ TYICNVNHKPSNT KVDKKVEPKSCD KTHTCPPCPAPEL LGGPSVFLFPPKP KDTLMISRTPEVT CVVVDVSHEDPE VKFNWYVDGVEV HNAKTKPREEQY ASTYRVVSVLTVL	GAGGTGCAGCTGGTGGAGAGCGGA GGAGGACTGGTGCAACCCGGAGGC AGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGA CATGAGCTGGGTGAGACAGGCCCT GGCAAAGCCTGGAGTGGGTGGCCA CCATCTCCGATGCGGGCGGCTACAT CTATTACTCCGACAGCGTGAAGGGC AGGTTACCATCAGCAGGGAACAACG CCAAGAACAGCCTGTACCTGCAGAT GAACAGCCTGAGGGATGAGGACACC GCCGTGTACATCTGCGCCAGGGAGT TCGGCAAAGGTACGCCCTGGACTA CTGGGGCCAGGGCACAACCGTGACC GTGAGCAGCgctAgcAccAAgGGCCCC TCTGTGTTCCCTCTGGCCCCCTTCCTC TAAATCCACCTCTGGCGGAACCGCT GCTCTGGGCTGTCTGGTCAAGGACT ACTTCCCTGAGCCCGTGACCGTGTC TTGGAATTCTGGCGCTCTGACCAGC GGAGTGCACACCTTTCCAGCTGTGC TGCAGTCCTCCGGCCTGTACTCTCT GTCCTCTGTCTGTGACAGTGCCCTTCCA GCTCTCTGGGCACCCAGACCTACAT CTGCAACGTGAACCACAAGCCCTCC

			HQDWLNGKEYKC KVSNKALPAPIEK TISKAKGQPREPQ VYTLPPSREEMTK NQVSLTCLVKGFY PSDIAVEWESNG QPENNYKTTTPVL DSDGSFFLYSKLT VDKSRWQQGNV FSCSVMHEALHN HYTQKSLSLSPGK (206)	AACACCAAGGTGGACAAGAAGGTGG AACCCAAGTCCTGCGACAAGACCCA CACCTGTCCTCCATGTCCTGCTCCAG AACTGCTGGGCGGACCCTCCGTGTT CCTGTTCCCTCCAAAGCCTAAGGACA CCCTGATGATCTCCCGGACCCCTGA AGTGACCTGCGTGGTGGTGGATGTG TCCCACGAGGATCCAGAAGTGAAGT TCAATTGGTACGTGGACGGCGTGGA AGTGACACAATGCCAAGACCAAGCCT AGAGAGGAACAGTACGCCTCCACCT ACAGAGTGGTGTCCGTGCTGACTGT GCTGCACCAGGATTGGCTGAACGGC AAAGAGTACAAGTGCAAGGTGTCCA ACAAGGCCCTGCCTGCTCCTATCGA AAAGACCATCAGCAAGGCCAAGGGC CAGCCTAGGGAACCCCAGGTTTACA CCCTGCCTCCAAGCCGGGAAGAGAT GACCAAGAACCAGGTGTCCCTGACC TGCCTCGTGAAGGGCTTCTACCCTTC CGATATCGCCGTGGAATGGGAGAGC AATGGCCAGCCTGAGAACAACCTACA AGACAACCCCTCCTGTGCTGGACTC CGACGGCTCATTCTTCTGTACTCCA AGCTGACCGTGGACAAGTCCAGATG GCAGCAGGGCAACGTGTTCTCCTGC TCCGTGATGCACGAGGCCCTGCACA ATCACTACACCCAGAAGTCCCTGTCT CTGAGCCCTGGAAAAGGCGGCGGA GGATCTGGCGGAGGTGGTAGCGGA GGCGGTGGATCTCAGTCTGTTCTGA CCCAGCCTCCTTCCGCTTCTGGCAC CCCTGGAGAGAGAGTGACCATCTCTT GCTCCGGCTCCTCCTCCAACATCGG CAACAACCTACGTGACCTGGTATCAGC AGCTGCCCCGGCACAGTCCCAAACT GCTGATCTACGCCGACTCTCACAGA CCTTCCGGCGTGCCCGATAGATTCT CCGGCTCTAAGTCTGGCACCTCTGC CAGCCTGGCTATCAGCGGCCTGAGA TCTGAGGACGAGGCCGACTACTACT GCGCCACCTGGGATTATTCCTGTC CGGCTACGTGTTCCGGCTGCGGCACA AAACTGACAGTGCTCGGAGGCGGAG GAAGTGGTGGCGGAGGTTTCAGGTGG TGGTGGTAGTGGCGGAGGCGGATCA GAAGTTCAGCTGTTGGAGTCAGGTG GCGGCTTGGTGCAACCAGGTGGAAG TCTGAGACTCAGCTGTGCTGCCAGC GGCTTTACCTTCAGTCTCTACGACAT GAGCTGGGTTCGACAAGCTCCCGGA
	Linker		GGGGSGGGGSG GGGS (207)	
scFv of 41B0 1.03	VL		QSVLTQPPSASG TPGQRTVISC SGS SSNIGNNYVTWY QQLPGTAPKLLIY ADSHRPSGVPDR FSGSKSGTSASLA ISGLRSEDEADYY CATWDYSLSGYV FGCGTKLTVL (208)	
	Linker		GGGGSGGGGSG GGGSGGGGS (209)	
	VH		EVQLLESGGGLV QPGGSLRLSCAA SGFTFSS YDMSW VRQAPGKCLEWV SWISYSGGSIYYA DSVKGRFTISRDN SKNTLYLQMNSL RAEDTAVYYCAR DAQRNSMREFDY WGQGTLLTVSS (210)	

				AAGTGCTTGGAGTGGGTTTCCTGGA TCTCCTACTCCGGCGGCAGCATCTAT TACGCCGACAGCGTGAAAGGCCGGT TTACCATCTCTCGGGATAACAGCAAG AATACCCTCTACCTCCAAATGAACTC TCTGAGAGCCGAGGACACTGCTGTG TACTATTGCGCCAGAGATGccCAGCG GAACTCCATGAGAGAGTTCGACTACT GGGGACAAGGCACCCTGGTCACCGT GTCTAGTTGA (211)
Light compo nent	Light chain of H12	DIQMTQSPSSLSA SVGDRVITITCKAS QDVTPAVAWYQ QKPGKAPKLLIYS TSSRYTGVPSRF SGSGSGTDFTFTI SSLQPEDIATYYC QQHYTTPLTFGQ GTKLEIKRTVAAP SVFIFPPSDEQLK SGTASVVCLLNNF YPREAKVQWKVD NALQSGNSQESV TEQDSKDYSTYLS STLTLSKADYEKH KVYACEVTHQGL SSPVTKSFNRGE C (30)	GACATCCAGATGACCCAGAGCCCTA GCAGCCTGAGCGCTAGCGTGGGCG ACAGGGTGACCATCACCTGCAAGGC CAGCCAGGATGTGACCCCTGCCGTG GCCTGGTACCAGCAGAAGGCCGGCA AGGCCCCCAAGCTGCTGATCTACAG CACCAGCAGCAGGTACACCGGCGTG CCCAGCAGGTTTAGCGGAAGCGGCA GCGGCACCGACTTCACCTTCACCAT CAGCAGCCTGCAGCCCGAGGACATC GCCACCTACTACTGCCAGCAGCACT ACACCACCCCTCTGACCTTCGGCCA GGGCACCAAGCTGGAGATCAAGAGA ACCGTGGCCGCTCCCTCCGTGTTCA TCTTCCCACCATCTGACGAGCAGCT GAAGTCCGGCACCGCTTCTGTCTGT TGCCTGCTGAACACTTCTACCCTCG GGAAGCCAAGGTGCAGTGGAAGGTG GACAATGCCCTGCAGTCCGGCAACT CCCAAGAGTCTGTGACCGAGCAGGA CTCCAAGGACAGCACCTACTCCCTG TCCTCTACCCTGACCCTGTCCAAGG CCGACTACGAGAAGCACAAGGTGTA CGCCTGCGAAGTGACCCACCAGGGA CTGTCTAGCCCCGTGACCAAGTCCTT CAACAGAGGCGAGTGCTGA (212)	
ABLPNB.03 (bispecific antibody comprising the anti-PD-L1 H12 clone in IgG form and the anti-4-1BB 41B02 clone in scFv form)				
		Amino acid sequence (N'→C') (Seq ID No.)	Nucleotide Sequence (5'→3') (Seq ID No.)	
Heavy compo nent	Heavy chain of H12	EVQLVESGGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKSLEWV ATISDAGGYIYS DSVKGRFTISRDN AKNSLYLQMNSL	GAGGTGCAGCTGGTGGAGAGCGGA GGAGGACTGGTGCAACCCGGAGGC AGCCTGAGACTGAGCTGCGCTGCCA GCGGCTTCACCTTCAGCAGCTACGA CATGAGCTGGGTGAGACAGGCCCT GGCAAAAGCCTGGAGTGGGTGGCCA CCATCTCCGATGCGGGCGGCTACAT	

			RDEDTAVYICARE FGKRYALDYWG QGTTVTVSSASTK GPSVFPLAPSSKS TSGGTAALGCLV KDYFPEPVTVSW NSGALTSGVHTF PAVLQSSGLYSLS SVVTVPSSSLGTQ TYICNVNHKPSNT KVDKKVEPKSCD KTHTCPPCPAPEL LGGPSVFLFPPKP KDTLMISRTPEVT CVVVDVSHEDPE VKFNWYVDGVEV HNAKTKPREEQY ASTYRVVSVLTVL HQDWLNGKEYKC KVSNAKALPAIEK TISKAKGQPREPQ VYTLPPSREEMTK NQVSLTCLVKGFY PSDIAVEWESNG QPENNYKTTTPVL DSDGSFFLYSKLT VDKSRWQQGNV FSCSVMHEALHN HYTQKSLSLSPGK (213)	CTATTACTCCGACAGCGTGAAGGGC AGGTTCACCATCAGCAGGGACAACG CCAAGAACAGCCTGTACCTGCAGAT GAACAGCCTGAGGGATGAGGACACC GCCGTGTACATCTGCGCCAGGGAGT TCGGCAAAAGGTACGCCCTGGACTA CTGGGGCCAGGGCACAACCGTGACC GTGAGCAGCgctAgcAccAAgGGCCCC TCTGTGTTCCCTCTGGCCCCCTTCCTC TAAATCCACCTCTGGCGGAACCGCT GCTCTGGGCTGTCTGGTCAAGGACT ACTTCCCTGAGCCCCGTGACCGTGTCT TTGGAATTCTGGCGCTCTGACCAGC GGAGTGCACACCTTTCCAGCTGTGC TGCAGTCCTCCGGCCTGTACTCTCT GTCCTCTGTCTGACAGTGCCTTCCA GCTCTCTGGGCACCCAGACCTACAT CTGCAACGTGAACCACAAGCCCTCC AACACCAAGGTGGACAAGAAGGTGG AACCCAAGTCCTGCGACAAGACCCA CACCTGTCCTCCATGTCCTGCTCCAG AACTGCTGGGCGGACCCCTCCGTGTT CCTGTTCCCTCCAAAGCCTAAGGACA CCCTGATGATCTCCCGGACCCCTGA AGTGACCTGCGTGGTGGTGGATGTG TCCCACGAGGATCCCGAAGTGAAGT TCAATTGGTACGTGGACGGCGTGGA AGTGACAACGCCAAGACCAAGCCT AGAGAGGAACAGTACgctTCCACCTA CCGGGTGGTGTCCGTGCTGACCGTT CTGCACCAGGATTGGCTGAACGGCA AAGAGTACAAGTGCAAGGTGTCCAA CAAGGCCCTGCCTGCCCTATCGAA AAGACCATCTCTAAGGCCAAGGGCC AGCCCCGGGAACCTCAAGTGTACAC CTTGCCCTCCAGCCGGGAAGAGATG ACCAAGAACCAGGTGTCCCTGACCT GCCTGGTTAAGGGCTTCTACCCCTC CGATATCGCCGTGGAATGGGAGTCT AACGGCCAGCCCGAGAACAACACTACA AGACCACCCCTCCTGTGCTGGACTC CGACGGCTCATTCTTCCTGTACTCCA AGCTGACCGTGGACAAGTCTCGGTG GCAGCAGGGCAACGTGTTCTCCTGC TCTGTGATGCACGAGGCCCTGCACA ACCACTACACCCAGAAGTCCCTGTC CCTGTCTCCCGGCAAGGTGGGGGG GGATCTGGTGGTGGTGGATCAGGGG GTGGGGGGTCTCAAAGCGTACTCAC CCAACCTCCATCTGCATCCGGTACAC CTGGTCGGCGAGTAACCATCTCCTG
	Linker		GGGGSGGGGSG GGGS (214)	
	scFv of 41B0 2	VL	QSVLTQPPSASG TPGRRVTISCSGS SSNIGNNYVTWY QQLPGTAPKLLIY ADSHRPSGVPDR FSGSKSGTSASLA ISGLRSEDEADYY CATWDYSLSGYV FGCGTKLTVL (215)	
		Linker	GGGGSGGGGSG GGGSGGGGS (216)	
		VH	EVQLLESGGGLV QPGGSLRLSCAA SGFTFSGYDMSW VRQAPGKCLEWV SVIYPDDGNTYYA DSVKG RFTISRDN	

		SKNTLYLQMNSL RAEDAAVYYCAK HGGQKPTTKSSS AYGMDGWGQGT LVTVSS (217)	CTCTGGGAGCTCTTCTAATATTGGTA ACAACTATGTCACCTGGTATCAGCAG TTGCCTGGGACAGCACCCAACTTCT TATATATGCCGATAGCCATCGGCCTT CCGGCGTACCCGATCGCTTCTCCGG GTCAAAATCTGGAACATCTGCCTCAC TCGCAATTAGTGGATTGCGATCTGAG GATGAAGCAGATTATTATTGCGCTAC CTGGGATTATTCACCTTCTGGCTACG TCTTTGGTgtGGAACAAACTTACCG TGTGGGCGGCGGAGGAAGCGGAG GCGGCGGTTCTGGTGGTGGCGGTA GCGGAGGTGGTGGATCTGAGGTTCA ACTGTTGGAGTCAGGTGGCGGACTT GTCCAGCCTGGCGGGTCTCTGAGGC TGAGTTGCGCTGCTTCTGGGTTTACT TTTTCAGGATATGACATGAGTTGGGT ACGTCAGGCTCCAGGTAAGtgCCTCG AATGGGTCTCCGTTATCTATCCCGAT GATGGAAATACTTACTACGCTGACAG TGTGAAAGGCAGGTTTACAATCAGTA GGGACAATTCTAAAAATACACTCTAC CTCCAGATGAACTCACTTCGAGCCG AGGACGCCGCCGTATATTACTGTGC CAAACACGGCGGGCAAAAACCCACT ACTAAATCCAGTAGTGCTTACGGGAT GGATGGCTGGGGACAGGGGACATTG GTCACGTATCTTCCtga (218)
Light compo nent	Light chain of H12	DIQMTQSPSSLSA SVGDRVITITCK AS QDVTPAVAWYQ QKPGKAPKLLIYS TSSRYTGVPSRF SSGSGSGTDFTFI SSLQPEDIATYYC QQHYTTPLTFGQ GTKLEIKRTVAAP SVFIFPPSDEQLK SGTASVVCLLNNF YPREAKVQWKVD NALQSGNSQESV TEQDSKDSTYSLS STLTLISKADYEKH KVYACEVTHQGL SSPVTKSFNRGE C (32)	GACATCCAGATGACCCAGAGCCCTA GCAGCCTGAGCGCTAGCGTGGGCG ACAGGGTGACCATCACCTGCAAGGC CAGCCAGGATGTGACCCCTGCCGTG GCCTGGTACCAGCAGAAAGCCCGGCA AGGCCCCCAAGCTGCTGATCTACAG CACCAGCAGCAGGTACACCGGCGTG CCCAGCAGGTTTAGCGGAAGCGGCA GCGGCACCGACTTCACCTTCACCAT CAGCAGCCTGCAGCCCGAGGACATC GCCACCTACTACTGCCAGCAGCACT ACACCACCCCTCTGACCTTCGGCCA GGGCACCAAGCTGGAGATCAAGAGA ACCGTGGCCGCTCCCTCCGTGTTCA TCTTCCACCATCTGACGAGCAGCT GAAGTCCGGCACCGCTTCTGTCGTG TGCCTGCTGAACAACTTCTACCCTCG GGAAGCCAAGGTGCAGTGGAAGGTG GACAATGCCCTGCAGTCCGGCAACT CCAAGAGTCTGTGACCGAGCAGGA CTCCAAGGACAGCACCTACTCCCTG TCCTCTACCCTGACCCTGTCCAAGG CCGACTACGAGAAGCACAAGGTGTA

			CGCCTGCGAAGTGACCCACCAGGGA CTGTCTAGCCCCGTGACCAAGTCCTT CAACAGAGGCGAGTGCTGA (219)
ABLPNB.04 (bispecific antibody comprising the anti-PD-L1 B6 clone in IgG form and the anti-4-1BB 41B01 clone in scFv form)			
		Amino acid sequence (N'→C') (Seq ID No.)	Nucleotide Sequence (5'→3') (Seq ID No.)
Heavy compo nent	Heavy chain of B6	EVQLVESGGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKSLEWV ATISDAGGYIYR DSVKGRFTISRDN AKNSLYLQMNSL RDEDTAVYICARE LPWRYALDYWG QGTTVTVSSASTK GPSVFPLAPSSKS TSGGTAALGCLV KDYFPEPVTVSW NSGALTSGVHTF PAVLQSSGLYSLS SVVTPSSSLGTQ TYICNVNHKPSNT KVDKKVEPKSCD KTHTCPPCPAPEL LGGPSVFLFPPKP KDTLMISRTPEVT CVVVDVSHEDPE VKFNWYVDGVEV HNAKTKPREEQY ASTYRVVSVLTVL HQDWLNGKEYKC KVSNAKALPAIEK TISKAKGQPREPQ VYTLPPSREEMTK NQVSLTCLVKGFY PSDIAVEWESNG QPENNYKTTTPVL DSDGSFFLYSKLT VDKSRWQQGNV FSCSVMHEALHN HYTQKSLSLSPGK (220)	GAAGTGCAGCTGGTTGAATCTGGCG GCGGATTGGTTCAGCCTGGCGGATC TCTGAGACTGTCTTGTGCCGCCTCC GGCTTCACCTTCTCCAGCTACGATAT GTCCTGGGTCCGACAGGCCCTGGC AAGTCTTTGGAATGGGTGCGCCACCAT CTCTGACGCTGGCGGCTACATCTAC TACCGGGACTCTGTGAAGGGCAGAT TCACCATCAGCCGGGACAACGCCAA GAACTCCCTGTACCTGCAGATGAACA GCCTGCGCGACGAGGATACCGCCGT GTACATCTGTGCTAGAGAGCTGCCTT GGAGATACGCCCTGGATTATTGGGG CCAGGGCACCACAGTGACCGTGTCC TCTGCTTCTACCAAGGGACCCAGCG TGTTCCCTCTGGCTCCTTCCAGCAAG TCTACCTCTGGCGGAACAGCTGCTC TGGGCTGCCTGGTCAAGGACTACTT TCCTGAGCCTGTGACAGTGTCTGG AACTCTGGCGCTCTGACATCTGGCG TGACACACCTTTCCAGCAGTGCTGCA GTCCTCCGGCCTGTACTCTCTGTCTC CTGTCGTGACCGTGCTTCCAGCTC TCTGGGAACCCAGACCTACATCTGC AATGTGAACCACAAGCCTTCCAACAC CAAGGTGGACAAGAAGGTGGAACCC AAGTCCTGCGACAAGACCCACACCT GTCCTCCATGTCCTGCTCCAGAACTG CTCGGCGGACCTTCCGTGTTCTCTGT TTCCTCCAAAGCCTAAGGACACCCTG ATGATCTCTCGGACCCCTGAAGTGA CCTGCGTGGTGGTGGATGTGTCCCA CGAGGATCCAGAAGTGAAGTTCAATT GGTACGTGGACGGCGTGGAAGTGCA CAATGCCAAGACCAAGCCTAGAGAG GAACAGTACGCCTCCACCTACAGAG TGGTGTCCGTGCTGACTGTGCTGCA CCAGGATTGGCTGAACGGCAAAGAG TACAAGTGCAAGGTGTCCAACAAGG
	Linker	GGGGSGGGGSG GGGS (221)	

	scFv of 41B0 1	VL	QSVLTQPPSASG TPGRRVTISCSGS SSNIGNNYVTWY QQLPGTAPKLLIY ADSHRPSGVPDR FSGSKSGTSASLA ISGLRSEDEADYY CATWDYSLSGYV FGCGTKLTVL (222)	CCCTGCCTGCTCCTATCGAAAAGAC CATCAGCAAGGCCAAGGGCCAGCCT AGGGAACCCCAGGTTTACACCCTGC CTCCAAGCCGGAAGAGATGACCAA GAACCAGGTGTCCCTGACCTGCCTC GTGAAGGGCTTCTACCCTTCCGATAT CGCCGTGGAATGGGAGAGCAATGGC CAGCCTGAGAACAACTACAAGACAA CCCCTCCTGTGCTGGACTCCGACGG CTCATTCTTCCTGTACTCCAAGCTGA CCGTGGACAAGTCCAGATGGCAGCA GGGCAACGTGTTCTCCTGTCCGTG ATGCACGAGGCCCTGCACAATCACT ACACCCAGAAGTCCCTGTCTCTGAG CCCTGGAAAAGGCGGCGGAGGATCT GGCGGAGGTGGTAGCGGAGGCGGT GGATCTCAGTCTGTTCTGACCCAGC CTCCTTCCGCTTCTGGCACCCCTGG AAGAAGAGTGACCATCTCTTGCTCCG GCTCCTCCTCCAACATCGGCAACAA CTACGTGACCTGGTATCAGCAGCTG CCCGGCACAGCTCCCAAAGTCTGTA TCTACGCCGACTCTCAGACACCTTCC GGCGTGCCCGATAGATTCTCCGGCT CTAAGTCTGGCACCTCTGCCAGCCT GGCTATCAGCGGCCTGAGATCTGAG GACGAGGCCGACTACTACTGCGCCA CCTGGGATTATTCCCTGTCCGGCTAC GTGTTCCGGCTGCGGCACAAAAGTGA CAGTGCTCGGAGGCGGAGGAAGTG GTGGCGGAGGTTTCAAGTGGTGGTG GTAGTGGCGGAGGCGGATCAGAAGT TCAGCTGTTGGAGTCAGGTGGCGGC TTGGTGCAACCAGGTGGAAGTCTGA GACTCAGCTGTGCTGCCAGCGGCTT TACCTTCAGCTCCTACGACATGAGCT GGGTTTCGACAAGCTCCCGGAAAGTG CTTGAGTGGGTTTCTTGATCTCCT ACTCCGGCGGCAGCATCTATTACGC CGACAGCGTGAAAGGCCGTTTACC ATCTCTCGGGATAACAGCAAGAATAC CCTCTACCTCCAAATGAACTCTCTGA GAGCCGAGGACACTGCTGTGTACTA TTGCGCCAGAGATGGCCAGCGGAAC TCCATGAGAGAGTTTCACTACTGGG GACAAGGCACCCTGGTCACCGTGTC TAGTTGA (225)
		Linker	GGGSGGGGSG GGGSGGGGS (223)	
		VH	EVQLLESGGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKCLEWV SWISYSGGSIYYA DSVKG RFTISRDN SKNTLYLQMNSL RAEDTAVYYCAR DGQRNSMREFDY WGQGTLLTVSS (224)	
Light compo nent	Light chain of B6		DIQMTQSPSSLSA SVGDRVITICKAS QDVT PAVAWYQ QKPGKAPKLLIYS	GACATCCAGATGACCCAGAGCCCTA GCAGCCTGAGCGCTAGCGTGGGCG ACAGGGTGACCATCACCTGCAAGGC CAGCCAGGATGTGACCCCTGCCGTG

		TSSRYTGVPSRF SSGSGTDFTFTI SSLQPEDIATYYC QQHYTTPLTFGQ GTKLEIKRTVAAP SVFIFPPSDEQLK SGTASVVCLLNNF YPREAKVQWKVD NALQSGNSQESV TEQDSKDYSTLS STLTLSKADYEKH KVVACEVTHQGL SSPVTKSFNRGE C (34)	GCCTGGTACCAGCAGAAGCCCGGCA AGGCCCCCAAGCTGCTGATCTACAG CACCAGCAGCAGGTACACCGGCGTG CCCAGCAGGTTTAGCGGAAGCGGCA GCGGCACCGACTTCACCTTCACCAT CAGCAGCCTGCAGCCCGAGGACATC GCCACCTACTACTGCCAGCAGCACT ACACCACCCCTCTGACCTTCGGCCA GGGCACCAAGCTGGAGATCAAGAGA ACCGTGGCCGCTCCCTCCGTGTTCA TCTTCCCACCATCTGACGAGCAGCT GAAGTCCGGCACCGCTTCTGTCTGTG TGCCTGCTGAACAACCTTCTACCTCG GGAAGCCAAGGTGCAGTGAAGGTG GACAATGCCCTGCAGTCCGGCAACT CCCAAGAGTCTGTGACCGAGCAGGA CTCCAAGGACAGCACCTACTCCCTG TCCTCTACCCTGACCCTGTCCAAGG CCGACTACGAGAAGCACAAGGTGTA CGCCTGCGAAGTGACCCACCAGGGA CTGTCTAGCCCCGTGACCAAGTCCTT CAACAGAGGCGAGTGCTGA (226)
ABLPNB.05 (bispecific antibody comprising the anti-PD-L1 B6 clone in IgG form and the anti-4-1BB 41B01.01 clone in scFv form)			
		Amino acid sequence (N'→C') (Seq ID No.)	Nucleotide Sequence (5'→3') (Seq ID No.)
Heavy component	Heavy chain of B6	EVQLVESGGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKSLEWV ATISDAGGYIYYR DSVKGRFTISRDN AKNSLYLQMNSL RDEDTAVYICARE LPWRYALDYWG QGTTTVTVSSASTK GPSVFPLAPSSKS TSGGTAALGCLV KDYFPEPVTVSW NSGALTSGVHTF PAVLQSSGLYSLS SVVTVPSSSLGTQ TYICNVNHKPSNT KVDKKVEPKSCD KTHTCPPCPAPEL LGGPSVFLFPPKP KDTLMISRTP EVT CVVVDVSHEDPE	GAAGTGCAGCTGGTTGAATCTGGCG GCGGATTGGTTCAGCCTGGCGGATC TCTGAGACTGTCTTGTCGCGCTCC GGCTTCACCTTCTCCAGCTACGATAT GTCCTGGGTCCGACAGGCCCTGGC AAGTCTTTGGAATGGGTGCGCCACCAT CTCTGACGCTGGCGGCTACATCTAC TACCGGGA CTCTGTGAAGGGCAGAT TCACCATCAGCCGGGACAACGCCAA GAACTCCCTGTACCTGCAGATGAACA GCCTGCGCGACGAGGATACCGCCGT GTACATCTGTGCTAGAGAGCTGCCTT GGAGATACGCCCTGGATTATTGGGG CCAGGGCACCAAGTGACCGTGTCC TCTGCTTCTACCAAGGGACCCAGCG TGTTCCCTCTGGCTCCTTCCAGCAAG TCTACCTCTGGCGGAACAGCTGCTC TGGGCTGCCTGGTCAAGGACTACTT TCCTGAGCCTGTGACAGTGTCTGG AACTCTGGCGCTCTGACATCTGGCG TGCACACCTTTCCAGCAGTGCTGCA GTCCTCCGGCCTGTACTCTCTGCTCT

		VKFNWYVDGVEV HNAKTKPREEQY ASTYRVVSVLTVL HQDWLNGKEYKC KVSNAKALPAIEK TISKAKGQPREPQ VYTLPPSREEMTK NQVSLTCLVKGFY PSDIAVEWESNG QPENNYKTTTPVL DSDGSFFLYSKLT VDKSRWQQGNV FSCSVMHEALHN HYTQKSLSLSPGK (227)	CTGTCGTGACCGTGCCTTCCAGCTC TCTGGGAACCCAGACCTACATCTGC AATGTGAACCACAAGCCTTCCAACAC CAAGGTGGACAAGAAGGTGGAACCC AAGTCCTGCGACAAGACCCACACCT GTCCTCCATGTCCTGCTCCAGAAGT CTCGGCGGACCTTCCGTGTTCCCTGT TTCCTCCAAAGCCTAAGGACACCCCTG ATGATCTCTCGGACCCCTGAAGTGA CCTGCGTGGTGGTGGATGTGTCCCA CGAGGATCCAGAAGTGAAGTTCAATT GGTACGTGGACGGCGTGGAAAGTGCA CAATGCCAAGACCAAGCCTAGAGAG GAACAGTACGCCTCCACCTACAGAG TGGTGTCCGTGCTGACTGTGCTGCA CCAGGATTGGCTGAACGGCAAAGAG TACAAGTGCAAGGTGTCCAACAAGG CCCTGCCTGCTCCTATCGAAAAGAC CATCAGCAAGGCCAAGGGCCAGCCT AGGGAACCCAGGTTTACACCCTGC CTCCAAGCCGGGAAGAGATGACCAA GAACCAGGTGTCCCTGACCTGCCTC GTGAAGGGCTTCTACCCTTCCGATAT CGCCGTGGAATGGGAGAGCAATGGC CAGCCTGAGAACAACCTACAAGACAA CCCCTCCTGTGCTGGACTCCGACGG CTCATTCTTCCTGTACTCCAAGCTGA CCGTGGACAAGTCCAGATGGCAGCA GGGCAACGTGTTCTCCTGCTCCGTG ATGCACGAGGCCCTGCACAATCACT ACACCCAGAAGTCCCTGTCTCTGAG CCCTGGAAAAGGCGGCGGAGGATCT GGCGGAGGTGGTAGCGGAGGCGGT GGATCTCAGTCTGTTCTGACCCAGC CTCCTTCCGCTTCTGGCACCCCTGG AAGAAGAGTGACCATCTCTTGCTCCG GCTCCTCCTCCAACATCGGCAACAA CTACGTGACCTGGTATCAGCAGCTG CCCGGCACAGCTCCCAAAGTCTGA TCTACGCCGACTCTCACAGACCTTCC GGCGTGCCCGATAGATTCTCCGGCT CTAAGTCTGGCACCTCTGCCAGCCT GGCTATCAGCGGCCTGAGATCTGAG GACGAGGCCGACTACTACTGCGCCA CCTGGGATTATTCCCTGTCCGGCTAC GTGTTCCGGCTGCGGCACAAAAGTGA CAGTGCTCGGAGGCGGAGGAAGTG GTGGCGGAGGTTCAAGTGGTGGTG GTAGTGGCGGAGGCGGATCAGAAGT TCAGCTGTTGGAGTCAGGTGGCGGC TTGGTGCAACCAGGTGGAAGTCTGA
	Linker	GGGGSGGGSG GGGS (228)	
scFv of 41B0 1.01	VL	QSVLTQPPSASG TPGRRVTISCSGS SSNIGNNYVTWY QQLPGTAPKLLIY ADSHRPSGVPDR FSGSKSGTSASLA ISGLRSEDEADYY CATWDYSLSGYV FGCGTKLTVL (229)	
	Linker	GGGGSGGGSG GGGSGGGGS (230)	
	VH	EVQLLES GGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKCLEWV SWISYSGGSIYYA DSVKGRFTISRDN SKNTLYLQMNSL RAEDTAVYYCAR DAQRNSMREFDY WGQGTLLVTVSS (231)	

				GACTCAGCTGTGCTGCCAGCGGCTT TACCTTCAGCTCCTACGACATGAGCT GGGTTCGACAAGCTCCCGGAAAGTG CTTGGAGTGGGTTTCCTGGATCTCCT ACTCCGGCGGCAGCATCTATTACGC CGACAGCGTGAAAGGCCGGTTTACC ATCTCTCGGGATAACAGCAAGAATAC CCTCTACCTCCAAATGAACTCTCTGA GAGCCGAGGACACTGCTGTGTACTA TTGCGCCAGAGATGccCAGCGGAACT CCATGAGAGAGTTTCGACTACTGGGG ACAAGGCACCCTGGTCACCGTGTCT AGTTGA (232)
Light compo nent	Light chain of B6	DIQMTQSPSSLSA SVGDRVITICKAS QDVT PAVAWYQ QKPGKAPKLLIYS TSSRY TGVPSRF SGSGSGTDFTFTI SSLQPEDIATYYC QQHYTT PLTFGQ GTKLEIKRTVAAP SVFIFPPSDEQLK SGTASVVCLLNNF YPREAKVQWKVD NALQSGNSQESV TEQDSKDYSL STLTLSKADYEKH KVVACEVTHQGL SSPVTKSFNRGE C (36)	GACATCCAGATGACCCAGAGCCCTA GCAGCCTGAGCGCTAGCGTGGGCG ACAGGGTGACCATCACCTGCAAGGC CAGCCAGGATGTGACCCCTGCCGTG GCCTGGTACCAGCAGAAGCCCGGCA AGGCCCCCAAGCTGCTGATCTACAG CACCAGCAGCAGGTACACCGGCGTG CCCAGCAGGTTTAGCGGAAGCGGCA GCGGCACCGACTTCACCTTCACCAT CAGCAGCCTGCAGCCCGAGGACATC GCCACCTACTACTGCCAGCAGCACT ACACCACCCCTCTGACCTTCGGCCA GGGCACCAAGCTGGAGATCAAGAGA ACCGTGGCCGCTCCCTCCGTGTTCA TCTTCCCACCATCTGACGAGCAGCT GAAGTCCGGCACCGCTTCTGTCTGTG TGCCTGCTGAACAATTCTACCCTCG GGAAGCCAAGGTGCAGTGGAAGGTG GACAATGCCCTGCAGTCCGGCAACT CCCAAGAGTCTGTGACCGAGCAGGA CTCCAAGGACAGCACCTACTCCCTG TCCTCTACCCTGACCCTGTCCAAGG CCGACTACGAGAAGCACAAGGTGTA CGCCTGCGAAGTGACCCACCAGGGA CTGTCTAGCCCCGTGACCAAGTCCTT CAACAGAGGCGAGTGCTGA (233)	
ABLPNB.06 (bispecific antibody comprising the anti-PD-L1 B6 clone in IgG form and the anti-4-1BB 41B01.02 clone in scFv form)				
		Amino acid sequence (N'→C') (Seq ID No.)	Nucleotide Sequence (5'→3') (Seq ID No.)	
Heavy compo nent	Heavy chain of B6	EVQLVESGGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKSLEWV	GTGCAGCTGGTTGAATCTGGCGGCG GATTGGTTCAGCCTGGCGGATCTCT GAGACTGTCTTGCGCCCTCCGGC TTCACCTTCTCCAGCTACGATATGTC	

		ATISDAGGYIYYR DSVKGRFTISRDN AKNSLYLQMNSL RDEDTAVYICARE LPWRYALDYWG QGTTVTVSSASTK GPSVFPLAPSSKS TSGGTAALGCLV KDYFPEPVTVSW NSGALTSGVHTF PAVLQSSGLYSLS SVVTVPSSSLGTQ TYICNVNHKPSNT KVDKKVEPKSCD KTHTCPPCPAPEL LGGPSVFLFPPKP KDTLMISRTPEVT CVVVDVSHEDPE VKFNWYVDGVEV HNAKTKPREEQY ASTYRVVSVLTVL HQDWLNGKEYKC KVSNAKALPAIEK TISKAKGQPREPQ VYTLPPSREEMTK NQVSLTCLVKGFY PSDIAVEWESNG QPENNYKTTTPVL DSDGSFFLYSKLT VDKSRWQQGNV FSCSVMHEALHN HYTQKSLSLSPGK (234)	CTGGGTCCGACAGGCCCTGGCAAG TCTTTGGAATGGGTCGCCACCATCTC TGACGCTGGCGGCTACATCTACTAC CGGGACTCTGTGAAGGGCAGATTCA CCATCAGCCGGGACAACGCCAAGAA CTCCCTGTACCTGCAGATGAACAGC CTGCGCGACGAGGATACCGCCGTGT ACATCTGTGCTAGAGAGCTGCCTTG GAGATACGCCCTGGATTATTGGGGC CAGGGCACCACAGTGACCGTGTCTC CTGCTTCTACCAAGGGACCCAGCGT GTTCCCTCTGGCTCCTTCCAGCAAGT CTACCTCTGGCGGAACAGCTGCTCT GGGCTGCCTGGTCAAGGACTACTTT CCTGAGCCTGTGACAGTGTCTGGA ACTCTGGCGCTCTGACATCTGGCGT GCACACCTTTCCAGCAGTGCTGCAG TCCTCCGGCCTGTACTCTCTGCTCCT TGTCGTGACCGTGCCTTCCAGCTCT CTGGGAACCCAGACCTACATCTGCA ATGTGAACCACAAGCCTTCCAACACC AAGGTGGACAAGAAGGTGGAACCCA AGTCCTGCGACAAGACCCACACCTG TCCTCCATGTCCTGCTCCAGAACTGC TCGGCGGACCTTCCGTGTTCTGTTT CCTCCAAAGCCTAAGGACACCCCTGA TGATCTCTCGGACCCCTGAAGTGAC CTGCGTGGTGGTGGATGTGTCCAC GAGGATCCAGAAGTGAAGTTCAATTG GTACGTGGACGGCGTGGAAGTGCAC AATGCCAAGACCAAGCCTAGAGAGG AACAGTACGCCTCCACCTACAGAGT GGTGTCCGTGCTGACTGTGCTGCAC CAGGATTGGCTGAACGGCAAAGAGT ACAAGTGCAAGGTGTCCAACAAGGC CCTGCCTGCTCCTATCGAAAAGACCA TCAGCAAGGCCAAGGGCCAGCCTAG GGAACCCAGGTTTACACCCTGCCT CCAAGCCGGGAAGAGATGACCAAGA ACCAGGTGTCCCTGACCTGCCTCGT GAAGGGCTTCTACCCTTCCGATATCG CCGTGGAATGGGAGAGCAATGGCCA GCCTGAGAACAACATAAGACAACC CCTCCTGTGCTGGACTCCGACGGCT CATTCTTCTGTACTCCAAGCTGACC GTGGACAAGTCCAGATGGCAGCAGG GCAACGTGTTCTCCTGCTCCGTGAT GCACGAGGCCCTGCACAATCACTAC ACCCAGAAGTCCCTGTCTCTGAGCC CTGGAAAAGGCGGCGGAGGATCTGG CGGAGGTGGTAGCGGAGGCGGTGG
Linker		GGGGSGGGGSG GGGS (235)	
scFv of 41B0 1.02	VL	QSVLTQPPSASG TPGRRVTISCSGS SSNIGNNYVTWY QQLPGTAPKLLIY ADSHRPSGVPDR FSGSKSGTSASLA ISGLRSEDEADYY CATWDYSLSGYV FGCGTKLTVL (236)	
	Linker	GGGGSGGGGSG GGGSGGGGS (237)	
	VH	EVQLLES GGGLV QPGGSLRLSCAA	

		SGFTFSSYDMSW VRQAPGKCLEWV SWISYSGGSIYYA DSVKGRFTISRDN SKNTLYLQMNSL RAEDTAVYYCAR DAQRQSMREFDY WGQGTLLTVSS (238)	ATCTCAGTCTGTTCTGACCCAGCCTC CTTCCGCTTCTGGCACCCCTGGAAG AAGAGTGACCATCTCTTGCTCCGGCT CCTCCTCCAACATCGGCAACAACCTAC GTGACCTGGTATCAGCAGCTGCCCG GCACAGCTCCCAAACCTGCTGATCTAC GCCGACTCTCACAGACCTTCCGGCG TGCCCGATAGATTCTCCGGCTCTAAG TCTGGCACCTCTGCCAGCCTGGCTA TCAGCGGCCTGAGATCTGAGGACGA GGCCGACTACTACTGCGCCACCTGG GATTATTCCCTGTCCGGCTACGTGTT CGGCTGCGGGCACAAAACCTGACAGTG CTCGGAGGCGGAGGAAGTGGTGGC GGAGGTTTCAAGTGGTGGTGGTAGTG GCGGAGGCGGATCAGAAGTTCAGCT GTTGGAGTCAGGTGGCGGCTTGGTG CAACCAGGTGGAAGTCTGAGACTCA GCTGTGCTGCCAGCGGCTTTACCTT CAGCTCCTACGACATGAGCTGGGTT CGACAAGCTCCCGGAAAGTGCTTGG AGTGGGTTTTCTGGATCTCCTACTCC GGCGGCAGCATCTATTACGCCGACA GCGTGAAAGGCCGGTTTACCATCTC TCGGGATAACAGCAAGAATACCCTCT ACCTCCAAATGAACTCTCTGAGAGCC GAGGACACTGCTGTGTACTATTGCG CCAGAGATGccCAGCGGCAATCCATG AGAGAGTTCGACTACTGGGGACAAG GCACCCTGGTCACCGTGTCTAGTTG A (239)
Light compo nent	Light chain of B6	DIQMTQSPSSLSA SVGDRVITITCKAS QDVTPAVAWYQ QKPGKAPKLLIYS TSSRYTGVPSRF SGSGSGTDFTFTI SSLQPEDIATYYC QQHYTTPLTFGQ GTKLEIKRTVAAP SVFIFPPSDEQLK SGTASVVCLLNNF YPREAKVQWKVD NALQSGNSQESV TEQDSKDYSL STLTLSKADYEKH KVYACEVTHQGL SSPVTKSFNRGE C (38)	GACATCCAGATGACCCAGAGCCCTA GCAGCCTGAGCGCTAGCGTGGGCG ACAGGGTGACCATCACCTGCAAGGC CAGCCAGGATGTGACCCCTGCCGTG GCCTGGTACCAGCAGAAGCCCGGCA AGGCCCCCAAGCTGCTGATCTACAG CACCAGCAGCAGGTACACCGGCGTG CCCAGCAGGTTTAGCGGAAGCGGCA GCGGCACCGACTTCACCTTCACCAT CAGCAGCCTGCAGCCCGAGGACATC GCCACCTACTACTGCCAGCAGCACT ACACCACCCCTCTGACCTTCGGCCA GGGCACCAAGCTGGAGATCAAGAGA ACCGTGGCCGCTCCCTCCGTGTTCA TCTTCCCACCATCTGACGAGCAGCT GAAGTCCGGCACCGCTTCTGTCTGTG TGCTGCTGAACAATTCTACCCTCG GGAAGCCAAGGTGCAGTGGAAGGTG GACAATGCCCTGCAGTCCGGCAACT CCCAAGAGTCTGTGACCGAGCAGGA

			CTCCAAGGACAGCACCTACTCCCTG TCCTCTACCCTGACCCTGTCCAAGG CCGACTACGAGAAGCACAAGGTGTA CGCCTGCGAAGTGACCCACCAGGGA CTGTCTAGCCCCGTGACCAAGTCCTT CAACAGAGGCGAGTGCTGA (240)
ABLPNB.07 (bispecific antibody comprising the anti-PD-L1 B6 clone in IgG form and the anti-4-1BB 41B01.03 clone in scFv form)			
		Amino acid sequence (N'→C') (Seq ID No.)	Nucleotide Sequence (5'→3') (Seq ID No.)
Heavy compo nent	Heavy chain of B6	EVQLVESGGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKSLEWV ATISDAGGYIYR DSVKGRFTISRDN AKNSLYLQMNSL RDEDTAVYICARE LPWRYALDYWG QGTTVTVSSASTK GPSVFPLAPSSKS TSGGTAALGCLV KDYFPEPVTVSW NSGALTSGVHTF PAVLQSSGLYSLS SVVTVPSSSLGTQ TYICNVNHKPSNT KVDKKVEPKSCD KTHTCPPCPAPEL LGGPSVFLFPPKP KDTLMISRTPEVT CVVVDVSHEDPE VKFNWYVDGVEV HNAKTKPREEQY ASTYRVVSVLTVL HQDWLNGKEYKC KVSNAKALPAIEK TISKAKGQPREPQ VYTLPPSREEMTK NQVSLTCLVKGFY PSDIAVEWESNG QPENNYKTTTPVL DSDGSFFLYSKLT VDKSRWQQGNV FSCSVMHEALHN HYTQKSLSLSPGK (241)	GAAGTGCAGCTGGTTGAATCTGGCG GCGGATTGGTTCAGCCTGGCGGATC TCTGAGACTGTCTTGTGCCGCCTCC GGCTTCACCTTCTCCAGCTACGATAT GTCCTGGGTCCGACAGGCCCTGGC AAGTCTTTGGAATGGGTGCGCCACCAT CTCTGACGCTGGCGGCTACATCTAC TACCGGGACTCTGTGAAGGGGCAGAT TCACCATCAGCCGGGACAACGCCAA GAACTCCCTGTACCTGCAGATGAACA GCCTGCGCGACGAGGATACCGCCGT GTACATCTGTGCTAGAGAGCTGCCTT GGAGATACGCCCTGGATTATTGGGG CCAGGGCACCACAGTGACCGTGTCC TCTGCTTCTACCAAGGGACCCAGCG TGTTCCCTCTGGCTCCTTCCAGCAAG TCTACCTCTGGCGGAACAGCTGCTC TGGGCTGCCTGGTCAAGGACTACTT TCCTGAGCCTGTGACAGTGTCTGG AACTCTGGCGCTCTGACATCTGGCG TGCACACCTTTCCAGCAGTGCTGCA GTCCTCCGGCCTGTACTCTCTGTCT CTGTCGTGACCGTGCCTTCCAGCTC TCTGGGAACCCAGACCTACATCTGC AATGTGAACCACAAGCCTTCCAACAC CAAGGTGGACAAGAAGGTGGAACCC AAGTCCTGCGACAAGACCCACACCT GTCCTCCATGTCCTGCTCCAGAAGT CTCGGCGGACCTTCCGTGTTCTGT TTCCTCCAAAGCCTAAGGACACCCTG ATGATCTCTCGGACCCCTGAAGTGA CCTGCGTGGTGGTGGATGTGTCCCA CGAGGATCCAGAAGTGAAGTTCAATT GGTACGTGGACGGCGTGGAAGTGCA CAATGCCAAGACCAAGCCTAGAGAG GAACAGTACGCCTCCACCTACAGAG TGGTGTCCGTGCTGACTGTGCTGCA

scFv of 41B0 1.03	Linker	GGGSGGGGSG GGGS (242)	CCAGGATTGGCTGAACGGCAAAGAG TACAAGTGCAAGGTGTCCAACAAGG CCCTGCCTGCTCCTATCGAAAAGAC CATCAGCAAGGCCAAGGGCCAGCCT AGGGAACCCCAGGTTTACACCCTGC CTCCAAGCCGGAAGAGATGACCAA GAACCAGGTGTCCCTGACCTGCCTC GTGAAGGGCTTCTACCCTTCCGATAT CGCCGTGGAATGGGAGAGCAATGGC CAGCCTGAGAACAACACTACAAGACAA CCCCTCCTGTGCTGGACTCCGACGG CTCATTCTTCCTGTACTCCAAGCTGA CCGTGGACAAGTCCAGATGGCAGCA GGGCAACGTGTTCTCCTGCTCCGTG ATGCACGAGGCCCTGCACAATCACT ACACCCAGAAGTCCCTGTCTCTGAG CCCTGGAAAAGGCGGCGGAGGATCT GGCGGAGGTGGTAGCGGAGGCGGT GGATCTCAGTCTGTTCTGACCCAGC CTCCTTCCGCTTCTGGCACCCCTGG AcAGAGAGTGACCATCTCTTGCTCCG GCTCCTCCTCCAACATCGGCAACAA CTACGTGACCTGGTATCAGCAGCTG CCCGGCACAGCTCCCAAACTGCTGA TCTACGCCGACTCTCACAGACCTTCC GGCGTGCCCGATAGATTCTCCGGCT CTAAGTCTGGCACCTCTGCCAGCCT GGCTATCAGCGGCCTGAGATCTGAG GACGAGGCCGACTACTACTGCGCCA CCTGGGATTATTCCCTGTCCGGCTAC GTGTTCCGGCTGCGGCACAAAAGTGA CAGTGCTCGGAGGCGGAGGAAGTG GTGGCGGAGGTTTCAGGTGGTGGTG GTAGTGGCGGAGGCGGATCAGAAGT TCAGCTGTTGGAGTCAGGTGGCGGC TTGGTGCAACCAGGTGGAAGTCTGA GACTCAGCTGTGCTGCCAGCGGCTT TACCTTCAGCTCCTACGACATGAGCT GGTTTCGACAAGCTCCCGGAAAGTG CTTGGAGTGGGTTTCCTGGATCTCCT ACTCCGGCGGCAGCATCTATTACGC CGACAGCGTGAAAGGCCGGTTTACC ATCTCTCGGGATAACAGCAAGAATAC CCTCTACCTCCAAATGAACTCTCTGA GAGCCGAGGACACTGCTGTGTACTA TTGCGCCAGAGATGccCAGCGGAACT CCATGAGAGAGTTCGACTACTGGGG ACAAGGCACCCTGGTCACCGTGTCT AGTTGA (246)
	VL	QSVLTQPPSASG TPGQRTVISC SGS SSNIGNNNYVTWY QQLPGTAPKLLIY ADSHRPSGVPDR FSGSKSGTSASLA ISGLRSEDEADYY CATWDYSLSGYV FGCGTKLTVL (243)	
	Linker	GGGSGGGGSG GGGSGGGGS (244)	
	VH	EVQLLES GGGLV QPGGSLRLSCAA SGFTF SSYDMSW VRQAPGKCLEWV SWISYSGGSIYYA DSVKGRFTISRDN SKNTLYLQMNSL RAEDTAVYYCAR DAQRNSMREFDY WGQGTLVTVSS (245)	

Light component	Light chain of B6	DIQMTQSPSSLSA SVGDRVITICKAS QDVTPAVAWYQ QKPGKAPKLLIYS TSSRYTGVPSRF SSGSGSGTDFTFI SSLQPEDIATYYC QQHYTTPLTFGQ GTKLEIKRTVAAP SVFIFPPSDEQLK SGTASVVCLLNNF YPREAKVQWKVD NALQSGNSQESV TEQDSKDYSL STLTLKADYEKH KVVACEVTHQGL SSPVTKSFNRGE C (40)	GACATCCAGATGACCCAGAGCCCTA GCAGCCTGAGCGCTAGCGTGGGCG ACAGGGTGACCATCACCTGCAAGGC CAGCCAGGATGTGACCCCTGCCGTG GCCTGGTACCAGCAGAAAGCCCGGCA AGGCCCCCAAGCTGCTGATCTACAG CACCAGCAGCAGGTACACCGGCGTG CCCAGCAGGTTTAGCGGAAGCGGCA GCGGCACCGACTTCACCTTCACCAT CAGCAGCCTGCAGCCCGAGGACATC GCCACCTACTACTGCCAGCAGCACT ACACCACCCCTCTGACCTTCGGCCA GGGCACCAAGCTGGAGATCAAGAGA ACCGTGGCCGCTCCCTCCGTGTTCA TCTTCCCACCATCTGACGAGCAGCT GAAGTCCGGCACCCTTCTGTCTGTG TGCCTGCTGAACAATTCTACCCTCG GGAAGCCAAGGTGCAGTGAAGGTG GACAATGCCCTGCAGTCCGGCAACT CCCAAGAGTCTGTGACCGAGCAGGA CTCCAAGGACAGCACCTACTCCCTG TCCTCTACCCTGACCCTGTCCAAGG CCGACTACGAGAAGCACAAGGTGTA CGCCTGCGAAGTGACCCACCAGGGA CTGTCTAGCCCCGTGACCAAGTCCTT CAACAGAGGCGAGTGCTGA (247)
ABLPNB.08 (bispecific antibody comprising the anti-PD-L1 B6 clone in IgG form and the anti-4-1BB 41B01.04 clone in scFv form)			
		Amino acid sequence (N'→C') (Seq ID No.)	Nucleotide Sequence (5'→3') (Seq ID No.)
Heavy component	Heavy chain of B6	EVQLVESGGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKSLEWV ATISDAGGYIYYR DSVKGRFTISRDN AKNSLYLQMNSL RDEDTAVYICARE LPWRYALDYWG QGTTTVTVSSASTK GPSVFPLAPSSKS TSGGTAALGCLV KDYFPEPVTVSW NSGALTSGVHTF PAVLQSSGLYSLS SVVTVPSSSLGTQ TYICNVNHKPSNT	GAAGTGCAGCTGGTTGAATCTGGCG GCGGATTGGTTCAGCCTGGCGGATC TCTGAGACTGTCTTGTGCCGCCTCC GGCTTCACCTTCTCCAGCTACGATAT GTCCTGGGTCCGACAGGCCCTGGC AAGTCTTTGGAATGGGTGCGCCACCAT CTCTGACGCTGGCGGCTACATCTAC TACCGGGACTCTGTGAAGGGCAGAT TCACCATCAGCCGGGACAACGCCAA GAACTCCCTGTACCTGCAGATGAACA GCCTGCGCGACGAGGATACCGCCGT GTACATCTGTGCTAGAGAGCTGCCTT GGAGATACGCCCTGGATTATTGGGG CCAGGGCACCACAGTGACCGTGCTC TCTGCTTCTACCAAGGGACCCAGCG TGTTCCCTCTGGCTCCTTCCAGCAAG TCTACCTCTGGCGGAACAGCTGCTC

		KVDKKVEPKSCD KTHTCPPCPAPEL LGGPSVFLFPPKP KDTLMISRTPEVT CVVVDVSHEDPE VKFNWYVDGVEV HNAKTKPREEQY ASTYRVVSVLTVL HQDWLNGKEYKC KVSNAKALPAIEK TISKAKGQPREPQ VYTLPPSREEMTK NQVSLTCLVKGFY PSDIAVEWESNG QPENNYKTTTPVL DSGDSFFLYSKLT VDKSRWQQGNV FSCSVMHEALHN HYTQKSLSLSPGK (248)	TGGGCTGCCTGGTCAAGGACTACTT TCCTGAGCCTGTGACAGTGTCTGCG AACTCTGGCGCTCTGACATCTGGCG TGACACACCTTTCCAGCAGTGCTGCA GTCCTCCGGCCTGTACTCTCTGTCTCCT CTGTCTGTGACCGTGCCTTCCAGCTC TCTGGGAACCCAGACCTACATCTGC AATGTGAACCACAAGCCTTCCAACAC CAAGGTGGACAAGAAGGTGGAACCC AAGTCCTGCGACAAGACCCACACCT GTCCTCCATGTCTGTCTCCAGAAGTG CTCGGCGGACCTTCCGTGTTCTGT TTCCTCCAAAGCCTAAGGACACCCCTG ATGATCTCTCGGACCCCTGAAGTGA CCTGCGTGGTGGTGGATGTGTCCCA CGAGGATCCAGAAGTGAAGTTCAATT GGTACGTGGACGGCGTGGAAAGTGCA CAATGCCAAGACCAAGCCTAGAGAG GAACAGTACGCCTCCACCTACAGAG TGGTGTCCGTGCTGACTGTGCTGCA CCAGGATTGGCTGAACGGCAAAGAG TACAAGTGCAAGGTGTCCAACAAGG CCCTGCCTGCTCCTATCGAAAAGAC CATCAGCAAGGCCAAGGGCCAGCCT AGGGAACCCCAAGTTTACACCCTGC CTCCAAGCCGGGAAGAGATGACCAA GAACCAGGTGTCCCTGACCTGCCTC GTGAAGGGCTTCTACCCTTCCGATAT CGCCGTGGAATGGGAGAGCAATGGC CAGCCTGAGAACAACACTACAAGACAA CCCCTCCTGTGCTGGACTCCGACGG CTCATTCTTCCTGTACTCCAAGCTGA CCGTGGACAAGTCCAGATGGCAGCA GGGCAACGTGTTCTCCTGCTCCGTG ATGCACGAGGCCCTGCACAATCACT ACACCCAGAAGTCCCTGTCTCTGAG CCCTGGAAAAGGCGGCGGAGGATCT GGCGGAGGTGGTAGCGGAGGCGGT GGATCTCAGTCTGTTCTGACCCAGC CTCCTTCCGCTTCTGGCACCCCTGG AcAGAGAGTGACCATCTCTTGCTCCG GCTCCTCCTCCAACATCGGCAACAA CTACGTGACCTGGTATCAGCAGCTG CCCGGCACAGCTCCCAAAGTCTGA TCTACGCCGACTCTCACAGACCTTCC GGCGTGCCCGATAGATTCTCCGGCT CTAAGTCTGGCACCTCTGCCAGCCT GGCTATCAGCGGCCTGAGATCTGAG GACGAGGCCGACTACTACTGCGCCA CCTGGGATTATTCCCTGTCCGGCTAC GTGTTCCGCTGCGGCACAAAAGTGA
	Linker	GGGGSGGGGSG GGGS (249)	
scFv of 41B0 1.04	VL	QSVLTQPPSASG TPGQRTVITSCSGS SSNIGNNYVTWY QQLPGTAPKLLIY ADSHRPSGVPDR FSGSKSGTSASLA ISGLRSEDEADYY CATWDYSLSGYV FGCGTKLTVL (250)	
	Linker	GGGGSGGGGSG GGGSGGGGS (251)	
	VH	EVQLLES GGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKCLEWV SWISYSGGSIYYA DSVKGRFTISRDN SKNTLYLQMNSL RAEDTAVYYCAR DAQRQSMREFDY WGQGTLLTVSS (252)	

				CAGTGCTCGGAGGCGGAGGAAGTG GTGGCGGAGGTTCAGGTGGTGGTG GTAGTGGCGGAGGCGGATCAGAAGT TCAGCTGTTGGAGTCAGGTGGCGGC TTGGTGCAACCAGGTGGAAGTCTGA GACTCAGCTGTGCTGCCAGCGGCTT TACCTTCAGCTCCTACGACATGAGCT GGGTTTCGACAAGCTCCCGGAAAGTG CTTGGAGTGGGTTTCCTGGATCTCCT ACTCCGGCGGCAGCATCTATTACGC CGACAGCGTGAAAGGCCGGTTTACC ATCTCTCGGGATAACAGCAAGAATAC CCTCTACCTCCAAATGAACTCTCTGA GAGCCGAGGACACTGCTGTGTACTA TTGCGCCAGAGATGccCAGCGGCAAT CCATGAGAGAGTTTCGACTACTGGGG ACAAGGCACCCTGGTCACCGTGTCT AGTTGA (253)
Light compo nent	Light chain of B6	DIQMTQSPSSLSA SVGDRVITITCKAS QDVTPAVAWYQ QKPGKAPKLLIYS TSSRYTGVPSRF SGSGSGTDFTFTI SSLQPEDIATYYC QQHYTTPLTFGQ GTKLEIKRTVAAP SVFIFPPSDEQLK SGTASVVCLLNMF YPREAKVQWKVD NALQSGNSQESV TEQDSKDSYSL STLTLSKADYEKH KYYACEVTHQGL SSPVTKSFNRGE C (42)	GACATCCAGATGACCCAGAGCCCTA GCAGCCTGAGCGCTAGCGTGGGCG ACAGGGTGACCATCACCTGCAAGGC CAGCCAGGATGTGACCCCTGCCGTG GCCTGGTACCAGCAGAAGCCCCGGCA AGGCCCCCAAGCTGCTGATCTACAG CACCAGCAGCAGGTACACCGGCGTG CCCAGCAGGTTTAGCGGAAGCGGCA GCGGCACCGACTTCACCTTCACCAT CAGCAGCCTGCAGCCCGAGGACATC GCCACCTACTACTGCCAGCAGCACT ACACCACCCCTCTGACCTTCGGCCA GGGCACCAAGCTGGAGATCAAGAGA ACCGTGGCCGCTCCCTCCGTGTTCA TCTTCCCACCATCTGACGAGCAGCT GAAGTCCGGCACCGCTTCTGTCTGT TGCCTGCTGAACAATTCTACCCTCG GGAAGCCAAGGTGCAGTGGAAGGTG GACAATGCCCTGCAGTCCGGCAACT CCCAAGAGTCTGTGACCGAGCAGGA CTCCAAGGACAGCACCTACTCCCTG TCCTTACCCTGACCCTGTCCAAGG CCGACTACGAGAAGCACAAGGTGTA CGCCTGCGAAGTGACCCACCAGGGA CTGTCTAGCCCCGTGACCAAGTCCTT CAACAGAGGCGAGTGCTGA (254)	
ABLPNB.09 (bispecific antibody comprising the anti-PD-L1 B6 clone in IgG form and the anti-4-1BB 41B02 clone in scFv form)				
		Amino acid sequence (N'→C')	Nucleotide Sequence (5'→3') (Seq ID No.)	

		(Seq ID No.)	
Heavy component	Heavy chain of B6	EVQLVESGGGLV QPGGSLRLSCAA SGFTFSSYDMSW VRQAPGKSLEWV ATISDAGGYIYYR DSVKGRFTISRDN AKNSLYLQMNSL RDEDTAVYICARE LPWRYALDYWG QGTTTVTVSSASTK GPSVFPLAPSSKS TSGGTAALGCLV KDYFPEPVTVSW NSGALTSGVHTF PAVLQSSGLYSLS SVVTVPSSSLGTQ TYICNVNHKPSNT KVDKKVEPKSCD KTHTCPPCPAPEL LGGPSVFLFPPKP KDTLMISRTPEVT CVVVDVSHEDPE VKFNWYVDGVEV HNAKTKPREEQY ASTYRVVSVLTVL HQDWLNGKEYKC KVSNAKALPAIEK TISKAKGQPREPQ VYTLPPSREEMTK NQVSLTCLVKGFY PSDIAVEWESNG QPENNYKTTTPVL DSDGSFFLYSKLT VDKSRWQQGNV FSCVMHEALHN HYTQKSLSLSPGK (255)	GAAGTGCAGCTGGTTGAATCTGGCG GCGGATTGGTTCAGCCTGGCGGATC TCTGAGACTGTCTTGCCGCCTCC GGCTTCACCTTCTCCAGCTACGATAT GTCCTGGGTCCGACAGGCCCTGGC AAGTCTTTGGAATGGGTGCGCCACCAT CTCTGACGCTGGCGGCTACATCTAC TACCGGGACTCTGTGAAGGGCAGAT TCACCATCAGCCGGGACAACGCCAA GAACTCCCTGTACCTGCAGATGAACA GCCTGCGCGACGAGGATACCGCCGT GTACATCTGTGCTAGAGAGCTGCCTT GGAGATACGCCCTGGATTATTGGGG CCAGGGCACCAAGTGACCGTGTCC TCTGCTTCTACCAAGGGACCCAGCG TGTTCCCTCTGGCTCCTTCCAGCAAG TCTACCTCTGGCGGAACAGCTGCTC TGGGCTGCCTGGTCAAGGACTACTT TCCTGAGCCTGTGACAGTGTCTGG AACTCTGGCGCTCTGACATCTGGCG TGCACACCTTTCCAGCAGTGCTGCA GTCCTCCGGCCTGTACTCTGTCTCT CTGTCGTGACCGTGCTTCCAGCTC TCTGGGCACCCAGACCTACATCTGC AACGTGAACCACAAGCCCTCCAACA CCAAGGTGGACAAGAAGGTGGAACC CAAGTCCTGCGACAAGACCCACACC TGTCTCCATGTCTGCTCCAGAACT GCTGGGCGGACCCCTCCGTGTTCTCG TTCCTCCAAAGCCTAAGGACACCCT GATGATCTCCCGGACCCCTGAAGT ACCTGCGTGTTGGTGATGTGTCC ACGAGGATCCCGAAGTGAAGTTCAA TTGGTACGTGGACGGCGTGGAAGTG CACAACGCCAAGACCAAGCCTAGAG AGGAACAGTACgacTCCACCTACCGG GTGGTGTCCGTGCTGACCGTTCTGC ACCAGGATTGGCTGAACGGCAAAGA GTACAAAGTGCAAGGTGTCCAACAAG GCCCTGCCTGCCCCTATCGAAAAGA CCATCTCTAAGGCCAAGGGCCAGCC CCGGGAACCTCAAGTGACACCTTG CCTCCCAGCCGGGAAGAGATGACCA AGAACCAGGTGTCCCTGACCTGCCT GGTTAAGGGCTTCTACCCCTCCGATA TCGCCGTGGAATGGGAGTCTAACGG CCAGCCCGAGAACAACATAAGACC ACCCCTCCTGTGCTGGACTCCGACG GCTCATTCTTCTGTACTCCAAGCTG ACCGTGGACAAGTCTCGGTGGCAGC
	Linker	GGGGSGGGGSG GGGS (256)	
	scFv of 41B0 2	VL QSVLTQPPSASG TPGRRVTISCSGS SSNIGNNYVTWY QQLPGTAPKLLIY ADSHRPSGVPDR FSGSKSGTSASLA ISGLRSEDEADYY CATWDYSLSGYV FGCGTKLTVL (257)	

		Linker	GGGSGGGGSG GGGSGGGGS (258)	AGGGCAACGTGTTCTCCTGCTCTGT GATGCACGAGGCCCTGCACAACCAC TACACCCAGAAGTCCCTGTCCCTGTC TCCCGGCAAAGGTGGGGGGGGATCT GGTGGTGGTGGATCAGGGGGTGGG GGGTCTCAAAGCGTACTCACCCAAC CTCCATCTGCATCCGGTACACCTGGT CGGCGAGTAACCATCTCCTGCTCTG GGAGCTCTTCTAATATTGGTAACAAC TATGTCACCTGGTATCAGCAGTTGCC TGGACAGCACCCAACTTCTTATAT ATGCCGATAGCCATCGGCCTTCCGG CGTACCCGATCGCTTCTCCGGGTCA AAATCTGGAACATCTGCCTCACTCGC AATTAGTGGATTGCGATCTGAGGATG AAGCAGATTATTATTGCGCTACCTGG GATTATTCACTTTCTGGCTACGTCTTT GGTgtGGAACAAAACCTACCGTGTTG GGCGGCGGAGGAAGCGGAGGCGGC GGTCTGGTGGTGGCGGTAGCGGAG GTGGTGGATCTGAGGTTCAACTGTT GGAGTCAGGTGGCGGACTTGTCCAG CCTGGCGGGTCTCTGAGGCTGAGTT GCGCTGCTTCTGGGTTTACTTTTTCA GGATATGACATGAGTTGGGTACGTC AGGCTCCAGGTAAAGtgcCTCGAATGG GTCTCCGTTATCTATCCCGATGATGG AAATACTTACTACGCTGACAGTGTGA AAGGCAGGTTTACAATCAGTAGGGA CAATTCTAAAAATACACTCTACCTCC AGATGAACTCACTTCGAGCCGAGGA CGCCGCCGTATATTACTGTGCCAAAC ACGGCGGGCAAAAACCCACTACTAA ATCCAGTAGTGCTTACGGGATGGAT GGCTGGGGACAGGGGACATTGGTCA CTGTATCTTCCTga (260)
		VH	EVQLLESGGGLV QPGGSLRLSCAA SGFTFSGYDMSW VRQAPGKCLEWV SVIYPDDGNTYYA DSVKGRFTISRDN SKNTLYLQMNSL RAEDAAVYYCAK HGGQKPTTKSSS AYGMDGWGQGT LVTVSS (259)	
Light compo nent	Light chain of B6		DIQMTQSPSSLSA SVGDRVITITCKAS QDVTPAVAWYQ QKPGKAPKLLIYS TSSRYTGVPSTRF SGSGSGTDFTFTI SSLQPEDIATYYC QQHYTTPLTFGQ GTKLEIKRTVAAP SVFIFPPSDEQLK SGTASVVCLLNNF YPREAKVQWKVD NALQSGNSQESV TEQDSKDYSL STLTLSKADYEKH	GACATCCAGATGACCCAGAGCCCTA GCAGCCTGAGCGCTAGCGTGGGCG ACAGGGTGACCATCACCTGCAAGGC CAGCCAGGATGTGACCCCTGCCGTG GCCTGGTACCAGCAGAAGCCCGGCA AGGCCCCCAAGCTGCTGATCTACAG CACCAGCAGCAGGTACACCGGCGTG CCCAGCAGGTTTAGCGGAAGCGGCA GCGGCACCGACTTCACCTTCACCAT CAGCAGCCTGCAGCCCGAGGACATC GCCACCTACTACTGCCAGCAGCACT ACACCACCCCTCTGACCTTCGGCCA GGGCACCAAGCTGGAGATCAAGAGA ACCGTGGCCGCTCCCTCCGTGTTCA TCTTCCACCATCTGACGAGCAGCT

		KVYACEVTHQGL SSPVTKSFNERGE C (44)	GAAGTCCGGCACCGCTTCTGTCGTG TGCCTGCTGAACAACCTTCTACCCTCG GGAAGCCAAGGTGCAGTGGAAGGTG GACAATGCCCTGCAGTCCGGCAACT CCCAAGAGTCTGTGACCGAGCAGGA CTCCAAGGACAGCACCTACTCCCTG TCCTCTACCCTGACCCTGTCCAAGG CCGACTACGAGAAGCACAAGGTGTA CGCCTGCGAAGTGACCCACCAGGGA CTGTCTAGCCCCGTGACCAAGTCCTT CAACAGAGGCGAGTGCTGA (261)
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The constructed vectors were transiently expressed in ExpiCHO-S™ cells (Thermo Fisher, A29127) using (ExpiFectamine™CHO Kit, Thermo, A29129), cultured in ExpiCHO™ Expression medium (Thermo, A29100-01) under the conditions of 30 to 37°C for 7 to 15 days in a CO₂ incubator equipped with rotating shaker. Plasmid DNA (250 µg) and ExpiFectamin CHO Reagent (800 µL) were mixed with Opti-MEM® I medium (20 mL final volume) and allowed to stand at room temperature for 5 min. The mixed solution was added to 6 x 10⁶ ExpiCHO cells cultured in ExpiCHO Expression Medium and gently mixed in a shaker incubator at 37°C with a humidified atmosphere of 8% CO₂ in air. At 18 hours post-transfection, 1.5 mL of ExpiFectamin CHO Transfection Enhancer 1 and 60 mL of ExpiFectamin CHO Transfection Feed were added to each flask.

Each BsAb was purified from the cell culture supernatant by recombinant Protein A affinity chromatography (Hitrap Mabselect Sure, GE Healthcare, 28-4082-55) and gel filtration chromatography with a HiLoad 26/200 Superdex200 prep grade column (GE Healthcare, 28-9893-36). SDS-PAGE (NuPage 4-12% Bis-Tris gel, NP0321) and size exclusion HPLC (Agilent, 1200 series) analysis with SE-HPLC column (SWXL SE-HPLC column, TOSOH, G3000SWXL) were performed to detect and confirm the size and purity of each BsAb. Purified proteins were concentrated in PBS by ultrafiltration using a Amicon Ultra 15 30K device (Merck, UFC903096), and protein concentrations were estimated using a nanodrop (Thermo, Nanodrop One). When a two-vector system is applied, the ratio between light to heavy chain could be 1:1 to 1:3 by weight. Alternatively, a one-vector system that contains both chains in one single vector can also be used.

The prepared anti-PD-L1/anti-4-1BB bispecific antibodies are named as H12x41B01(ABLPNB.01), H12x41B01.03(ABLPNB.02), H12x41B02(ABLPNB.03), B6x41B01(ABLPNB.04), B6x41B01.01(ABLPNB.05), B6x41B01.01(ABLPNB.06), B6x41B01.03(ABLPNB.07), B6x41B01.04(ABLPNB.08), and B6x41B02(ABLPNB.09), respectively, wherein the former refers to the clone in the IgG form and the latter refers to the clone in the scFv form.

Example 4. Characterization of bispecific antibodies PD-L1x4-1BB

4.1. Binding of the bispecific antibodies

To evaluate the binding activity to PD-L1 and 4-1BB of the bispecific antibodies prepared in Example 3, the BsAbs(ABLPNB.01, ABLPNB.03, ABLPNB.04 and ABLPNB.07), were subjected to DACE (Dual antigen captured ELISA) test. Briefly, microtiter plates were coated with 100 ng/well of human PD-L1-Fc protein (Sinobio, 10084-H02H) in PBS at 4°C overnight, then blocked with 100 µl/well of 1% BSA for 2 hours at 37°C. Three-fold dilutions of each of the BsAbs starting from 100 nM were added to each well and incubated for 2 hours at 37°C. The plates were washed with PBS/Tween and then incubate with 50 ng/well of human 4-1BB-His protein (Sinobio, 16498-H08H) in 1% BSA for 1 hour at 37°C. The plates were washed with PBS/Tween and then incubate with Anti-His HRP (Roche, Cat: 11965085001) for 1 hour at 37°C. After washing, the plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-650nm. The results are shown in FIGS. 7A and 7B. As shown in FIGS. 7A and 7B, all the BsAbs tested can bind to both of human PD-L1 and human 4-1BB proteins with high activities.

4.2 Serum stability of bispecific antibody

To evaluate the serum stability to PD-L1 and 4-1BB of the BsAb (ABLPNB.05, ABLPNB.06, ABLPNB.07 and ABLPNB.08), BsAb was incubated in human serum for 3, 7, 14 days at 37°C. Binding activity was analyzed to DACE (Dual antigen captured ELISA) test. Briefly, microtiter plates were coated with 100 ng/well of human PD-L1-Fc protein (Sinobio, 10084-H02H) in PBS at 4°C

overnight, then blocked with 100 μ l/well of 1% BSA for 2 hours at 37°C. Three-fold dilutions of each of the BsAbs starting from 100 nM were added to each well and incubated for 2 hours at 37°C. The plates were washed with PBS/Tween and then incubate with 50 ng/well of human 4-1BB-His protein (Sinobio, 16498-H08H) in 1% BSA for 1 hour at 37°C. The plates were washed with PBS/Tween and then incubate with Anti-His HRP (Roche, Cat: 11965085001) for 1 hour at 37°C. After washing, the plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-650 nm. The binding activity of each of the BsAbs (ABLPNB.05 to ABLPNB.08) to both antigens was comparable to each sample. It means that BsAbs are stable in human serum for 2 weeks at 37°C. The representative data is as shown in FIG. 8.

4.3. Developability of bispecific antibodies

The developability regarding the physicochemical properties to PD-L1 and 4-1BB of the BsAbs (ABLPNB.02 and ABLPNB.07) was assessed. The quality attributes for the BsAbs were evaluated by several analytical methods. Briefly, the purity was measured by Size exclusion-high performance liquid chromatography (SE-HPCL) and both of the BsAb showed the high purity over 99%. The thermal stability by Protein thermal shift (PTS) with fluorescence labeled Real time-polymerase chain reaction (RT-PCR) was analyzed. Their melting temperature was observed over 67°C which indicated that the test articles have stable structural integrity. To evaluate solubility of the molecules, the proteins were concentrated to 20 mg/mL using ultrafiltration (Amicon Ultra-15 spin concentrator). As a result, the visible particles were not observed by visual inspection and no increment of aggregates was confirmed by SE-HPLC. The Isoelectric point (pI) of ABLPNB.02 and ABLPNB.07 measured by capillary isoelectric focusing (cIEF) were 8.26 and 8.35, respectively. This pI range is appropriate to proceed downstream process and formulation development. Overall, as shown in Table 19. It showed that the tested BsAbs (ABLPNB.02 and ABLPNB.07) have proper physicochemical properties for the successful development.

[Table 19]

Content	Method	ABLPNB.07	ABLPNB.02
Purity	SEC	99.6	99.8
Thermal Stability	PTS	67.0	67.1
		76.8	80.5
Solubility	Visual inspection	Easy to concentrate upto 20 mg/mL, clear	Easy to concentrate upto 20 mg/mL, clear
pI	cIEF	8.26	8.35

4.4. Activity of the bispecific antibodies to promote 4-1BB signal

To test the ability of bispecific antibodies (ABLPNB.01, ABLPNB.03, ABLPNB.07 and ABLPNB.08) to promote 4-1BB signal, cell-based 4-1BB assay was used. In this assay, GloResponse™ NFκB-luc2/4-1BB Jurkat cell line (Promega, cat# CS196004) was used as effector cells and PD-L1-expressing or not expressing cancer cell line as target cells. GloResponse™ NFκB-luc2/4-1BB Jurkat cell line is genetically modified to stably express 4-1BB and luciferase downstream of a response element. Luciferase expression is induced upon antibody binding to the 4-1BB receptor. In brief, plate HCC1954 (expressing PD-L1) or BT474 or NCI-N87 (not expressing PD-L1) at 2.5×10^4 cells per well in a white 96-well assay plate in 100 μL culture medium (RPMI1640 + 10% FBS). Culture overnight in 37°C + 5% CO₂ humidified incubator. After overnight culture, remove 100 μL of culture medium and dispense 25 μL of Assay Medium (RPMI1640 + 1% FBS) to pre-plated target cells. 25 μL of bispecific antibodies (starting from 15 nM diluted for 8-fold or 1.5 nM diluted for 4-fold) and BMUR or monoclonal antibody 41B01 (starting from 20 nM for 10-fold or 133 nM for 6-fold) were added to the plate. Harvest GloResponse™ NFκB-luc2/4-1BB Jurkat cell line and resuspend with Assay Medium. Dispense 25 μL of GloResponse™ NFκB-luc2/4-1BB Jurkat cell line per well to make 2.5×10^4 cells per well to plate. Culture 6hrs in 37°C + 5% CO₂ humidified incubator. During incubation time reconstitute Bio-Glo™ reagent according to the manufacturer's instruction. After 6 hrs

incubation, add 75 μ l per well of Bio-Glo™ Reagent to the assay plate. Wait 5 minutes and measure luminescence using microplate reader. Four-parameter logistic curve analysis was performed with GraphPad software. The results are shown in FIGS. 9A-9F. As shown in FIGS. 9A-9B, anti-4-1BB monoclonal antibody showed very limited 4-1BB signal activation comparing to BUMR. 41B01 (assayed in Fc-cross linked form) showed only weak activation of 4-1BB signal (FIG. 9B). In case of bispecific antibody, co-cultured with PD-L1 negative cancer cells (FIG. 9C and 9D), PD-L1x4-1BB bispecific antibody showed no activation of 4-1BB signal (FIG. 9C) and weak 4-1BB signal upon Fc-cross linking as presented in FIGS. 9C and 9D, respectively. In the presence of PD-L1, i.e., when co-cultured with PD-L1 positive target cells (FIGS. 9E and 9F), PD-L1x4-1BB bispecific antibody showed 4-1BB signal activation, which was depending on the presence of tumor antigen (PD-L1).

4.5. Activity of the bispecific antibodies to promote human T cell immune response

To test the ability of bispecific antibodies to stimulate human PBMCs response, cytokine production assay was used. Human PBMCs stimulated with human anti-CD3 antibody were used as the effector cells. HCC1954 cells which express PD-L1 was used as the target cells. In this system, PBMCs (3×10^4) were co-cultured with HCC1954 (1×10^4) in the presence of human anti-CD3 antibody. Bispecific antibodies (ABLPNB.01 and ABLPNB.04) (starting from 20 nM (=4 μ g/mL) diluted for 10 dose) and their counterpart monoclonal antibodies (41B01 and H12) (starting from 26.67 nM (=4 μ g/mL) diluted for 10 dose) were added to the mixed culture. As shown in FIGS. 10A and 10B, only bispecific antibody can activate T cell in the presence of PD-L1 expressing tumor cells (See FIG. 10A). Moreover, bispecific antibodies activated T-cell in dose dependent manner (See FIG. 10B).

4.6. Tumor growth inhibition of the bispecific antibodies (*in vivo* assay)

Humanized mice that express the extracellular domain of human 4-1BB were used. Mouse colon adenocarcinoma cells (MC38) were engineered to express human PD-L1. Humanized mice (h4-1BB) were subcutaneously implanted with MC38-hPD-L1 cells. Mice were intraperitoneally administered Q3D for 5 times (five time injection of the antibody every three days) with following antibodies: isotype control (10 mg/kg), anti-PD-L1 antibody (H12, 10 mg/kg), anti-4-1BB antibody (41B01, 10 mg/kg), combination of anti-PD-L1 (H12, 10 mg/kg) and anti-4-1BB (41B01, 10 mg/kg) and PD-L1x4-1BB Bispecific antibody (BsAb) {BLPNB.01, 13.3 mg/kg}. Tumor volumes were monitored by caliper measurement twice per week for the duration of the experiment. Tumor growth inhibition induced by ABLPNB.01 was significantly greater than that observed with the combination of each targeting monoclonal antibodies (See FIG. 11).

The present disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present disclosure without departing from the spirit or scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Claims

[Claim 1]

An anti-PD-L1/anti-4-1BB bispecific antibody, comprising an anti-PD-L1 antibody or an antigen-binding fragment thereof and an anti-4-1BB antibody or an antigen-binding fragment thereof, wherein

the anti-PD-L1 antibody or antigen-binding fragment thereof is capable of specifically binding to an immunoglobulin C (Ig C) domain of a human Programmed death-ligand 1 (PD-L1) protein, wherein the Ig C domain consists of amino acid residues 133-225;

the anti-PD-L1 antibody or antigen-binding fragment thereof comprises a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 2 and 3; a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 5, 262, 263, 264, 265, 266 and 267; a VL CDR1 having an amino acid sequence of SEQ ID NOS: 6, 268 and 269; a VL CDR2 having an amino acid sequence of SEQ ID NO: 7; and a VL CDR3 having an amino acid sequence of SEQ ID NOS: 8, 270, 271 and 272; and

the anti-4-1BB antibody or antigen-binding fragment thereof comprises a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 10 and 11; a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 12 and 13; a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 14, 15, 16 and 17; a VL CDR1 having an amino acid sequence of SEQ ID NO: 18; a VL CDR2 having an amino acid sequence of SEQ ID NO: 19; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 20.

[Claim 2]

The anti-PD-L1/anti-4-1BB bispecific antibody of claim 1, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof is capable of binding to at least one of amino acid residues Y134, K162, or N183 of the PD-L1 protein.

[Claim 3]

The anti-PD-L1/anti-4-1BB bispecific antibody of claim 2, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof is capable of binding to amino acid residues Y134, K162, and N183 of the PD-L1 protein.

[Claim 4]

The anti-PD-L1/anti-4-1BB bispecific antibody of any of claims 1-3, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof does not bind to an immunoglobulin V (Ig V) domain of the PD-L1 protein, wherein the Ig V domain consists of amino acid residues 19-127.

[Claim 5]

The anti-PD-L1/anti-4-1BB bispecific antibody of any of claims 1-4, wherein the anti-PD-L1/anti-4-1BB bispecific antibody activates 4-1BB signaling depending on PD-L1 expressed on cell surfaces.

[Claim 6]

The anti-PD-L1/anti-4-1BB bispecific antibody of any of claims 1-5, wherein each of the anti-PD-L1 antibody or antigen-binding fragment thereof and the anti-4-1BB antibody or antigen-binding fragment thereof is independently a chimeric antibody, a humanized antibody, or a fully human antibody.

[Claim 7]

An anti-PD-L1/anti-4-1BB bispecific antibody, comprising an anti-PD-L1 antibody or an antigen-binding fragment thereof and an anti-4-1BB antibody or an antigen-binding fragment thereof, wherein

the anti-PD-L1 antibody or antigen-binding fragment thereof comprises:

- (1) a VH CDR1 having an amino acid sequence of SEQ ID NO: 1;
- (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 2 and 3;
- (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 5, 262, 263, 264, 265, 266 and 267;
- (4) a VL CDR1 having an amino acid sequence of SEQ ID NOS: 6, 268, and 269;
- (5) a VL CDR2 having an amino acid sequence of SEQ ID NO: 7; and

(6) a VL CDR3 having an amino acid sequence of SEQ ID NOS: 8, 270, 271 and 272, and

the anti-4-1BB antibody or antigen-binding fragment thereof comprises:

(i) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 10 and 11;

(ii) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 12 and 13;

(iii) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 14, 15, 16 and 17;

(iv) a VL CDR1 having an amino acid sequence of SEQ ID NO: 18;

(v) a VL CDR2 having an amino acid sequence of SEQ ID NO: 19; and

(vi) a VL CDR3 having an amino acid sequence of SEQ ID NOS: 20.

[Claim 8]

The anti-PD-L1/anti-4-1BB bispecific antibody of claim 7, wherein each of the anti-PD-L1 antibody or antigen-binding fragment thereof and the anti-4-1BB antibody or antigen-binding fragment thereof is independently a chimeric antibody, a humanized antibody, or a fully human antibody.

[Claim 9]

The anti-PD-L1/anti-4-1BB bispecific antibody of claim 8, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof and the anti-4-1BB antibody or antigen-binding fragment thereof are humanized antibodies.

[Claim 10]

The anti-PD-L1/anti-4-1BB bispecific antibody of any one of claims 7-9, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 103, and 104, or a polypeptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 103, and 104.

[Claim 11]

The anti-PD-L1/anti-4-1BB bispecific antibody of any one of claims 7-10, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof comprises a

light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 105, and 106, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 105, and 106.

[Claim 12]

The anti-PD-L1/anti-4-1BB bispecific antibody of any one of claims 7-11, wherein the anti-4-1BB antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 21, 22, 23, and 24, or a polypeptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 21, 22, 23, and 24.

[Claim 13]

The anti-PD-L1/anti-4-1BB bispecific antibody of any one of claims 7-12, wherein the anti-4-1BB antibody or antigen-binding fragment thereof comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 25 and 26, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 25 and 26.

[Claim 14]

The anti-PD-L1/anti-4-1BB bispecific antibody of any of claims 7-13, wherein the anti-PD-L1/anti-4-1BB bispecific antibody activates 4-1BB signaling depending on PD-L1 expressed on cell surfaces.

[Claim 15]

The anti-PD-L1/anti-4-1BB bispecific antibody of any one of claims 1-14, which is in the form of IgG-scFv form.

[Claim 16]

A pharmaceutical composition for treating or preventing a disease associated with PD-L1, 4-1BB, or both thereof, the composition comprising the anti-PD-L1/anti-4-1BB bispecific antibody of any one of claims 1-15 and a pharmaceutically acceptable carrier.

[Claim 17]

The pharmaceutical composition of claim 16, wherein the disease associated with PD-L1, 4-1BB, or both thereof is cancer.

[Claim 18]

The pharmaceutical composition of claim 17, wherein the cancer is a solid tumor.