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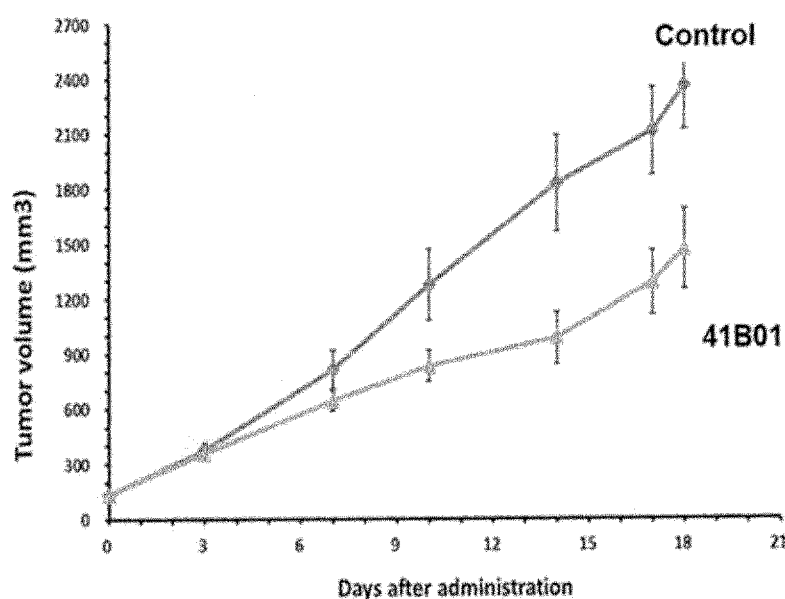
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(54) Title: ANTI-4-IBB ANTIBODY AND USE THEREOF

FIG. 6



(57) Abstract: Provided is an anti-4-IBB antibody or an antigen-binding fragment thereof, and uses thereof.

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[TITLE]**ANTI-4-1BB ANTIBODY AND USE THEREOF****[BACKGROUND OF THE INVENTION]****5 1. Field**

Provided is an anti-4-1BB antibody or an antigen-binding fragment thereof, and uses thereof.

2. Description of the Related Art

10 4-1BB 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. 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.
15 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.

20 [SUMMARY]

An embodiment provides an anti-4-1BB antibody or an antigen-binding fragment thereof 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
25 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.

Another embodiment provides an anti-4-1BB antibody or an antigen-binding fragment thereof comprising:

30 a CDR(complementarity determining region)-H1 comprising an amino acid sequence of SEQ ID NO: 1, 2, or 3;

a CDR-H2 comprising an amino acid sequence of SEQ ID NO: 4, 5, or 6;

a CDR-H3 comprising an amino acid sequence of SEQ ID NO: 7, 8, 9, 10, or 11;

a CDR-L1 comprising an amino acid sequence of SEQ ID NO: 12 or 13;

a CDR-L2 comprising an amino acid sequence of SEQ ID NO: 14 or 15;

5 and

a CDR-L3 comprising an amino acid sequence of SEQ ID NO: 16 or 17.

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

Heavy Chain CDRs		
CDR-H1	CDR-H2	CDR-H3
SYDMS (SEQ ID NO: 1)	WISYSGGSIYYADS	DGQRNSMREFDY (SEQ ID NO: 7)
GYDMS (SEQ ID NO: 2)	VIYPDDGNTYYADS	DAQRNSMREFDY (SEQ ID NO: 8)
SNVMN (SEQ ID NO: 3)	VKG (SEQ ID NO: 4)	DAQRQSMREFDY (SEQ ID NO: 9)
	VKG (SEQ ID NO: 5)	HGGQKPTTKSSSAYGMDG (SEQ ID NO: 10)
	EISHSGSTNYPNPSL	GAGNLGY (SEQ ID NO: 11)
	KS (SEQ ID NO: 6)	
Light Chain CDRs		
CDR-L1	CDR-L2	CDR-L3
SGSSSNIGNNYVT (SEQ ID NO: 12)	ADSHRPS (SEQ ID NO: 14)	ATWDYSLSGYV (SEQ ID NO: 16)
QASQDISNYLN (SEQ ID NO: 13)	GASSRAT (SEQ ID NO: 15)	QQYNSYPIT (SEQ ID NO: 17)

10 In an specific embodiment, the anti-4-1BB antibody or an antigen-binding fragment thereof may comprise;

a polypeptide comprising an amino acid sequence of SEQ ID NO: 40 as a H-FR1;

15 a polypeptide comprising an amino acid sequence of SEQ ID NO: 41 as a H-FR2;

a polypeptide comprising an amino acid sequence of SEQ ID NO: 42, 43, 44, or 45 as a H-FR3;

a polypeptide comprising an amino acid sequence of SEQ ID NO: 46 as a H-FR4;

20 a polypeptide comprising an amino acid sequence of SEQ ID NO: 47, 48,

or 49 as a L-FR1;

a polypeptide comprising an amino acid sequence of SEQ ID NO: 50 or 51 as a L-FR2;

a polypeptide comprising an amino acid sequence of SEQ ID NO: 52 or 53 as a L-FR3; and/or

a polypeptide comprising an amino acid sequence of SEQ ID NO: 54 or 55 as a L-FR4.

Another embodiment provides an anti-4-1BB antibody or an antigen-binding fragment thereof comprising a heavy chain variable region comprising a CDR-H1 comprising an amino acid sequence of SEQ ID NO: 1, 2, or 3, a CDR-H2 comprising an amino acid sequence of SEQ ID NO: 4, 5, or 6, and a CDR-H3 comprising an amino acid sequence of SEQ ID NO: 7, 8, 9, 10, or 11; and a light chain variable region comprising a CDR-L1 comprising an amino acid sequence of SEQ ID NO: 12 or 13, a CDR-L2 comprising an amino acid sequence of SEQ ID NO: 14 or 15, and a CDR-L3 comprising an amino acid sequence of SEQ ID NO: 16 or 17.

Another embodiment provides an anti-4-1BB antibody or an antigen-binding fragment thereof comprising a heavy chain variable region comprising or consisting essentially of SEQ ID NO: 18, 19, 20, 21, 22, 23, 39, 57, 58, 59, or 60; and a light chain variable region comprising or consisting essentially of an amino acid sequence of SEQ ID NO: 24, 25, 26, 61, or 62.

Another embodiment provides an anti-4-1BB antibody or an antigen-binding fragment thereof comprising a heavy chain comprising or consisting essentially of an amino acid sequence of SEQ ID NO: 27, 28, 29, 30, 31, or 32; and a light chain comprising or consisting essentially of an amino acid sequence of SEQ ID NO: 33, 34, or 35.

In an embodiment, the anti-4-1BB antibody or antigen-binding fragment described herein may further comprise at least one polypeptide targeting a bioactive material other than 4-1BB.

Another embodiment provides a pharmaceutical composition comprising the anti-4-1BB antibody or an antigen-binding fragment thereof. The pharmaceutical composition may further comprise a pharmaceutically

acceptable carrier. The pharmaceutical composition may be used for enhancing immune response, and/or for treating and/or preventing a cancer. The enhancing immune response may be 4-1BB signal activation, T-cell activation, or both of them.

5 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 anti-4-1BB antibody or an antigen-binding fragment thereof or the pharmaceutical composition. The method may further step of identifying the subject in need of treating and/or preventing a
10 cancer, prior to the administering step.

 Another embodiment provides a method of enhancing immune response in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of the anti-4-1BB antibody or an antigen-binding fragment thereof or the pharmaceutical composition. The method may
15 further step of identifying the subject in need of enhancing immune response, prior to the administering step. The enhancing immune response may be 4-1BB signal activation, T-cell activation, or both of them.

 Another embodiment provides a use of the anti-4-1BB antibody or an antigen-binding fragment thereof or the pharmaceutical composition in treating
20 and/or preventing a cancer. Another embodiment provides a use of the anti-4-1BB antibody or an antigen-binding fragment thereof in preparing a pharmaceutical composition for treating and/or preventing a cancer.

 Another embodiment provides a use of the anti-4-1BB antibody or an antigen-binding fragment thereof or the pharmaceutical composition enhancing
25 immune response. Another embodiment provides a use of the anti-4-1BB antibody or an antigen-binding fragment thereof in preparing a pharmaceutical composition for enhancing immune response. The enhancing immune response may be 4-1BB signal activation, T-cell activation, or both of them.

 An embodiment provides a polynucleotide encoding the anti-4-1BB
30 antibody or an antigen-binding fragment thereof. In particular, an embodiment provides a first polynucleotide encoding at least one of CDR-H1, CDR-H2, and CDR-H3, a heavy chain variable region, or a heavy chain of the anti-4-1BB

antibody or an antigen-binding fragment thereof, as described above. Another embodiment provides a second polynucleotide encoding at least one of CDR-L1, CDR-L2, and CDR-L3, a light chain variable region, or a light chain of the anti-4-1BB antibody or an antigen-binding fragment thereof, as described above.

5 An embodiment provides a recombinant vector comprising the first polynucleotide, the second polynucleotide, or a combination thereof. Another embodiment provides a recombinant cell transfected with the recombinant vector.

10 Another embodiment provides a method of preparing the anti-4-1BB antibody or an antigen-binding fragment thereof, comprising expressing the first polynucleotide and the second polynucleotide 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
15 isolating and/or purifying the anti-4-1BB antibody or an antigen-binding fragment thereof from the cell culture, after the step of expressing or culturing.

 The terms "antigen-binding fragment", as used herein, is a portion of an antibody with a capability of recognizing and/or binding to an antigen, which is selected from the group consisting of F(ab')₂, F(ab)₂, Fab', Fab, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VK or VH domain, fragments produced by a Fab expression library, and anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to
20 LIGHT antibodies disclosed herein). Immunoglobulin (e.g., a human immunoglobulin) or antibody molecules of the disclosure can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, IgY, and the like), class (e.g., IgG1, IgG2, IgG3, IgG4, IgG5, IgA1, IgA2, and the like), or subclass of immunoglobulin molecule.

 The term "antibody" may encompass 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
30 epsilon (γ , μ , α , δ , ϵ) with some subclasses among them (e.g., γ 1- γ 4), and light chains are classified as either kappa or lambda (K, λ). 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.

Antibodies and antigen-binding fragments of the disclosure may include, but not be limited to, polyclonal or monoclonal; monospecific or multispecific; and/or human, humanized, animal (e.g., mouse, rabbit, etc.), or chimeric antibodies.

The term "subject" may refer to any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects may 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.

In one aspect, antibody or fragment thereof is chemically or recombinantly synthesized (not naturally occurring).

Given that each of antibodies can bind to 4-1BB such as human 4-1BB, the CDR sequences, or V_L (heavy chain variable region) and V_L (light chain variable region) sequences as disclosed herein can be "mixed and matched" to create other anti-4-1BB binding molecules.

[BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS]

FIGS. 1A-1C show the results of analysis (ELISA) of the binding capacity to the 4-1BB antigen of the anti-4-1BB antibody prepared according to one embodiment of the present invention. It shows that each anti-4-1BB monoclonal antibody specifically binds to the extracellular domain 4-1BB antigen.

FIG. 2 is the result of measurement (FACS) of the binding capacity to the 4-1BB antigen of the anti-4-1BB antibody prepared according to one embodiment of the present invention. It shows that each anti-4-1BB monoclonal antibody specifically binds to the 4-1BB expressed on the cell surface.

FIG. 3 is the graph that shows the 4-1BB signal activation by the anti-4-1BB antibody prepared according to one embodiment of the present invention.

FIG. 4 is the graph that shows the T-cell activation by the anti-4-1BB antibody prepared according to one embodiment of the present invention.

FIG. 5 is the graph that shows the 4-1BB signal activation by the anti-4-1BB antibody prepared according to one embodiment of the present invention,
5 in the form of bispecific antibody.

FIG. 6 is the graph that shows a cancer-inhibiting efficacy in a mouse tumor syngeneic model, of the anti-4-1BB antibody prepared according to one embodiment of the present invention. It was shown that each anti-4-1BB antibody inhibited the growth of cancer in human 4-1BB knock-in mouse. This
10 result means that the 4-1BB antibody of the present invention binds to immune cell expressing human 4-1BB, thereby inhibiting the growth of cancer, and being usefully used as a cancer therapeutic agent.

FIG. 7 is a schematic view of an anti-PD-L1/anti-4-1BB bispecific antibody.

FIG. 8A is a schematic view showing binding sites of an anti-4-1BB antibody (41B01) on 4-1BB.
15

FIG. 8B is a schematic view showing binding sites of an anti-4-1BB antibody (41B02) on 4-1BB.

FIGS. 9A-9E show cross-competition between Urelumab and
20 candidates (41B01 and 41B02).

FIGS. 10A-10E show cross-competition between Utomilumab and candidates (41B01 and 41B02).

FIGS. 11A-11D show cross-competition between 41B01 and 41B02.

FIGS. 12A-12C show cross-competition between recombinant human
25 41BB-ligand and 41B01.

[DETAILED DESCRIPTION]

Definitions

Herein, "polynucleotide" or "nucleic acid" includes a single or double
30 strand nucleotide polymer. The nucleotide comprising such a polynucleotide may be a ribonucleotide or deoxyribonucleotide or their modified forms.

Unless otherwise stated herein, the left end of the polynucleotide stated

herein is 5' end and its right end represents 3' end.

Herein, "isolated nucleic acid molecule" means DNA or RNA of genomic origin or mRNA, cDNA of synthetic origin or their combinations, which is linked to the polynucleotide that all or a portion of it is not associated with a polynucleotide present in nature, or it is not observed in nature. On the purpose of the present invention, the nucleic acid molecule comprising a specific nucleic acid sequence does not comprise an intact chromosome. Instead, the isolated nucleic acid molecule comprising a specific nucleic acid sequence may comprise at least several additional protein coding sequences, in addition to its specific sequence, or may further comprise a regulatory sequence and/or vector for expression of the specific nucleic acid sequence.

Herein, the term "regulatory sequence" means a polynucleotide sequence which can affect the expression and processing of a coding sequence by being operably connected thereto. This property of the regulatory sequence may be influenced by kinds of hosts. For example, the regulatory sequence applicable in a prokaryotic cell may include a promoter, occasionally an operator, a ribosome-binding site and a transcription termination sequence. In a eukaryotic cell, the regulatory sequence may comprise a promoter comprising multiple recognition sites, a transcription enhancer, a polyadenylation sequence and a transcription termination sequence. The regulatory sequence may further comprise a reader sequence and/or a fusion partner sequence.

Herein, "vector" means any molecule used for delivering a nucleic acid molecule encoding a protein to a host cell, comprising for example, a nucleic acid, a plasmid, a bacteriophage or a virus.

Herein, "expression vector or recombinant vector" means a vector which is suitable for transformation of a host cell and comprises a nucleic acid sequence that is operably connected to an expression vector and regulates the expression of heterologous sequences encoding a targeting protein. This expression vector may be also operably connected to the coding sequence, and in case of transcription, translation and that an intron is present, it may comprise a sequence regulating RNA splicing or affecting it.

Herein, "operably (or operatively) connected (or linked)" means that

nucleic acid sequences to be connected are positioned so as to perform a targeting function under an appropriate condition. For example, if the transcription of the coding sequence is affected by the regulatory sequence under an appropriate condition in a vector comprising a coding sequence and a regulatory sequence, it is operably connected.

Herein, "host cell" means a cell which can express a target gene that is transformed or to be transformed by a targeting nucleic acid sequence. The term includes progeny of the host cell, as long as expressing the targeting gene, regardless of identity of host cell and form and genetic makeup.

Herein, "transduction" commonly means movement of a nucleic acid from one bacterium to another bacterium by a bacteriophage. For example, it includes movement of a nucleic acid to a eukaryotic cell using a retrovirus which cannot replicate.

Herein, "transfection" means that a cell takes a foreign or exogenous DNA, and in this case, DNA is introduced in a cell through a cell membrane. This may refer methods known in the art, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012), Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates.

Herein, "transformation" means a change of genetic properties of a cell, which are modified so that a cell comprises a new DNA or RNA. For example, a cell may be transformed as its genetic properties are changed, by introducing a new genetic material through transduction, transfection, or other techniques. The DNA transformed by methods including transduction or transfection, etc. may be present by being physically integrated in a chromosome of a cell, or may be temporarily present as an episome form without replication or a replicable plasmid. When the transformed DNA is replicated with division of a host cell, it is considered as stably transformed.

Herein, "amino acid" includes the common meaning understood in the art. Twenty natural-occurring amino acids and their abbreviations are as those commonly used in the art (*Immunology-A Synthesis*, 2nd Edition, E. S. Golub and D. R. Green, eds., Sinauer Associates: Sunderland, Mass. 1991). The

amino acid includes typical amino acids, stereoisomers of typical 20 amino acids (D-amino acids), non-natural amino acids, for example, α,α -disubstituted amino acids, N-alkyl amino acids, and other non-typical amino acids. As examples of non-typical amino acids, 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ -N-methylarginine and other similar amino acids and imino acids (for example, 4-hydroxyproline). In the mark of polypeptide used herein, as commonly used in the art, the left of a sequence is an amino terminal and the right represents a carboxy terminal.

Herein, "polypeptide" or "protein" means a polymer of an amino acid residue, and it is used interchangeably herein. This also includes not only polymers of naturally occurring amino acid residues but also polymers of their analogues or mimetics. In addition, the polypeptide or protein may comprise modification such as addition of carbohydrates for phosphorylation or glycosylation, etc. Moreover, the polypeptide or protein may be produced in a recombinant or naturally found cell. Furthermore, the polypeptide or protein may include those in which a portion of a wild type sequence or the amino acid sequence is deleted, added and/or substituted. In addition, the polypeptide or protein includes an antibody, for example, an anti-4-1BB antibody (or named as 4-1BB antibody), 4-1BB binding protein, or an antigen-binding fragment, or a sequence in which one or more amino acids in the sequence are deleted, added and/or substituted. Moreover, "polypeptide fragment" means a polypeptide having an amino terminal deletion, a carboxyl terminal deletion and/or an internal deletion, compared to a full-length protein. This fragment may also include modified amino acids compared to a full-length protein. In one embodiment, the fragment may be about 5 to 500 amino acids in length, for example, at least 5, 6, 8, 10, 14, 20, 50, 70, 100, 110, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850 or more amino acids in length. Considering the purpose of the present invention, the useful polypeptide fragment includes an immunological functional fragment of an antibody comprising an antigen-binding domain. In case of 4-1BB binding antibody, such a useful fragment includes a CDR sequence comprising 1, 2, or 3 of heavy

chains or light chains, or all or a portion of antibody chain comprising a variable region or constant region of a heavy chain or light chain, but not limited thereto.

Herein, "isolated polypeptide, antibody or protein" is that there is not any other protein to be found together with them commonly and at least about 50% or more of lipids, carbohydrates and polynucleotides naturally connected to them are removed. Typically, the isolated protein, polypeptide or antibody comprises at least about 5%, at least about 10%, at least about 25% or at least about 50%, in a certain composition. This polypeptide may be encoded by genome DNA, cDNA, mRNA or other RNA of synthetic origins or any combinations thereof. In particular, the isolated protein, polypeptide or antibody is substantially free of contaminants of other proteins or other polypeptides, which interfere with its therapeutic, diagnostic and prophylactic researches or application for other uses.

The antibody and/or its antigen-binding fragment described herein may include "variants" thereof, wherein the variant may refer a polypeptide in which one or more amino acid residues are inserted, deleted, added and/or substituted from the polypeptide sequence, so long as maintain the desired biological activity and/or structure of the antibody and/or its antigen-binding fragment, and may include a fusion polypeptide by linking to other polypeptide. In addition, the variant may include one modified by protein enzyme cutting, phosphorylation, and/or other posttranslational modification, but maintaining biological activity of the antibody and/or its antigen-binding fragment disclosed herein, for example, binding to 4-1BB and specificity. The variant may be have 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%, for example, a sequence identity of about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, or 80%, to the sequence of the antibody or its antigen-binding fragment disclosed herein. The percent identity (%) or homology may be calculated with reference to the following description.

In one embodiment, the percent homology or identity may be calculated as $100 \times [(\text{identical position}) / \min(\text{TGA}, \text{TGB})]$, and in the formula, TGA, TGB are the sum of the number of residues of sequences A and B compared and the

internal gap position (Russell et al., J. Mol Biol., 244: 332-350 (1994)).

Herein, the conservative amino acid substitution means the substitution which does not substantially affect the activity or antigenicity of a polypeptide. The polypeptide may comprise one or more conservative substitutions. Non-limiting examples are disclosed in the following Table 3.

The "derivative" of the polypeptide herein means a polypeptide chemically modified in one or more residues through conjugation with other chemical moiety, which is different from an insertion, deletion, addition or substitution variant.

The 4-1BB (Receptor Tyrosine Kinase-Like Orphan Receptor), that is recognized by the antibody or antigen-binding fragment thereof described herein, may refer to a transmembrane protein of an RTK (Receptor Tyrosine Kinase) family. In one embodiment, it particularly recognizes an extracellular domain. The 4-1BB which the antibody recognizes may be an extracellular domain which is present in a cell membrane or is not present in a cell membrane. The human protein of 4-1BB consists of 937 amino acids, and the amino acid sequence is NCBI Reference Sequence ID: NP_005003.2, and the nucleic acid sequence is NM_005012.3. Unless apparent from the context used herein, the 4-1BB refers to a human h4-1BB, but the antibody has the binding capacity to mouse 4-1BB specifically. The mouse 4-1BB amino acid sequence is represented by GenBank: BAA75480.1.

Herein "identity" means the sequence similarity of two or more polypeptides or two or more polynucleotides, which are determined by arranging and comparing two or more polypeptides or two or more polynucleotides. This identity between sequences is commonly represented by "identity percent", and this means the ratio of identical amino acids or nucleotides between molecules to be compared, and it is calculated on the basis of the smallest size of molecule, among molecules to be compared.

When the identity percent is calculated, sequences to be compared are arranged in the way of providing the maximum matching between sequences, and in the arranged sequences, gap, matching and mis-match may be present, and this is treated by a specific mathematical model or a computer algorithm. In

one embodiment, this identity percent may be determined using a GCG program package including a GAP program which arranges two sequences in the way of maximizing the match between sequences to be compared and minimizing the number of gaps, using Needleman and Wunsch algorithm
5 (Devereux et al., 1984, Nucl. Acid Res. 12:387; Genetics Computer Group, University of Wisconsin, Madison, WI, USA). The computer algorithm GAP determines "matching span" by arranging two sequences in the way of maximizing the match between them and minimizing the number of gaps in two polypeptide or polynucleotide sequences to be compared. The algorithm also
10 uses a gap opening penalty [this is calculated as 3 x average diagonal, wherein "average diagonal" is the average of diagonals of comparison matrix to be used; and "diagonal" is a score or number assigned for each complete amino acid match by a specific comparison matrix] and a gap extension penalty (this is commonly 1/10 fold of the gap opening penalty), and a comparison matrix, for
15 example, PAM 250 or BLOSUM 62 together. In a specific embodiment, a standard comparison matrix (refer to Dayhoff et al., 1978, Atlas of Protein Sequence and Structure 5:345-352 for PAM 250 comparison matrix; refer to Henikoff et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:10915-10919 for BLOSUM 62 comparison matrix) is used. In one embodiment, parameters recommended
20 for determining the identity percent of polypeptides or polynucleotides in which the GAP program is used are as follows: algorithm: Needleman et al., 1970, J. Mol. Biol. 48:443-453; comparison matrix: BLOSUM 62 (Henikoff et al., 1992, supra); gap penalty: 12 (no penalty for a terminal gap); gap length penalty: 4; similarity threshold: 0.

25 When two sequences are arranged using specific parameters, although there is no significant relation between two sequences, the result that they are matched with high identity in a short sequence region may be derived. In this case, in order that two sequences are arranged through at least 50 sequential amino acids, parameters of the algorithm used as the GAP program can be
30 corrected.

"Substantially pure" used herein is that a targeting molecule is present as a predominant species. In other words, it means that on the basis of mole,

the concentration is higher than any other individual species in the same mixture. In one embodiment, a substantially pure molecule is comprised as at least about 50% (based on mole), at least about 80%, about 85%, at least about 90%, or at least about 99%, among all polymers comprised in a composition. In
5 other embodiment, the targeting molecule is substantially homogeneously purified until any more contaminants are not detected by using a conventional method, and therefore the composition comprises one kind of homogeneous polymer material.

In one aspect, the present invention relates to a recombinant antibody
10 specifically binding to 4-1BB protein or its antigen-binding fragment. In this aspect, "recombinant protein" is a protein prepared using a recombination technique, namely, through the expression of the recombinant nucleic acid described in the present invention. The methods and techniques for production of a recombinant protein are widely known in the art.

15 Herein, "affinity" is the strength of interaction between an antibody or its antigen-binding fragment and an antigen, and it is determined by properties of the antigen such as size, shape and/or charge of antigen, and CDR sequences of the antibody or antigen-binding fragment. The methods for determining the affinity are known in the art, and the followings can be referred.

20 The antibody or its antigen-binding fragment is called "specifically binding" to its target such as an antigen, when a dissociation constant (KD) is 10^{-6} M or less. The antibody specifically binds to a target with "high affinity", when KD is 1×10^{-8} M or less.

Herein, "antigen-binding region or site" means a protein or a part of
25 protein specifically binding to a specific antigen. For example, a part of an antibody comprising an amino acid residue providing the antibody with specificity and affinity to an antigen, by interacting with the antigen. This antigen-binding region typically comprises one or more "complementary determining regions (CDR)". A specific antigen-binding region also comprises
30 one or more "framework (FR)" regions. The framework region helps to maintain an appropriate conformation of these CDRs, thereby facilitating binding between the antigen-binding region and an antigen.

Herein, "antibody" means an antigen-binding fragment which can compete to an intact antibody for binding to any isotype of intact immunoglobulin, or a target antigen. For example, it comprises chimeric, humanized, complete human and dual-specific antibodies or their antigen-binding fragments. The antibody is one kind of antigen binding proteins by itself. The intact antibody commonly comprises at least 2 full-length heavy chains and 2 full-length light chains, but in some cases as naturally found in Camelid animals, the antibody may comprise only heavy chains. The antibody or its antigen-binding fragment may be derived from only one source or chimeric. The chimeric antibody comprises a part derived from two kinds of different antibodies, and is described in more detail below. The antibody or its antigen-binding fragment can be produced by hybridoma, recombinant DNA technique or enzymatic or chemical cutting of an intact antibody. Unless otherwise stated, herein, the term, antibody includes an antibody comprising 2 full-length heavy chains and 2 full-length light chains, and its derivatives, variants, fragments, and mutants, and their examples are as described below.

Herein, "light chain" includes a full-length light chain having a variable region sequence enough to provide binding specificity to an antigen or epitope and its fragment. The full-length light chain comprises a variable region domain VL and a constant region domain CL. The variable region domain of light chain is present in an amino terminal of a light chain polypeptide. The kinds of light chains include kappa and lambda chains.

Herein, "heavy chain" includes a full-length heavy chain having a variable region sequence enough to provide binding specificity to an antigen or epitope and its fragment. The full-length heavy chain comprises a variable region domain VH and 3 constant region domains CH1, CH2 and CH3. The VH domain is present in an amino terminal of a heavy chain polypeptide and the CH domain is present in a carboxy terminal, and the CH3 is positioned closest to a carboxy terminal. The heavy chain comprises IgG (comprising IgG1, IgG2, IgG3 and IgG4 subtypes), IgA (comprising IgA1 and IgA2 subtypes), and isotypes of IgM and IgE.

Used herein, "antigen-binding fragment" of a chain (heavy chain or light

chain) of an antibody or immunoglobulin includes a part of an antibody which lacks some amino acids compared to a full-length chain, but can specifically bind to an antigen. This fragment can be considered as having biological activity, in an aspect that it can specifically bind to a target antigen, or can compete to other antibodies or an antigen-binding fragment to bind a specific epitope. In one aspect, this fragment comprises at least one CDR present in a full-length light chain or heavy chain, and in some embodiments, it comprises a short-chain heavy chain and/or light chain, or its part. This biological active fragment may be produced by a recombinant DNA technique or may be produced for example, by cutting an intact antibody enzymatically or chemically. An immunologically functional immunoglobulin fragment includes Fab, Fab', F(ab')₂, scFab, dsFv, Fv, scFV, scFV-Fc, diabody, minibody, scAb, and dAb, but not limited thereto, and may be derived from any mammal including human, mouse, rat, camelid or rabbit, but not limited thereto. The functional part of the antibody such as one or more CDRs described herein may be linked with a secondary protein or small molecular compound by a covalent bond, thereby being used as a target therapeutic agent to a specific target.

Herein, "Fc" region comprises two heavy chain fragments comprising CH2 and CH3 domains of an antibody. These 2 heavy chain fragments are combined each other by hydrophobic interaction of two or more of disulfide bonds and CH3 domain.

Herein, "Fab fragment" consists of 1 light chain and 1 heavy chain comprising a variable region and CH1 only. The heavy chain of Fab molecule cannot form a disulfide bond with other heavy chain molecule. scFab is one that two molecules of Fab is linked by a flexible linker.

Herein, "Fab' fragment" comprises a region between CH1 and CH2 domains of a heavy chain, in addition to Fab fragment, and it can form a disulfide bond between two heavy chains of two molecules of Fab' fragment, to form a F(ab')₂ molecule.

Herein, "F(ab')₂ fragment" comprises two light chains, and two heavy chains comprising a variable region, CH1 and a part of a constant region between CH1 and CH2 domains, as aforementioned, and thereby an intrachain

disulfide bond between 2 heavy chains is formed. Thus, the F(ab')₂ fragment consists of two Fab' fragments, and the two Fab' fragments are meeting each other by the disulfide bond between them.

Herein, "Fv region" is a fragment of an antibody which comprises each
5 variable region of a heavy chain and a light chain, but does not comprise a constant region. sdFV is one that a heavy chain and a light chain are linked by a disulfide bond. scFc is one that single stranded variable regions (Fv) of heavy chain and light chain are linked via a flexible linker. scFv-Fc is one that Fc is linked to scFV. The minibody is one that CH₃ is linked to scFV. The diabody
10 comprises two molecules of scFV.

Herein, "short-chain antibody (scAb)" is a single polypeptide chain comprising one variable region of a heavy chain or a light chain constant region in which a heavy chain and light chain variable region is linked by a flexible linker. The short-chain antibody may refer to for example, U.S. patent No.
15 5,260,203, and this is disclosed herein by reference.

Herein, "domain antibody (dAb)" is an immunologically functional immunoglobulin fragment comprising a variable region of heavy chain or a variable region of light chain only. In one embodiment, two or more of VH regions are linked by a covalent bond by a peptide linker, to form a bivalent
20 domain antibody. Two VH regions of this bivalent domain antibody may target the same or different antigen.

Herein, "bivalent antigen-binding protein" or "bivalent antibody" comprises 2 antigen-binding sites. Two antigen-binding sites comprised in this bivalent antibody may have the same antigen specificity or may be a dual-
25 specific antibody binding to different antigens separately.

Herein, "multi-specific antigen-binding protein" or "multi-specific antibody" is targeting two or more of antigens or epitopes.

Herein, "bispecific", "dual-specific" antigen-binding protein or antibody is a hybrid antigen-binding protein or antibody having 2 different antigen-binding
30 sites. This bispecific antibody is one kind of multi-specific antigen-binding protein or multi-specific antibody, and it can be produced by known various methods, for example, methods such as fusion of hybridoma or linking of Fab'

fragment. For example, Songsivilai and Lachmann, 1990, Clin. Exp. Immunol. 79:315-321; Kostelny et al., 1992, J. Immunol. 148:1547-1553, etc. may be referred. The 2 epitopes different each other to which 2 antigen-binding sites of the bispecific antigen-binding protein or antibody bind may be positioned on the
5 same or different protein target.

Herein, the term “antigen” or “immunogen” means a molecule or a part of molecule which for example, an antigen-binding protein (for example, antibody or its immunologically functional antigen-binding fragment) can bind to, and can be used for production of an antibody which can bind to an antigen in
10 an animal. The antigen may comprise one or more of epitopes which can interact with a different antibody or its fragment. In one embodiment, the antigen is an extracellular domain of 4-1BB protein.

Herein, “epitope” is a part of molecule which is bound by an antigen-binding protein or antibody or is recognized by them, and comprise any
15 determining factor which can specifically bind to an antigen-binding protein, such as for example, an antibody or a T-cell receptor. The epitope may be sequential or unsequential, and for example, in a polypeptide sequence, it is not sequential each other, but in an aspect of molecule, like a conformational epitope, it may be an amino acid residue that is bound by one antigen-binding
20 protein, but is positioned away each other. In one embodiment, the epitope comprises a three-dimensional structure similar to an epitope used for antibody production, but it may be a mimetic in an aspect that it can comprise no residue found in the epitope or can comprise some residues only. Commonly, the epitope is a protein, but it may be other kinds of materials such as a nucleic acid.
25 The epitope determining factor may be a chemically active group formed on a surface by a molecule such as an amino acid, a sugar side chain, a phosphoryl group or a sulfonyl group, or may have specific three-dimensional structural properties and/or specific charge properties. Commonly, an antibody which is specific to a specific target antigen recognizes an epitope of a target antigen
30 which is present in a complex of a protein and/or a polymer.

Herein, “therapeutic agent” means a molecule to be administered to a subject for a targeting therapeutic effect. The subject includes a non-human

mammal, for example, primates, or a human. The example of the therapeutic agent includes a protein comprising a peptide and a polypeptide, a nucleic acid, an antibody or a small molecular compound. In other aspect, the therapeutic agent can be used as a therapeutic agent of related diseases such as cancer,
5 by being bound to the antibody, or the antibody.

Herein, the term "treating" means alleviation or treatment of an injury, disease, or symptom of disease or morbid condition, including any objective or subjective parameters, including reduction, relief, alleviation of an injury, disease, or symptom of disease or condition, or making a patient better able to
10 withstand an injury, disease, or symptom of disease or morbid condition, slowing the deteriorating rate of an injury, disease, or symptom of disease or morbid condition, or improving the quality of life of a patient mentally or physically. This treatment or improvement of an injury, disease, or symptom of disease or morbid condition may be judged on the basis of results of physical
15 examination, examination of various indexes related to a disease and imaging examination.

Herein, "effective amount (dose)" commonly means an amount enough to reduce seriousness and/or occurrence frequency of symptoms due to a disease, particularly, a disease related to 4-1BB, remove symptoms due to a
20 disease, particularly, a disease related to 4-1BB and/or a root cause of disease occurrence, or prevent occurrence of symptoms due to a disease, particularly, a disease related to 4-1BB and/or a root cause, and/or improve or correct damages due to a disease, particularly, a disease related to 4-1BB. In some embodiments, the effective dose is a therapeutic effective dose or a
25 prophylactic effective dose. The "therapeutic or pharmaceutically effective dose" is an amount enough to treat a disease, particularly symptoms or conditions related to 4-1BB, or prevent, delay a disease, particularly symptoms or conditions related to 4-1BB, or reverse its progress. The "prophylactic effective dose" is an amount for prevent or delay occurrence or reoccurrence of a
30 disease, particularly, a disease related to 4-1BB, or symptoms related to a disease, particularly, a disease related to 4-1BB, and reduce its probability. The complete therapeutic or prophylactic effect can be caused by several times of

administration of dose, rather than by a single administration of dose. Therefore, the therapeutic or prophylactic effective dose may be delivered by once or more of administration.

5 **4-1BB**

4-1BB, which is also called as CD137, or TNFRSF9 (TNF Receptor 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. 4-1BB plays important
10 role in modulate the activity of various 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_001552.2) may be represented by the amino acid sequence (SEQ ID NO: 67), as follows:

```
15  1      mgnscyniva tillvInfer trslqdpesn cpagtfcdnn mqi cspcpp nsfssaggqr
    61      tcdicrqkg vfrtrkecss tsnaecdctp gfhclgagcs mceqdckqgg eltkkgckdc
    121     cfgtfndqkr gicrpwtncs ldgksvlvng tkerdvvcgp spadlspgas svtpapare
    181     pghspqiisf flaltstall flffltlrf svvkrgrkkl lyifkqpfmr pvqttqeedg
    241     cscrfpeeee ggcel
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20

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
25 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
30 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
5 biological function as the human 4-1BB of NCBI Accession No. NP_001552.

Anti-4-1BB antibodies

As demonstrated in the experimental examples, the anti-4-1BB antibodies disclosure herein show 4-1BB binding abilities, binding abilities to 4-
10 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, for instance, 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
15 increases 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.

The present invention discloses an antibody specifically binding to an
20 extracellular domain of 4-1BB protein, or its antigen-binding fragment. The antibody is a polypeptide comprising one or more of complementary determining regions or sites (CDR), as disclosed herein.

In some embodiments, a CDR is comprised in a "framework" region, and the framework orients a CDR(s) so that this CDR(s) can have appropriate
25 antigen-binding properties.

The antibody specifically binds to a human and mouse-derived 4-1BB extracellular domain, and it can specifically bind to an isolated form of extracellular domain or an extracellular domain of 4-1BB expressed on a cell surface.

30 The antibody disclosed herein binds to 4-1BB, in particular, human 4-1BB and mouse 4-1BB. The antibody disclosed herein can specifically bind to an 4-1BB extracellular domain or 4-1BB expressed on a cell surface, derived

from a human or mouse, and thereby it can be usefully used for target treatment of cancer targeting 4-1BB. For example, it can be used for treatment of a specific cancer, by combining the antibody and an anti-cancer agent. In addition, an on-target tox can be confirmed through a mouse experiment, since it binds to mouse 4-1BB, and in vivo efficacy can be confirmed through a syngeneic model using a mouse cancer cell line overexpressing mouse 4-1BB, and thereby it can be usefully used for development of various drugs related to 4-1BB.

In one embodiment, the antibody includes a monoclonal antibody, dual-specific antibody, double antibody, multi-specific antibody, multiple antibody, minibody, domain antibody, antibody mimetic (or synthetic antibody), chimeric antibody or antibody fusion (or antibody conjugate) and fragment thereof, but not limited thereto, and includes various forms of antibodies disclosed herein.

In one embodiment, the antibody fragment of the antibody disclosed herein may be Fab, Fab', F(ab')₂, scFab, Fv, dsFv, scFV, scFV-Fc, minibody, diabody, scAb, or dAb.

In other embodiment, the antibody disclosed herein may consist of a polypeptide of only light chains or only heavy chains comprising variable regions disclosed in Table 2a and Table 2b.

One antibody disclosed herein shares a specific region or sequence with another antibody disclosed herein. In one embodiment, it may share a constant region of the antibody or antigen-binding fragment. In another embodiment, it may share an Fc region. In another embodiment, it may share a frame of variable region.

In one embodiment, the antibody has a typical structure of an antibody found in nature. Camelid animals produces an antibody consisting of a single heavy chain, but the structural unit of this antibody commonly comprises a tetrameric polypeptide, and the tetramer comprises two of one pair of polypeptide chain bodies consisting of different 2 polypeptide chains. In a typical antibody, the one pair of polypeptide chain body comprises one full-length light chain (about 25kDa) and one full-length heavy chain (about 50 to 70kDa). Each chain shows a characteristic folding pattern, and consists of several immunoglobulin domains, consisting of about 90 to 110 amino acids.

These domains are basic units consisting of an antibody polypeptide. The amino-terminal part of each chain typically comprises a part called a variable region or V region that is a part recognizing an antigen. The carboxy-terminal part is conserved evolutionarily more than the amino-terminal, and it comprises

5 a part called a constant region or C region. The human light chain is commonly classified as kappa (κ) and lambda (λ) light chains, and these comprise one variable region and one constant region, respectively. The heavy chain is typically classified as mu (μ), delta (δ), gamma (γ), alpha (α) or epsilon (ϵ) chain, and these are defined as IgM, IgD, IgG, IgA and IgE isotypes, respectively. IgG

10 includes IgG1, IgG2, IgG3 and IgG4, but has unlimited numerous subtypes. IgM subtype includes IgM and IgM2. IgA subtype includes IgA1 and IgA2. In human, IgA and IgD isotypes comprise 4 heavy chains and 4 light chains; IgG and IgE isotypes comprise 2 heavy chains and 2 light chains, and IgM isotype comprises 5 heavy chains and 5 light chains. The heavy chain constant region

15 typically shows an effector function, but comprises one or more domains. The number of heavy chain constant region domains becomes different depending of isotypes. IgG heavy chain, for example, comprises 3 C region domains known as CH1, CH2 and CH3, respectively. The antibody disclosed herein may be any one of these isotypes and subtypes. In one embodiment, the antibody is

20 an IgG1, IgG2a, IgG2b, IgG3 or IgG4 subtype. In a further embodiment, the antibody of the present invention is an IgG1- or IgG2-type. In a further embodiment, the antibody of the present invention is an IgG1-type.

The heavy chain variable region and light chain variable region may be linked to at least a part of a human constant region. The selection of a constant

25 region may be determined by whether the antibody-dependent cell-mediated cytotoxicity, antibody-dependent cell phagocytosis, and/or complement-dependent cytotoxicity is required partially. For example, human isotype IgG1 and IgG3 have complement-dependent cytotoxicity and human isotype IgG2 and IgG4 do not have this cytotoxicity. In addition, human IgG1 and IgG3 induce

30 a cell-mediated effector function stronger than human IgG2 and IgG4. The light chain constant region may be lambda or kappa.

In one embodiment, the antibody may be a humanized or human antibody, and the heavy chain constant region may be an IgG1-, IgG2- IgG3- or IgG4-type. In a further embodiment, the antibody of the present invention is an IgG1- or IgG2-type.

5 In other embodiment, the antibody is a humanized or human antibody, and recognizes mouse 4-1BB specifically.

In full-length light chain and heavy chain, a variable region and a constant region are linked by "J" region that is about 12 or more of amino acids in length, and the heavy chain also comprises "D" region of about 10 or more of
10 amino acids. Typically, a variable region of light chain/heavy chain pair of an antibody forms an antigen-binding site.

A variable region of an immunoglobulin chain has the same overall structure commonly, and comprises a comparatively conserved framework region (FR) connected by 3 hypervariable regions called "complementary
15 determining site or region or domain" or CDR (Complementary Determining Region). The CDR of a variable region derived from each chain consisting of heavy chain/light chain pair is arranged by a framework region typically, thereby forming a structure specifically binding to a specific epitope of a target protein (4-1BB). These factors of naturally occurring light chain and heavy chain
20 regions are typically comprised from the N-terminal to the C-terminal in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The position of amino acid sequences corresponding to each of them in the variable region may be determined by Kabat (Kabat et al., (1983) U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest"), Chothia
25 (Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)) or a method related to OPAL library (Hye Young Yang et. al., 2009 Mol. Cells 27: 225). The CDRs determined by each definition may be a subset which is overlapped or that one comprises the other, when comparing each other. However, herein, all CDRs to be defined by each method are included in the scope of the present invention.
30 Those skilled in the art will easily select a CDR sequence by each definition among them, when the sequence of variable region of an antibody is provided.

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 CDR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, and 3; (ii) a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 5, and 6; (iii) a CDR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 7, 8, 9, 10, and 11; (iv) a VL CDR1 comprising an amino acid sequence of SEQ ID NO: 12 or 13; (v) a VL CDR2 comprising an amino acid sequence of SEQ ID NO: 14 or 15; and (vi) a VL CDR3 comprising an amino acid sequence of SEQ ID NO: 16 or 17.

In a specific embodiment, the anti-4-1BB antibody or fragment thereof may comprise: (a) a CDR-H1 of SEQ ID NO: 1, a CDR-H2 of SEQ ID NO: 4, a CDR-H3 of SEQ ID NO: 7, a VL CDR1 of SEQ ID NO: 12, a VL CDR2 of SEQ ID NO: 14, and a VL CDR3 of SEQ ID NO: 16;

(b) a CDR-H1 of SEQ ID NO: 1, a CDR-H2 of SEQ ID NO: 4, a CDR-H3 of SEQ ID NO: 8, a VL CDR1 of SEQ ID NO: 12, a VL CDR2 of SEQ ID NO: 14, and a VL CDR3 of SEQ ID NO: 16;

(c) a CDR-H1 of SEQ ID NO: 1, a CDR-H2 of SEQ ID NO: 4, a CDR-H3 of SEQ ID NO: 9, a VL CDR1 of SEQ ID NO: 12, a VL CDR2 of SEQ ID NO: 14, and a VL CDR3 of SEQ ID NO: 16;

(d) a CDR-H1 of SEQ ID NO: 2, a CDR-H2 of SEQ ID NO: 5, a CDR-H3 of SEQ ID NO: 10, a VL CDR1 of SEQ ID NO: 12, a VL CDR2 of SEQ ID NO: 14, and a VL CDR3 of SEQ ID NO: 16; or

(e) a CDR-H1 of SEQ ID NO: 3, a CDR-H2 of SEQ ID NO: 6, a CDR-H3 of SEQ ID NO: 11, a VL CDR1 of SEQ ID NO: 13, a VL CDR2 of SEQ ID NO: 15, and a VL CDR3 of SEQ ID NO: 17.

In a non-limiting embodiment, the anti-4-1BB antibody or an antigen-binding fragment thereof may comprise;

a polypeptide comprising an amino acid sequence of SEQ ID NO: 40 as a H-FR1;

a polypeptide comprising an amino acid sequence of SEQ ID NO: 41 as

a H-FR2;

a polypeptide comprising an amino acid sequence of SEQ ID NO: 42, 43, 44, or 45 as a H-FR3;

5 a H-FR4;

a polypeptide comprising an amino acid sequence of SEQ ID NO: 47, 48, or 49 as a L-FR1;

a polypeptide comprising an amino acid sequence of SEQ ID NO: 50 or 51 as a L-FR2;

10 a polypeptide comprising an amino acid sequence of SEQ ID NO: 52 or 53 as a L-FR3; and/or

a polypeptide comprising an amino acid sequence of SEQ ID NO: 54 or 55 as a L-FR4.

15 In a non-limiting example, the anti-4-1BB antibody or fragment thereof may comprise:

(1) a heavy chain variable region comprising or consisting essentially of a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 18, 19, 20, 21, 22, 23, 39, 57, 58, 59, and 60, or a polypeptide having a sequence identity of at least 80%, at least 85%, at least 20 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) a light chain variable region comprising or consisting essentially of a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 24, 25, 26, 61, and 62, or a polypeptide having a 25 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. In a non-limiting example, the anti-4-1BB antibody may comprise:

(1) a heavy chain comprising or consisting essentially of a polypeptide 30 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 27, 28, 29, 30, 31, and 32, 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) a light chain comprising or consisting essentially of a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ
5 ID NOS: 33, 34, and 35, 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.

The antibodies of the disclosure are characterized by particular functional features or properties of the antibodies. For example, the antibodies
10 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.

15 The binding of an antibody or its antigen-binding fragment 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
20 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.
25 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 1×10^{-6} M or less, 1×10^{-7} M or less, or 1×10^{-8} M or less, for example, 1.01×10^{-9} M or less.

30 Further, the antibodies of the disclosure, particularly when constructed in the form of bispecific antibody, has the ability to enhance immune cell proliferation, survival, secretion of cytokines and cytolytic activity CD8 T cells. In

certain embodiments, a bispecific antibody comprising an antibody of the disclosure and an anti-PD-L1 antibody, binds to human 4-1BB and exhibits an ability to activate T cells.

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

In some embodiments, the anti-4-1BB antibody or fragment thereof further comprises a heavy chain constant region (e.g., SEQ ID NO: 36), a light chain constant region (e.g., SEQ ID NO: 37 or 38), an Fc region, or the
10 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
15 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
20 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.

25 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
30 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.

5 An embodiment can be a polynucleotide encoding the 4-1BB antibody in a bispecific antibody. An embodiment can be a polynucleotide encoding a heavy chain of the bispecific antibody in an IgG-scFv form. Other embodiment can be a polynucleotide encoding a light chain of the bispecific antibody in the IgG-scFv form.

10 The IgG-scFv form may refer to a kind of a bispecific antibody comprising a full-length IgG antibody targeting (binding to) one of the two antigens 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.

15 In an embodiment, when the bispecific antibody in an IgG-scFv form comprises a full-length IgG antibody against a certain antigen named X 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 X 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; 20 and the polynucleotide encoding a light chain of the bispecific antibody may encode a light chain of the full-length IgG antibody against X.

25 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 a certain antigen ("X"), 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 X 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. A 30 representative embodiment (e.g., an anti-PD-L1/anti-4-1BB bispecific antibody) can be illustrated in FIG. 7.

The anti-4-1BB antibody or an antigen-binding fragment thereof may

applied to treating and/or preventing a cancer and/or enhancing immune response. The enhancing immune response by the anti-4-1BB antibody or an antigen-binding fragment thereof may refer that the level of immune response, when the anti-4-1BB antibody or an antigen-binding fragment thereof is treated
5 (or administered), is higher than that of the case when the anti-4-1BB antibody or an antigen-binding fragment thereof is not treated (or administered). The enhancing immune response may be 4-1BB signal activation, T-cell activation, or both of them.

In an embodiment, examples of the cancer may include blood cancer,
10 lung cancer, stomach cancer, liver cancer, bone cancer, pancreatic cancer, skin cancer, head and neck cancer, skin melanoma, uterine cancer, ovarian cancer, rectal cancer, colorectal cancer, colon cancer, breast cancer, uterine sarcoma, fallopian tube carcinoma, endometrial carcinoma, uterine cervical carcinoma, vaginal carcinoma, vulva carcinoma, esophageal cancer, laryngeal cancer,
15 small-intestine cancer, thyroid cancer, parathyroid cancer, soft-tissue sarcoma, urethral cancer, penile cancer, prostate cancer, chronic or acute leukemia, solid tumors in juvenile stage, differentiated lymphoma, bladder cancer, renal cancer, renal cell carcinoma, renal pelvic carcinoma, primary central nervous system lymphoma, spinal axis tumors, glioma, brain stem glioma, and pituitary
20 adenoma, but are not limited thereto.

The effective ingredient such as the anti-4-1BB antibody, the antigen-binding fragment, etc. or the pharmaceutical composition may be administered orally or parenterally. For parenteral administration, intravenous injection, subcutaneous injection, intramuscular injection, intraperitoneal injection,
25 endothelial administration, topical administration, intranasal administration, intrapulmonary administration, and intrarectal administration may be conducted. Since oral administration leads to digestion of proteins or peptides, an active ingredient in the compositions for oral administration may be coated or formulated to prevent digestion in the stomach. In addition, the composition
30 may be administered using an optional device that enables an active ingredient to be delivered to target cells.

The pharmaceutically effective amount of the anti-4-1BB antibody or the

antigen-binding fragment thereof may be prescribed in various amounts, depending on factors such as preparation (formulation) methods, method of administration, the patient's age, body weight, gender, pathologic conditions and diet, administration time, administration interval, administration route, excretion speed, and reaction sensitivity. For example, a daily dosage of the anti-4-1BB antibody or the antigen-binding fragment thereof may be within the range of 0.001 to 1000 mg/kg, particularly 0.01 to 100 mg/kg, more particularly 0.1 to 50 mg/kg, and even more particularly 0.1 to 20 mg/kg, but is not limited thereto. The daily dosage may be formulated into a single formulation in a unit dosage form or formulated in suitably divided dosage forms, or it may be manufactured to be contained in a multiple dosage container. The pharmaceutical composition may be administered in combination with other medications, and proper prescriptions may be made on the dose, the administration method, and kinds of the other medications, depending on patients' states.

The pharmaceutical composition may be formulated into a form of a solution in oil or an aqueous medium, a suspension, syrup, an emulsion, an extract, powder, granules, a tablet, or a capsule, and may further include a dispersing or a stabilizing agent for formulation.

Particularly, the pharmaceutical composition comprising the anti-4-1BB antibody or the antigen-binding fragment thereof may be formulated into an immunoliposome since it contains an antibody or an antigen-binding fragment. An antibody-containing liposome may be prepared using any of the methods widely known in the art. The immunoliposome may be a lipid composition including phosphatidylcholine, cholesterol, and polyethyleneglycol-derivatized phosphatidylethanolamine, and may be prepared by a reverse phase evaporation method. For example, Fab' fragments of an antibody may be conjugated to the liposome through a disulfide-exchange reaction.

The anti-4-1BB antibody or the antigen-binding fragment thereof, or the pharmaceutical composition may be administered to any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects may 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.

Another embodiment provides:

5 a polynucleotide coding for the heavy-chain complementarity determining region, a polynucleotide coding for the light-chain complementarity determining region, or a combination thereof; a polynucleotide coding for the heavy-chain variable region, a polynucleotide coding for the light-chain variable region, or a combination thereof; or a polynucleotide coding for the heavy chain, a polynucleotide coding for the light chain, or a combination thereof, wherein the
10 complementarity determining regions, the heavy- and light-chain variable regions, and the heavy and light chains are as described for the anti-4-1BB antibody above;

a recombinant vector carrying the polynucleotides or a combination thereof; and/or

15 a recombinant cell harboring the recombinant vector.

In one embodiment, the recombinant vector described above may contain polynucleotides coding respectively for a heavy-chain complementarity determining region and a light-chain complementarity determining region; for a heavy-chain variable region and a light-chain variable region; or for a heavy
20 chain and a light chain in the anti-4-1BB antibody, in a single vector or in separate vectors carrying each of the polynucleotides.

The term "vector" refers to a means for expressing a target gene in a host cell, as exemplified by a plasmid vector, a cosmid vector, and a viral vector such as a bacteriophage vector, an adenovirus vector, a retrovirus vector, and
25 an adeno-associated virus vector. The recombinant vector may be constructed from plasmids frequently used in the art (for example, pcDNA3.1, pcDNA3.4, pCHO1.0, pSC101, pGV1106, pACYC177, ColE1, pKT230, pME290, pBR322, pUC8/9, pUC6, pBD9, pHc79, pIJ61, pLAFR1, pHV14, pGEX series, pET series, and pUC19), phages (for example, λ gt4 λ B, λ -Charon, λ Δz1, and
30 M13) or by manipulating viruses (for example, SV40, etc.).

In the recombinant vector, the polynucleotide may be operatively linked to a promoter. The term "operatively linked" is intended to pertain to a

functional linkage between a nucleotide sequence of interest and an expression regulatory sequence (for example, a promoter sequence). When being "operatively linked", the regulatory element can control the transcription and/or translation of the nucleotide of interest.

5 The recombinant vector may be constructed typically as a cloning vector or an expression vector. For recombinant expression vectors, a vector generally available in the art for expressing a foreign protein in plant, animal, or microbial cells may be employed. Various methods well known in the art may be used for the construction of recombinant vectors.

10 For use in hosts, such as prokaryotic or eukaryotic cells, the recombinant vector may be constructed accordingly. For example, when a vector is constructed as an expression vector for use in a prokaryotic host, the vector typically includes a strong promoter for transcription (e.g., a pL λ promoter, a CMV promoter, a trp promoter, a lac promoter, a tac promoter, a T7
15 promoter, etc.), a ribosomal binding site for initiating translation, and transcriptional/translational termination sequences. On the other hand, an expression vector for use in a eukaryotic host includes an origin of replication operable in a eukaryotic cell, such as an f1 origin of replication, an SV40 origin of replication, a pMB1 origin of replication, an adeno origin of replication, an
20 AAV origin of replication, and a BBV origin of replication, but is not limited thereto. In addition, the expression vector typically includes a promoter derived from genomes of mammalian cells (for example, metallothionein promoter) or from mammalian viruses (for example, adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, cytomegalovirus promoter, and tk
25 promoter of HSV), and a polyadenylation sequence as a transcription termination sequence.

 The recombinant cell may be prepared by introducing the recombinant vector into a suitable host cell. As long as it allows the sequential cloning and expression of the recombinant vector in a stable manner, any host cell known in
30 the art may be employed in the present disclosure. Examples of the prokaryotic host cell available for the present disclosure include *E. coli*, *Bacillus* spp. such as *Bacillus subtilis* and *Bacillus thuringiensis*, and enterobacteriaceae

strains such as *Salmonella typhimurium*, *Serratia marcescens* and various *Pseudomonas* species. Eukaryotic host cells that may be used for transformation may include, but are not limited to, *Saccharomyces cerevisiae*, insect cells, and animal cells, such as Sp2/0, CHO (Chinese hamster ovary),
5 CHO-K1, CHO DG44, CHO-S, PER.C6, W138, BHK, COS-7, 293, HepG2, Huh7, 3T3, RIN, and MDCK.

The nucleic acid molecule or a recombinant vector carrying the same may be introduced (transfected) into a host cell using a method well known in the art,. This transfection may be carried out using a CaCl_2 or electroporation
10 method when the host cell is prokaryotic. For eukaryotic host cells, the genetic introduction may be achieved using, but not limited to, microinjection, calcium phosphate precipitation, electroporation, liposome-mediated transfection, or particle bombardment.

To select a transformed host cell, advantage may be taken of a
15 phenotype associated with a selection marker according to methods well known in the art. For example, when the selection marker is a gene conferring resistance to a certain antibiotic, the host cells may be grown in the presence of the antibiotic in a medium to select a transformant of interest.

Another aspect provides a method for production of an anti-4-1BB
20 antibody or an antigen-binding fragment thereof, the method comprising a step of expressing the polynucleotide or the recombinant vector in a pertinent host cell. In one embodiment, the production method may comprise culturing a recombinant cell harboring the polynucleotide or the recombinant vector thereat, and optionally isolating and/or purifying the antibody from the culture medium.

25

[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.

30

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

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

For panning of each of phage libraries (obtained from KBio Health and CUREBIO) against target molecules, A total of four rounds of panning were carried out using 4-1BB (NCBI Accession No. NP_001552.2; SEQ ID NO: 67) coated immunotubes.

5 Bacterial colonies from the 3 rounds of panning output were grown in SB-Carbenicillin in 96 deepwell plate until turbid, at which point 10^{11} 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.

10 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
15 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.

20 While any type of IgG form can be prepared, sequences of the obtained 4-1BB specific antibodies in IgG4 form are summarized in Tables 2-9:

[Table 2] 41B01

41B01	Amino acid sequence (N'→C')
Heavy Chain	SEQ ID NO: 27
Heavy Chain Variable Region (VH)	SEQ ID NO: 18
H-CDR1	SYDMS (SEQ ID NO: 1)
H-CDR2	WISYSGGSIYYADSVKG (SEQ ID NO: 4)
H-CDR3	DGQRNSMREFDY (SEQ ID NO: 7)
Light Chain	SEQ ID NO: 33
Light Chain Variable Region (VL)	SEQ ID NO: 24
L-CDR1	SGSSSNIGNNYVT (SEQ ID NO: 12)
L-CDR2	ADSHRPS (SEQ ID NO: 14)
L-CDR3	ATWDYSLSGYV (SEQ ID NO: 16)

[Table 3] 41B01.01

41B01.01	Amino acid sequence (N'→C')
Heavy Chain	SEQ ID NO: 28
Heavy Chain Variable Region (VH)	SEQ ID NO: 19
H-CDR1	SYDMS (SEQ ID NO: 1)
H-CDR2	WISYSGGSIYYADSVKG (SEQ ID NO: 4)
H-CDR3	DAQRNSMREFDY (SEQ ID NO: 8)
Light Chain	SEQ ID NO: 33
Light Chain Variable Region (VL)	SEQ ID NO: 24
L-CDR1	SGSSSNIGNNYVT (SEQ ID NO: 12)
L-CDR2	ADSHRPS (SEQ ID NO: 14)
L-CDR3	ATWDYSLSGYV (SEQ ID NO: 16)

[Table 4] 41B01.02

41B01.02	Amino acid sequence (N'→C')
Heavy Chain	SEQ ID NO: 29
Heavy Chain Variable Region (VH)	SEQ ID NO: 20
H-CDR1	SYDMS (SEQ ID NO: 1)
H-CDR2	WISYSGGSIYYADSVKG (SEQ ID NO: 4)
H-CDR3	DAQRQSMREFDY (SEQ ID NO: 9)
Light Chain	SEQ ID NO: 33
Light Chain Variable Region (VL)	SEQ ID NO: 24
L-CDR1	SGSSSNIGNNYVT (SEQ ID NO: 12)
L-CDR2	ADSHRPS (SEQ ID NO: 14)
L-CDR3	ATWDYSLSGYV (SEQ ID NO: 16)

5

[Table 5] 41B01.03

41B01.03	Amino acid sequence (N'→C')
Heavy Chain	SEQ ID NO: 28
Heavy Chain Variable Region (VH)	SEQ ID NO: 19
H-CDR1	SYDMS (SEQ ID NO: 1)

H-CDR2	WISYSGGSIYYADSVKG (SEQ ID NO: 4)
H-CDR3	DAQRNSMREFDY (SEQ ID NO: 8)
Light Chain	SEQ ID NO: 34
Light Chain Variable Region (VL)	SEQ ID NO: 25
L-CDR1	SGSSSNIGNNYVT (SEQ ID NO: 12)
L-CDR2	ADSHRPS (SEQ ID NO: 14)
L-CDR3	ATWDYSLSGYV (SEQ ID NO: 16)

[Table 6] 41B01.04

41B01.04	Amino acid sequence (N'→C')
Heavy Chain	SEQ ID NO: 29
Heavy Chain Variable Region (VH)	SEQ ID NO: 20
H-CDR1	SYDMS (SEQ ID NO: 1)
H-CDR2	WISYSGGSIYYADSVKG (SEQ ID NO: 4)
H-CDR3	DAQRQSMREFDY (SEQ ID NO: 9)
Light Chain	SEQ ID NO: 34
Light Chain Variable Region (VL)	SEQ ID NO: 25
L-CDR1	SGSSSNIGNNYVT (SEQ ID NO: 12)
L-CDR2	ADSHRPS (SEQ ID NO: 14)
L-CDR3	ATWDYSLSGYV (SEQ ID NO: 16)

[Table 7] 41B02

41B02	Amino acid sequence (N'→C')
Heavy Chain	SEQ ID NO: 30
Heavy Chain Variable Region (VH)	SEQ ID NO: 21
H-CDR1	GYDMS (SEQ ID NO: 2)
H-CDR2	VIYPDDGNTYYADSVKG (SEQ ID NO: 5)
H-CDR3	HGGQKPTTKSSSAYGMDG (SEQ ID NO: 10)
Light Chain	SEQ ID NO: 33
Light Chain Variable Region (VL)	SEQ ID NO: 24
L-CDR1	SGSSSNIGNNYVT (SEQ ID NO: 12)
L-CDR2	ADSHRPS (SEQ ID NO: 14)

L-CDR3	ATWDYSLSGYV (SEQ ID NO: 16)
--------	-----------------------------

[Table 8] 41B02.01

41B02.01	Amino acid sequence (N'→C')
Heavy Chain	SEQ ID NO: 31
Heavy Chain Variable Region (VH)	SEQ ID NO: 22
H-CDR1	GYDMS (SEQ ID NO: 2)
H-CDR2	VIYPDDGNTYYADSVKG (SEQ ID NO: 5)
H-CDR3	HGGQKPTTKSSSAYGMDG (SEQ ID NO: 10)
Light Chain	SEQ ID NO: 34
Light Chain Variable Region (VL)	SEQ ID NO: 25
L-CDR1	SGSSSNIGNNYVT (SEQ ID NO: 12)
L-CDR2	ADSHRPS (SEQ ID NO: 14)
L-CDR3	ATWDYSLSGYV (SEQ ID NO: 16)

[Table 9] 41B03

41B03	Sequence
Heavy Chain	SEQ ID NO: 32
Heavy Chain Variable Region (VH)	SEQ ID NO: 23
CDR-H1	SNVMN(SEQ ID NO: 3)
CDR-H2	EISHSGSTNYPNPSLKS(SEQ ID NO: 6)
CDR-H3	GAGNLGY(SEQ ID NO: 11)
Light Chain	SEQ ID NO: 35
Light Chain Variable Region (VL)	SEQ ID NO: 26
CDR-L1	QASQDISNYLN (SEQ ID NO: 13)
CDR-L2	GASSRAT(SEQ ID NO: 15)
CDR-L3	QQYNSYPIT (SEQ ID NO: 17)

5

1.2. Preparation of scFv antibodies against 4-1BB

Anti-4-1BB scFv antibodies with a structure of (N')-VL-linker-VH-(C') were prepared using the variable regions of the full human monoclonal antibodies against 4-1BB shown in the tables of Example 1.1, wherein the

amino acid residue "G" at the position 44 of a heavy chain variable region was substituted with "C", and the amino acid residue "G" at the position 103 of a light chain variable region was substituted with "C". Such amino acid substitution from "G" to "C" in scFv can contribute to increase in stabilities of bispecific antibodies comprising the scFv as one target-specific moiety. The amino acid sequences of the prepared anti-4-1BB scFvs were illustrated in the following tables, while skilled persons in the art may apply changes or modifications of amino acid sequences in the following embodiments to meet specific purposes, including applying various types of peptide linkers such as (GGGGS)₂, (GGGGS)₃, (GGGGS)₄, or (GS)₉.

Sequences of the obtained 4-1BB specific antibodies in scFv form are summarized in Tables 10-15:

[Table 10] 41B01 (scFv)

	Amino acid sequence (N'→C')
Light chain variable region (VL)	SEQ ID NO: 61
Linker	SEQ ID NO: 56
Heavy chain variable region (VH)	SEQ ID NO: 57

[Table 11] 41B01.01 (scFv)

	Amino acid sequence (N'→C')
Light chain variable region (VL)	SEQ ID NO: 61
Linker	SEQ ID NO: 56
Heavy chain variable region (VH)	SEQ ID NO: 58

[Table 12] 41B01.03 (scFv)

	Amino acid sequence (N'→C')
Light chain variable region (VL)	SEQ ID NO: 62
Linker	SEQ ID NO: 56
Heavy chain variable region (VH)	SEQ ID NO: 58

[Table 13] 41B01.04 (scFv)

	Amino acid sequence (N'→C')
Light chain variable region (VL)	SEQ ID NO: 62
Linker	SEQ ID NO: 56
Heavy chain variable region (VH)	SEQ ID NO: 39

[Table 14] 41B02 (scFv)

	Amino acid sequence (N'→C')
Light chain variable region (VL)	SEQ ID NO: 61

Linker	SEQ ID NO: 56
Heavy chain variable region (VH)	SEQ ID NO: 59

[Table 15] 41B02.01 (scFv)

	Amino acid sequence (N'→C')
Light chain variable region (VL)	SEQ ID NO: 62
Linker	SEQ ID NO: 56
Heavy chain variable region (VH)	SEQ ID NO: 60

1.4. Preparation of scFv antibodies against 4-1BB in the form of bispecific antibody

As a representative example of a bispecific antibody, PD-L1x4-1BB bispecific antibodies consisting of heavy components and light components as follows, were prepared:

(1) Heavy components (N'→C')

- 1) heavy chain of anti-PD-L1 antibody: SEQ ID NO: 63 or SEQ ID NO: 65,
- 2) linker: SEQ ID NO: 66 (GGGGSGGGSGGGGS), and
- 3) anti-4-1BB scFv, 41B01 (scFv) (Table 10), 41B01.01 (scFv) (Table 11), 41B01.02 (scFv), 41B01.03 (scFv) (Table 12), or 41B01.04 (scFv) (Table 13), prepared in Example 1.2; and

(2) Light components (N'→C')

Light chain of anti-PD-L1 antibody: SEQ ID NO: 64.

1.5. 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 0.1μg/ml in PBS, 100 μl/well at 4°C overnight, then blocked with 100 μl/well of 5% BSA. Serial dilutions of antibodies obtained in Example 1.1 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. The obtained results are shown in FIGS. 1A-1C. As shown in FIGS. 1A, 1B, and 1C, the anti-4-1BB antibodies tested showed high 4-1BB binding abilities.

5

(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
10 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. 2, 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.

15 As shown in FIG. 2, the anti-4-1BB antibodies tested show binding abilities to 4-1BB which expressed on cell surface.

(3) Protein kinetic for 4-1BB

To explore the binding kinetics of the humanized antibody, this example
20 performed the affinity ranking by using Octet Red 96. As shown in Table 16 below, the anti-4-1BB antibodies tested show high 4-1BB binding affinities.

[Table 16] 4-1BB Affinity Result of OCTET

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
41B03	7.90E-10	7.55E+05	5.97E-04	0.1213	0.9951

(4) Epitope Mapping

25 Epitope mapping was performed to identify the unique epitope of the 4-1BB antibodies. Almost all surface residues of 4-1BB (NCBI Accession No. NP_001552.2) were mutated screened by high-throughput flowcytometry to map epitope by Ranomics (Toronto, CA). A GFP tag was fused to the C-

terminus of 4-1BB, and each surface residue of the extracellular domain (24-186) of 4-1BB was mutated to Arg. If the residue is Arg, it was mutated to Glu. The mutated plasmid was pooled into a library and transfected to the HEK293 cell line. The resulting cell line was sorted against GFP and/or 41B01/41B02 binding. The population of each mutant in the sorted cell pool was analyzed by RNAseq. If a mutant shows a higher count in the GFP pool than the GFP/anti-4-1BB pool, the mutated site was considered as an epitope. The epitope residues are listed in Table 17:

[Table 17]

clone	Epitope residues of 4-1BB (NCBI Accession No. NP_001552.2)	Domain
41B01	Phe125, Gly150, Arg154, Asp155, Val157	CRD4
41B02	Trp136, Thr137, Leu147, Gly150, Arg154	CRD4

10

FIGS. 8A and 8B show the epitopes of the 41B01 clone and 41B02 clone. In the figures, 4-1BB protein is shown in ribbon as bound with 4-1BB ligand in molecular surface representation (PDB ID:6MGP). The identified epitope residues within 4-1BB for anti-4-1BB antibodies are shown in spheres. As shown in the figures, 41B01 and 41B02 bind to the epitopes within CDR4 of 4-1BB, which are far from the binding site of 4-1BBL within 4-1BB. As such, 41B01 and 41B02 do not compete with 4-1BBL.

15

These observations are in agreement with the finding in Example 1.5 (5) competition assay that 41B01 and 41B02 do not compete with 4-1BBL, while utomilulumab competes with 4-1BBL. Utomilulumab also competes with 41B01 or 41B02. Because utomilumab binds to an epitope within CRD3~4 (87-118) of 4-1BB, the binding site of utomilumab within 4-1BB is close to and overlaps with the binding site of 4-1BBL, 41B01, or 41B02.

20

25 **(5) Competition Assay**

Competition assay was performed to test if the 4-1BB antibodies have unique epitope.

A 96 well black microplate (greiner bio one) was installed in a biosensor

tray case, then 200 μ L of 1X Kinetic Buffer (1X KB) was added in 8 wells respectively. 8 Ni-NTA biosensors (ForteBio, USA) were hydrated by soaking in the wells with buffer for 10 min. Analytes (41B01, 41B02, Urelumab, Utomilumab, Recombinant Human 41BB Ligand) were diluted using 1X KB to the concentration of 100 nM. Recombinant Human 41BB-His tag protein (Sino Biological, 10041-H08H) was diluted with 1X KB buffer to the concentration of 2 μ g/mL which was then used for antigen fixation on the biosensor.

The experiment was performed by sequential association steps of two analytes based on the Table 18. 1X KB was added to a new 96 well black microplate according to the octet program template. Biosensors are soaked in the 1X KB 200 μ L to generate Baseline1, followed by placing biosensors to 200 μ L of the antigen, Recombinant Human 41BB His tag protein (2 μ g/mL) to fix antigen on the biosensor. After re-hydrating biosensors in 1X KB 200 μ L (Baseline2), first analyte (200 μ L) was associated with the antigen on the biosensors. Subsequently, second analyte (200 μ L) was associated with the molecules on the biosensors. The temperature of the experimental plate was controlled with 30 $^{\circ}$ C. After finishing the experiment, cross-competition between the first and second analytes was evaluated with the Octet Analysis 9.0 software.

[Table 18] List of Analytes

Run number	First Analyte	Second Analyte
1	Urelumab	Urelumab
2	Urelumab	41B02
3	41B01	Urelumab
4	Urelumab	41B02
5	41B02	Urelumab
6	Utomilumab	Utomilumab
7	Utomilumab	41B01
8	41B01	Utomilumab
9	Utomilumab	41B02
10	41B02	Utomilumab
11	41B01	41B01
12	41B01	41B02
13	41B02	41B01
14	41B02	41B02
15	Recombinant Human 41BB-Ligand	Recombinant Human 41BB-Ligand
16	Recombinant Human 41BB-Ligand	41B01
17	41B01	Recombinant Human 41BB-Ligand

The obtained results are shown in FIGS. 9A-12C.

FIGS. 9A-9E show cross-competition between Urelumab and candidates (41B01 and 41B02). Competition assay was performed between Urelumab and two candidates (41B01 and 41B02). Recombinant human 41BB-His tag protein was firstly fixed on the biosensor. Then primary and secondary antibodies were sequentially associated with the biosensor to detect binding competition between two antibodies. Overall, no binding competition was not observed between Urelumab and two candidates indicating that each antibody has unique epitope. FIG. 9A shows results of self-binding of Urelumab (control), FIG. 9B shows results of Urelumab as a 1st antibody and 41B01 as a 2nd antibody, FIG. 9C shows results of 41B01 as a 1st antibody and Urelumab as a 2nd antibody, FIG. 9D shows results of Urelumab as a 1st antibody and 41B02 as a 2nd antibody, and FIG. 9E shows results of 41B02 as a 1st antibody and Urelumab as a 2nd antibody.

FIGS. 10A-10E show cross-competition between Utomilumab and candidates (41B01 and 41B02). Competition assay was performed between Utomilumab and two candidates (41B01 and 41B02). Recombinant human 41BB-His tag protein was firstly fixed on the biosensor. Then primary and secondary antibodies were sequentially associated with the biosensor to detect binding competition between two antibodies. Overall, no binding competition was not observed between Utomilumab and two candidates, indicating that each antibody has unique epitope. FIG. 10A shows results of self-binding of Utomilumab (control), FIG. 10B shows results of Utomilumab as a 1st antibody and 41B01 as a 2nd antibody, FIG. 10C shows results of 41B01 as a 1st antibody and Utomilumab as a 2nd antibody, FIG. 10D shows results of Utomilumab as a 1st antibody and 41B02 as a 2nd antibody, and FIG. 10E shows results of 41B02 as a 1st antibody and Utomilumab as a 2nd antibody.

FIGS. 11A-11D show cross-competition between 41B01 and 41B02. Competition assay was performed between two candidates (41B01 and 41B02). Recombinant human 41BB-His tag protein was firstly fixed on the biosensor. Then primary and secondary antibodies were sequentially associated with the biosensor to detect binding competition between two antibodies. Overall,

binding competition was observed between 41B01 and 41B02, indicating that these two candidates have same epitope against 41BB. FIG. 11A shows results of self-binding of 41B01, FIG. 11B shows results of 41B01 as a 1st antibody and 41B02 as a 2nd antibody, FIG. 11C shows results of 41B02 as a 1st antibody and 41B01 as a 2nd antibody, and FIG. 11D shows results of self-binding of 41B02.

FIGS. 12A-12C show cross-competition between recombinant human 41BB-ligand and 41B01. Competition assay was performed between recombinant human 41BB-ligand and 41B01. Recombinant human 41BB-His tag protein was firstly fixed on the biosensor. Then recombinant human 41BB-ligand and 41B01 were sequentially associated with the biosensor to detect binding competition between them. Overall, binding competition was not observed between recombinant human 41BB-ligand and 41B01, indicating that 41B01 has unique binding site to 41BB compared to recombinant human 41BB-ligand. FIG. 12A shows results of self-binding of recombinant human 41BB-ligand, FIG. 12B shows results of recombinant human 41BB-ligand as a 1st antibody and 41B01 as a 2nd antibody, and FIG. 12C shows results of 41B01 as a 1st antibody and recombinant human 41BB-ligand as a 2nd antibody.

Example 2. Activities of Anti-4-1BB Antibodies

(1) Activity of the anti-4-1BB antibodies to promote 4-1BB signal

To test the ability of anti-4-1BB antibodies 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. GloResponse™ NFκB-luc2/4-1BB Jurkat cell line is genetically modified to stably express 4-1BB receptors and luciferase downstream of a response element. Luciferase expression is induced upon antibody binding to the 4-1BB receptors. In brief, 25 μL of antibodies crosslinked with anti-human IgG Fc secondary antibodies (starting from 500 nM diluted for 5-fold) were added to the plate. Harvest GloResponse™ NFκB-luc2/4-1BB Jurkat cell line and dispense 50 μL of GloResponse™ NFκB-luc2/4-1BB Jurkat cell line per well to make 5x10⁴ 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 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.

- 5 The obtained results are shown in FIG. 3. As shown in FIG. 3, the anti-4-1BB antibodies tested showed 4-1BB agonistic activity.

(2) Activity of the anti-4-1BB antibodies to promote human T cell immune response

- 10 To test the ability of anti-4-1BB antibodies to stimulated human PBMCs response, cytokine production assay was used. Human PBMCs stimulated with human anti-CD3 antibody were used as the effector cells. HCC-1954 cells which no expressed 4-1BB was used as the target cells. In this system, PBMCs (3×10^4) were co-cultured with HCC-1954 (1×10^4) in the presence of human anti-
15 CD3 antibody. Anti-4-1BB antibodies (starting from 100 nM (=15 ug/mL) diluted for 10 dose) and Fc crosslinked antibodies (starting from 500 nM (=75 ug/mL) diluted for 10 dose) were added to the mixed culture. The obtained results are shown in FIG. 4. As shown in FIG.4, anti-4-1BB antibodies induced cytokine release.

20

(3) Activities of PD-L1x4-1BB Antibodies to promote 4-1BB signal

The 4-1BB signal promotion by the 4-1BB antibody in the form of bispecific antibodies was measured using cell-based 4-1BB assay.

- 25 The bispecific antibody, PD-L1x4-1BB bispecific antibody, consisted of heavy components and light components as follows:

(1) Heavy components (N'→C')

1) heavy chain of anti-PD-L1 antibody: SEQ ID NO: 63,

2) linker: SEQ ID NO: 66 (GGGGSGGGGSGGGGS), and

3) anti-4-1BB scFv, 41B01 (scFv) (Table 10) or 41B02 (scFv) (Table 14),

- 30 prepared in Example 1.2; and

(2) Light components (N'→C')

Light chain of anti-PD-L1 antibody: SEQ ID NO: 64.

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) 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 each bispecific antibody (starting from 15 nM diluted for 8-fold or 1.5 nM diluted for 4-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 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 obtained results are shown in FIG. 5, wherein 41B01 indicates a PD-L1x4-1BB bispecific antibody comprising 41B01 (scFv) and 41B02 indicates a PD-L1x4-1BB bispecific antibody comprising 41B02 (scFv). As shown in FIG. 5, when the 4-1BB antibodies were constructed in the form of bispecific antibody (PD-L1x4-1BB bispecific antibody), the PD-L1x4-1BB bispecific antibodies tested showed stronger 4-1BB signal activation in the presence of tumor antigen (PD-L1), compared to anti PD-L1 monoclonal antibodies applied alone.

(4) In vivo efficacy of anti-4-1BB antibodies

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. Mouse were intraperitoneally administered Q3D for 5 times (five time injection of the antibody every three days) with following antibodies: human IgG type control (10 mg/kg), and anti-4-1BB antibody (41B01, 10 mg/kg).

Tumor volumes were monitored by caliper measurement twice per week for the duration of the experiment, and the obtained results are shown in FIG. 6. Tumor growth inhibition induced by the anti-4-1BB antibody using 41B01 was significantly greater than that observed with the combination of each targeting monoclonal antibodies (See FIG. 6).

As shown in FIG. 6, the anti-4-1BB antibody has increased *in vivo* anti-tumor effect compared to hIgG (control).

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

1. An anti-4-1BB antibody or an antigen-binding fragment thereof comprising:
- 5 a CDR(complementarity determining region)-H1 comprising an amino acid sequence of SEQ ID NO: 1, 2, or 3;
a CDR-H2 comprising an amino acid sequence of SEQ ID NO: 4, 5, or 6;
a CDR-H3 comprising an amino acid sequence of SEQ ID NO: 7, 8, 9, 10, or 11;
10 a CDR-L1 comprising an amino acid sequence of SEQ ID NO: 12 or 13;
a CDR-L2 comprising an amino acid sequence of SEQ ID NO: 14 or 15;
and
a CDR-L3 comprising an amino acid sequence of SEQ ID NO: 16 or 17.
- 15 2. The anti-4-1BB antibody or an antigen-binding fragment thereof according to claim 1, comprising:
- (a) a CDR-H1 of SEQ ID NO: 1, a CDR-H2 of SEQ ID NO: 4, a CDR-H3 of SEQ ID NO: 7, a VL CDR1 of SEQ ID NO: 12, a VL CDR2 of SEQ ID NO: 14, and a VL CDR3 of SEQ ID NO: 16;
- 20 (b) a CDR-H1 of SEQ ID NO: 1, a CDR-H2 of SEQ ID NO: 4, a CDR-H3 of SEQ ID NO: 8, a VL CDR1 of SEQ ID NO: 12, a VL CDR2 of SEQ ID NO: 14, and a VL CDR3 of SEQ ID NO: 16;
- (c) a CDR-H1 of SEQ ID NO: 1, a CDR-H2 of SEQ ID NO: 4, a CDR-H3 of SEQ ID NO: 9, a VL CDR1 of SEQ ID NO: 12, a VL CDR2 of SEQ ID NO: 14, 25 and a VL CDR3 of SEQ ID NO: 16;
- (d) a CDR-H1 of SEQ ID NO: 2, a CDR-H2 of SEQ ID NO: 5, a CDR-H3 of SEQ ID NO: 10, a VL CDR1 of SEQ ID NO: 12, a VL CDR2 of SEQ ID NO: 14, and a VL CDR3 of SEQ ID NO: 16; or
- (e) a CDR-H1 of SEQ ID NO: 3, a CDR-H2 of SEQ ID NO: 6, a CDR-H3 30 of SEQ ID NO: 11, a VL CDR1 of SEQ ID NO: 13, a VL CDR2 of SEQ ID NO: 15, and a VL CDR3 of SEQ ID NO: 17.

3. The anti-4-1BB antibody or an antigen-binding fragment thereof according to claim 1 or 2, comprising:

(1) a heavy chain variable region comprising a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 18, 19, 20, 21, 22, 23, 39, 57, 58, 59, and 60; and

(2) a light chain variable region comprising a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 24, 25, 26, 61, and 62.

4. The anti-4-1BB antibody or an antigen-binding fragment thereof according to any one of claims 1-3, comprising:

(1) a heavy chain comprising a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 27, 28, 29, 30, 31, and 32; and

(2) a light chain comprising a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 33, 34, and 35.

5. A pharmaceutical composition for treating or preventing a cancer comprising the anti-4-1BB antibody or an antigen-binding fragment thereof according to any one of claims 1-4.

6. A pharmaceutical composition for enhancing immune response comprising the anti-4-1BB antibody or an antigen-binding fragment thereof according to any one of claims 1-4.

7. The pharmaceutical composition of claim 6, wherein the enhancing immune response is 4-1BB signal activation, T-cell activation, or both of them.

8. A polynucleotide encoding the anti-4-1BB antibody or an antigen-binding fragment thereof according to any one of claims 1-4.

9. A recombinant vector comprising the polynucleotide according to claim 8.
10. A recombinant cell comprising the polynucleotide according to claim 8.
11. A method of preparing an anti-4-1BB antibody or an antigen-binding fragment thereof, comprising culturing the recombinant cell of claim 10.
12. A method of treating or preventing a cancer in a subject, comprising administering the anti-4-1BB antibody or an antigen-binding fragment thereof according to any one of claims 1-4 to the subject.
13. A method of enhancing immune response in a subject comprising administering the anti-4-1BB antibody or an antigen-binding fragment thereof according to any one of claims 1-4 to the subject.
14. The method of claim 13, wherein the enhancing immune response is 4-1BB signal activation, T-cell activation, or both of them.

FIG. 1A

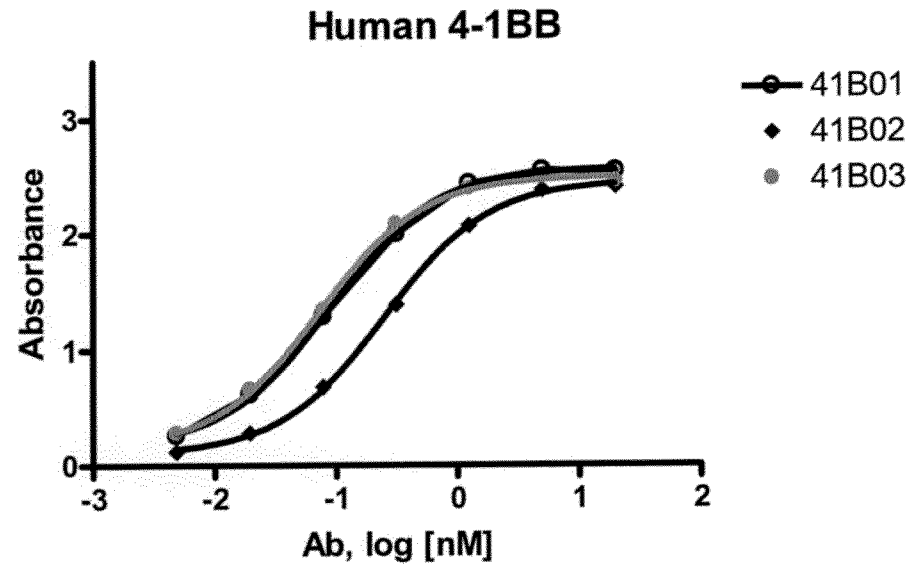


FIG. 1B

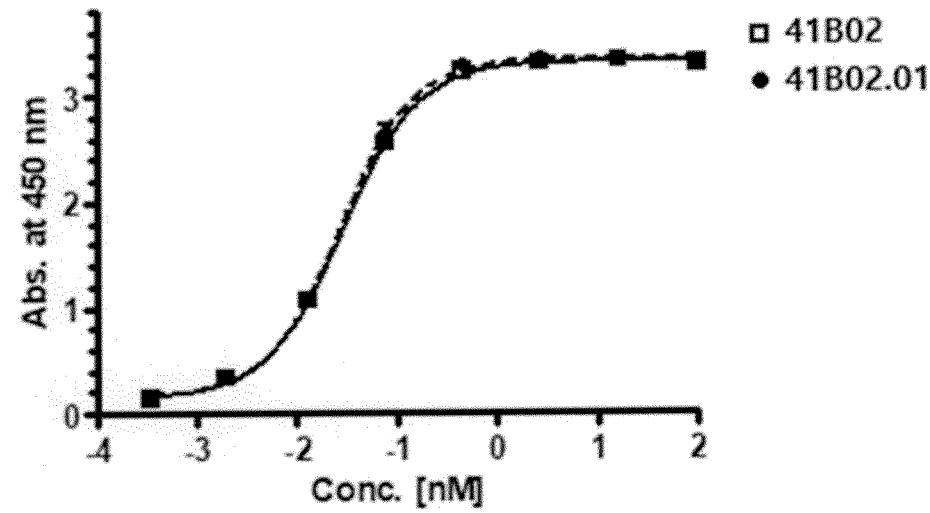


FIG. 1C

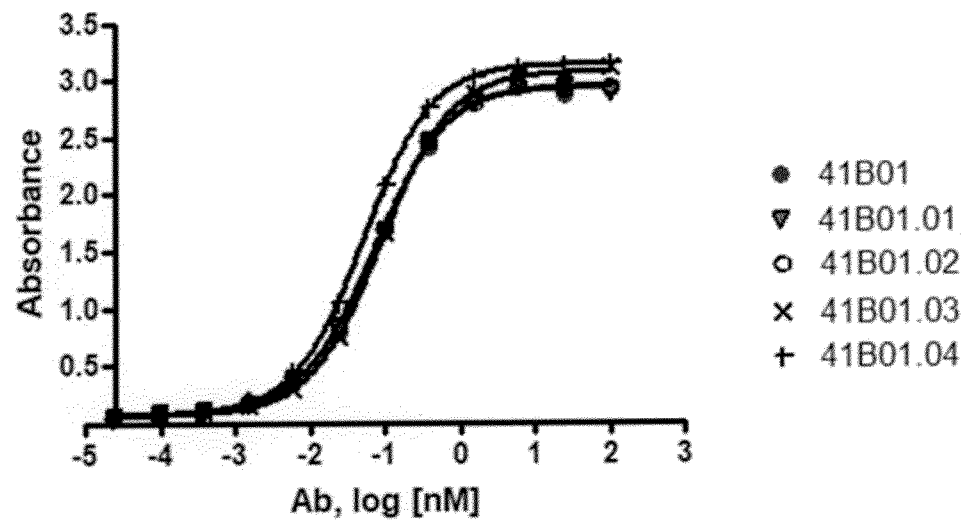


FIG. 2

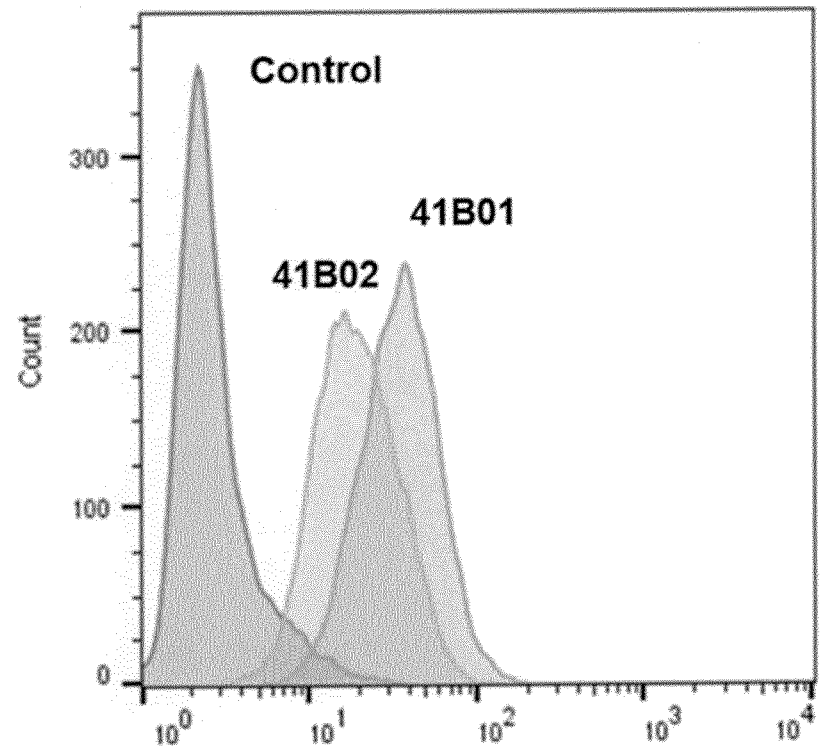


FIG. 3

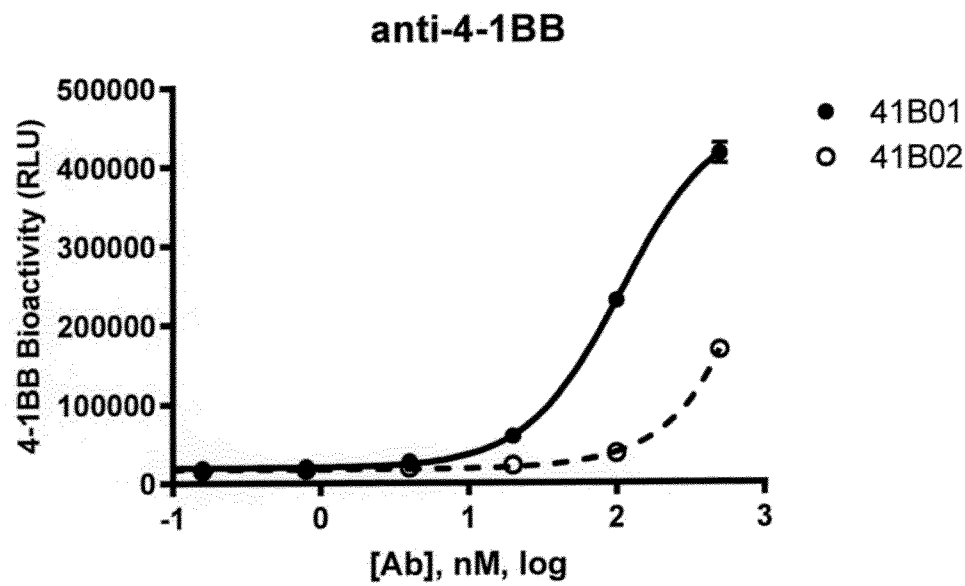


FIG. 4

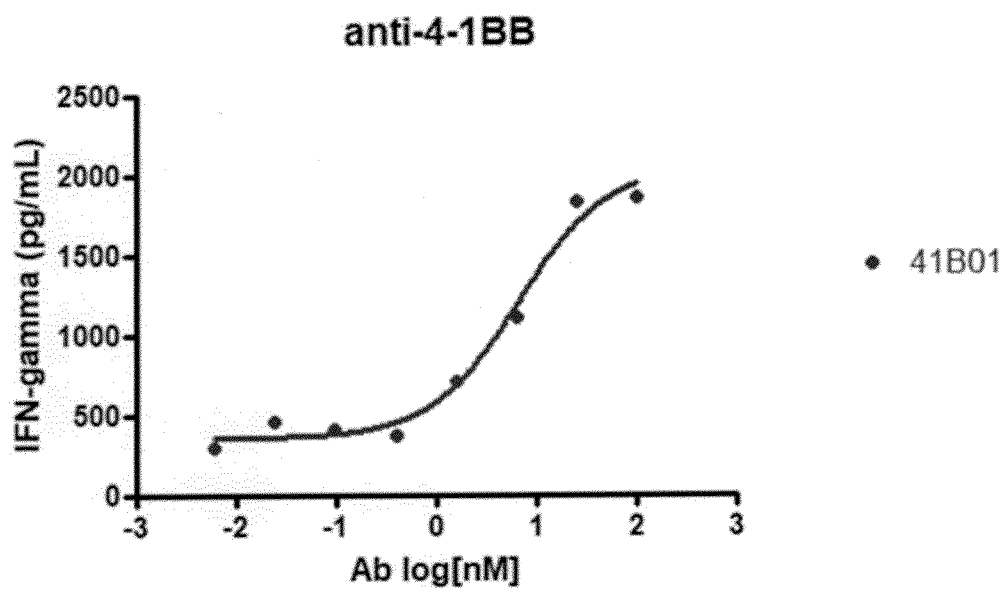


FIG. 5

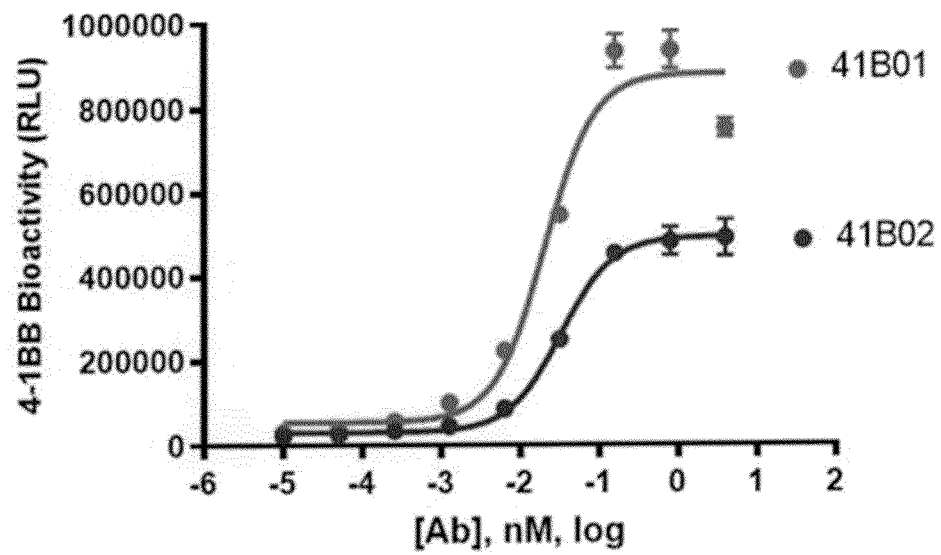


FIG. 6

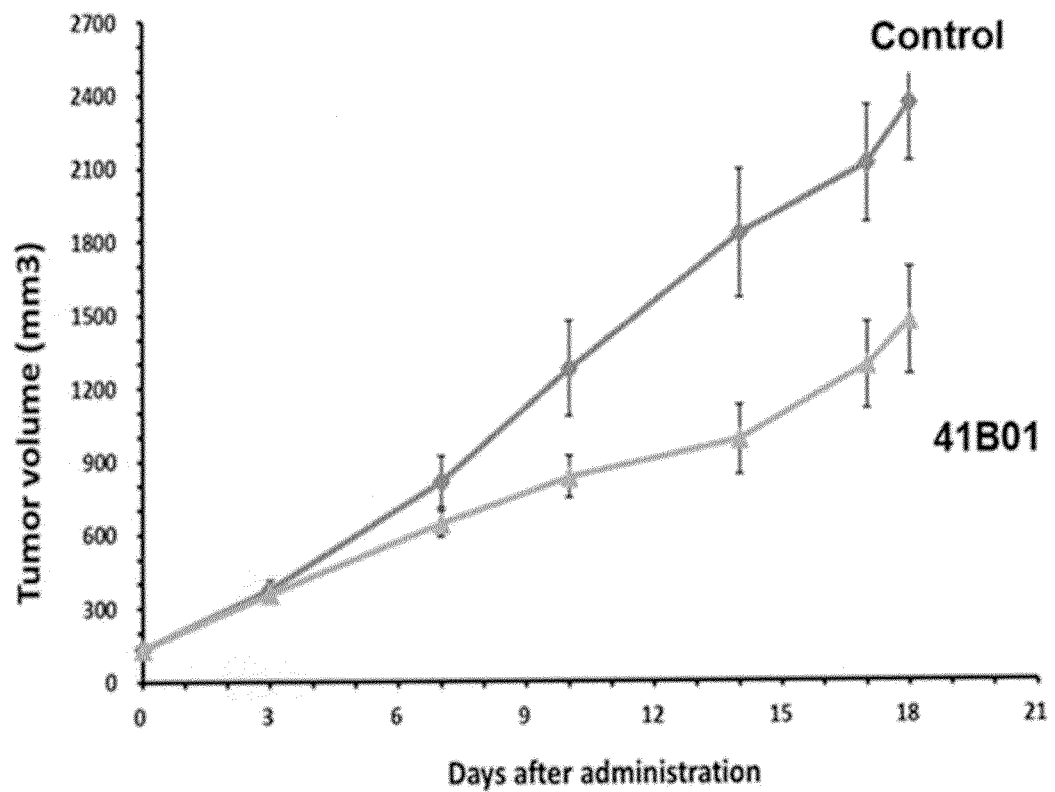


FIG. 7

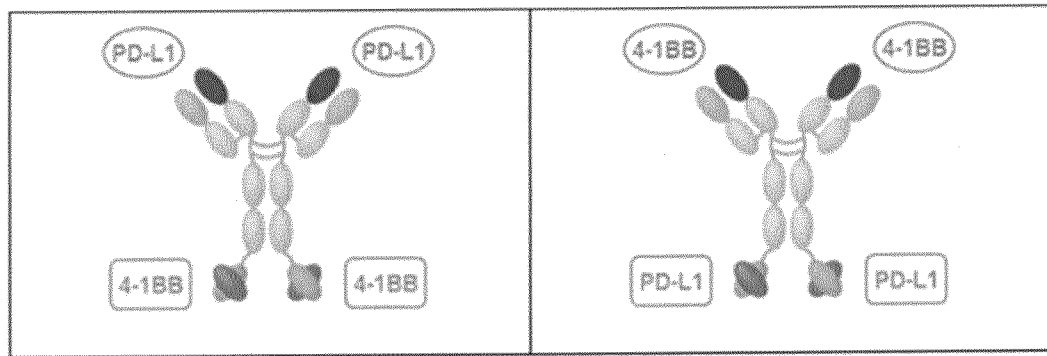


FIG. 8A

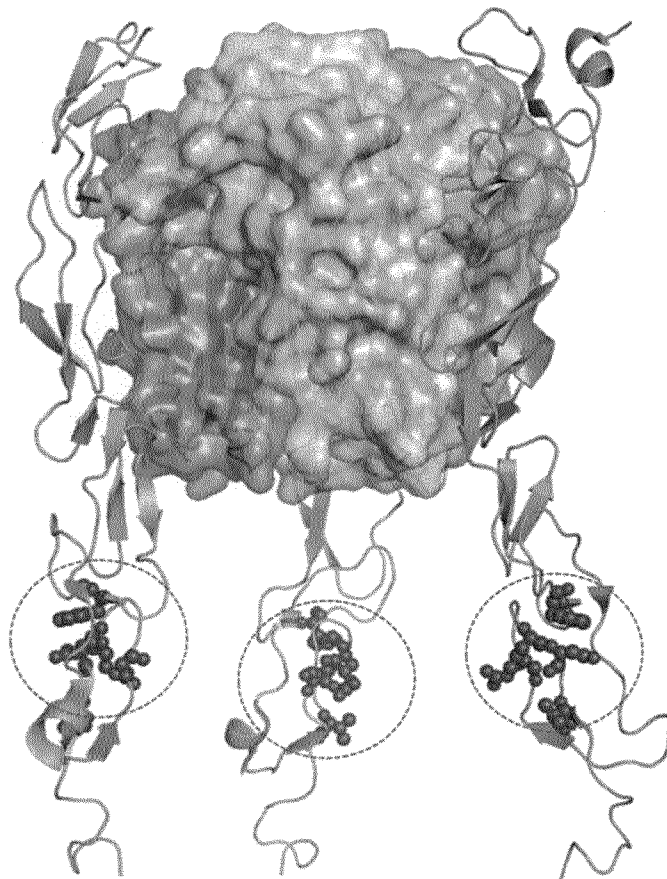


FIG. 8B

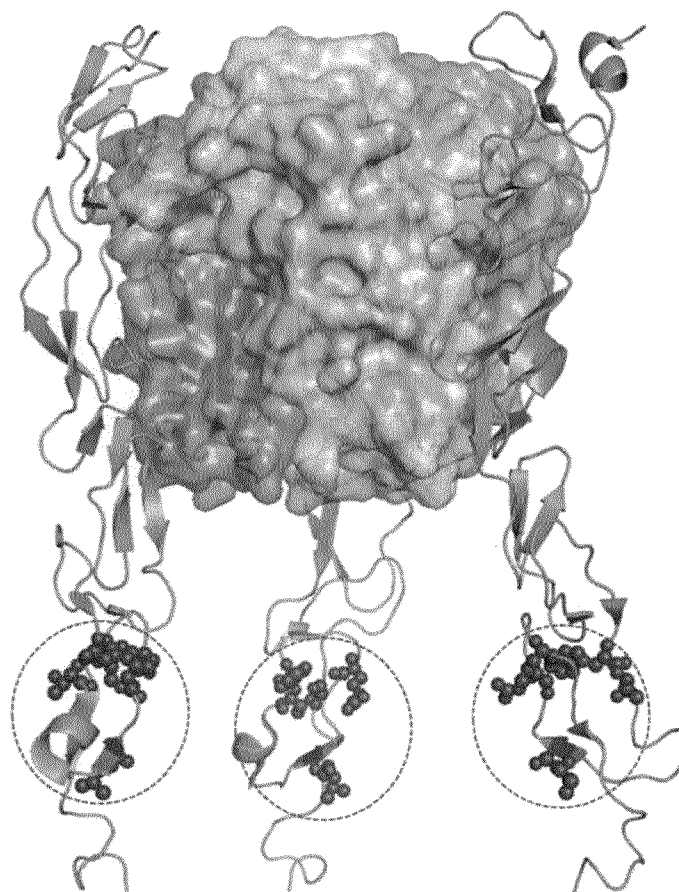


FIG. 9A

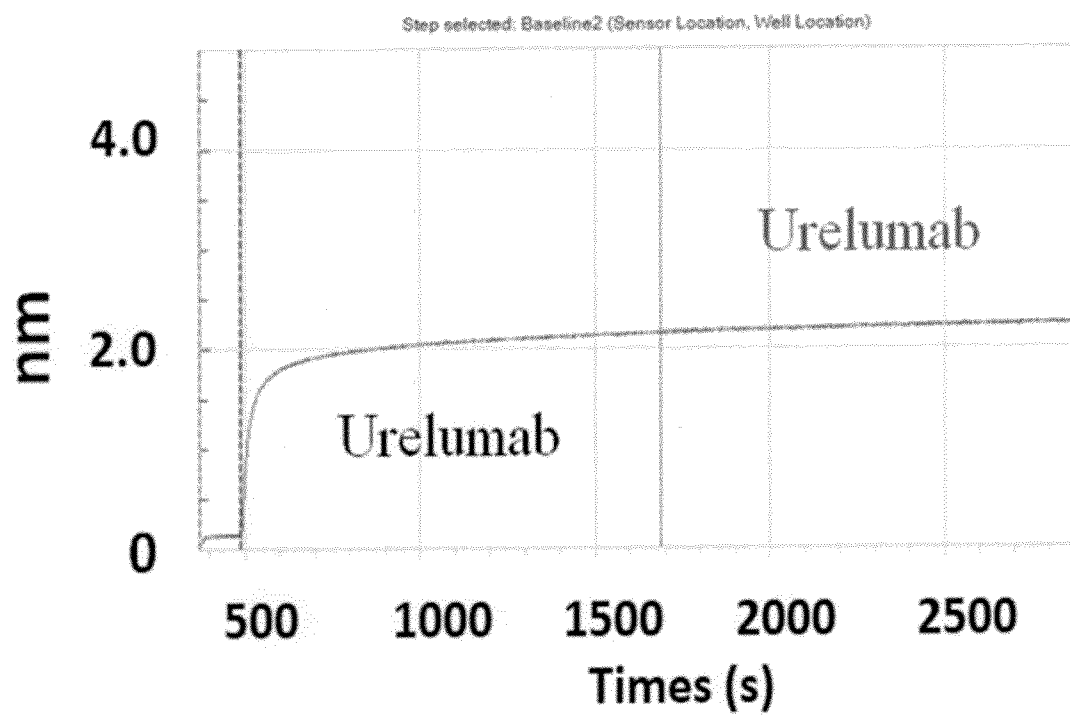


FIG. 9B

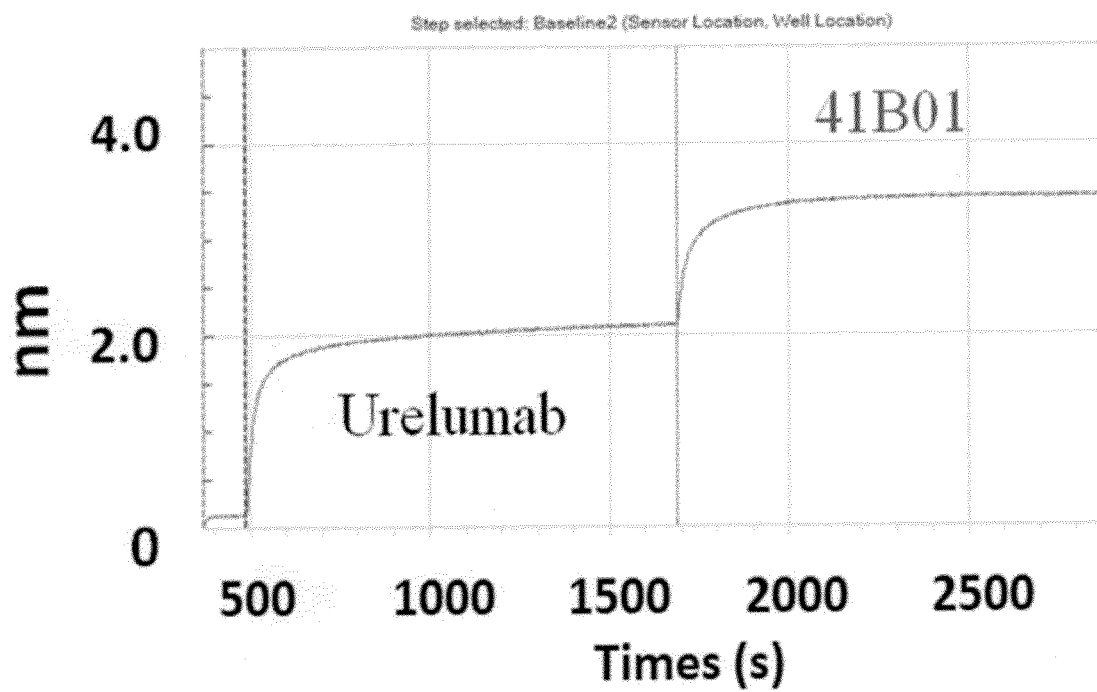


FIG. 9C

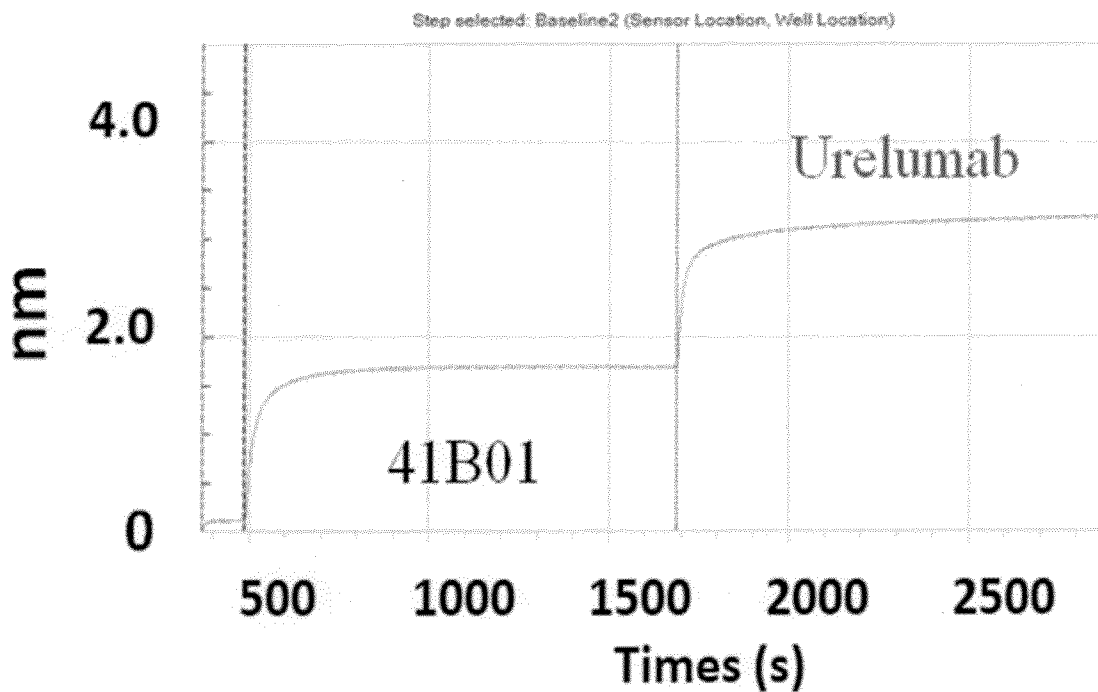


FIG. 9D

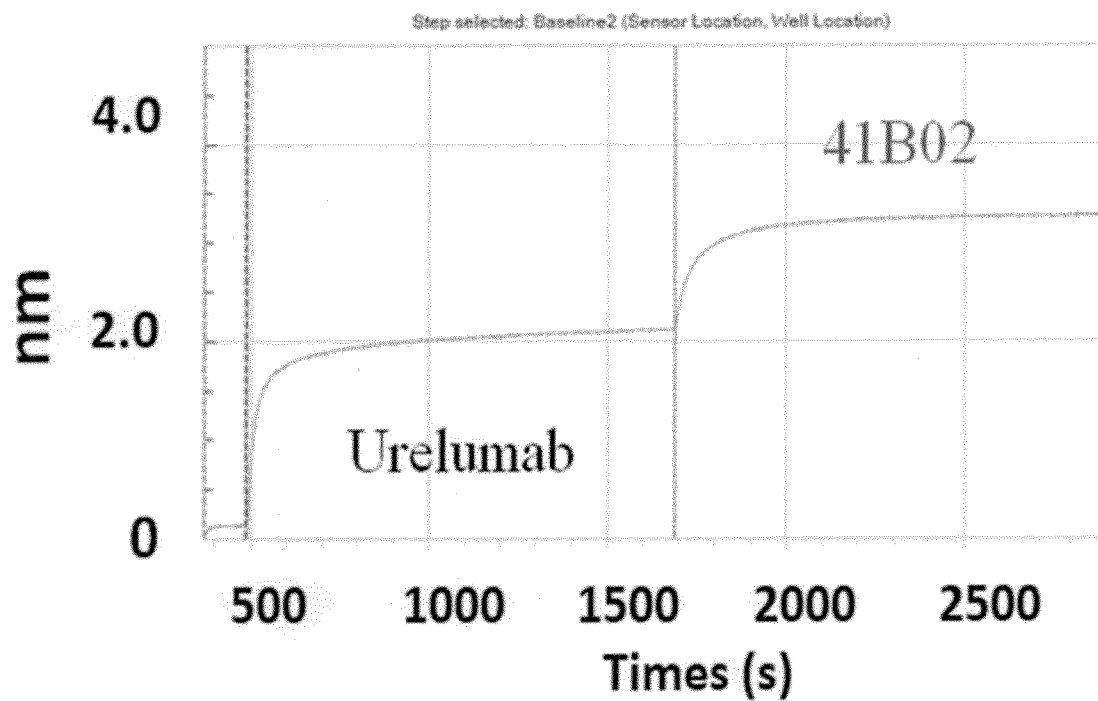


FIG. 9E

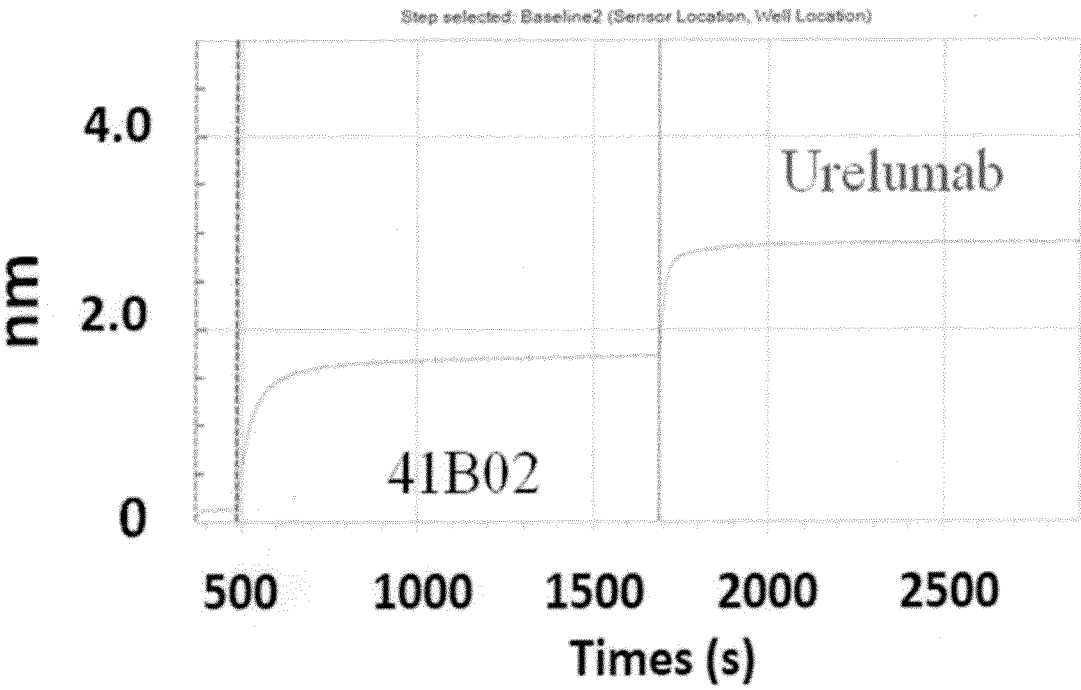


FIG. 10A

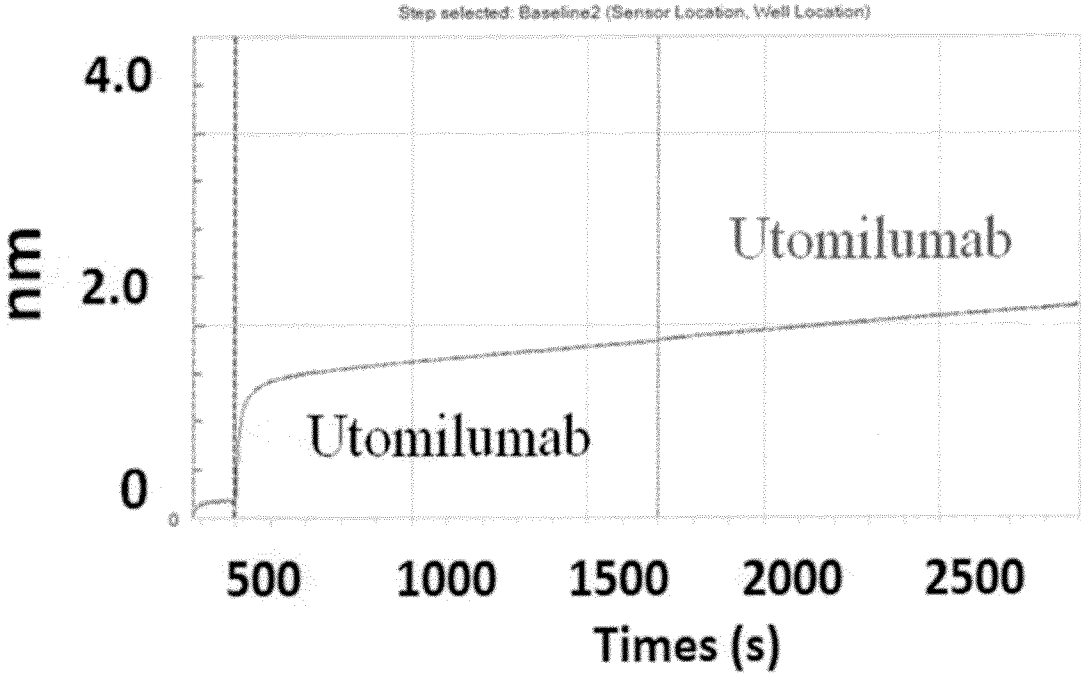


FIG. 10B

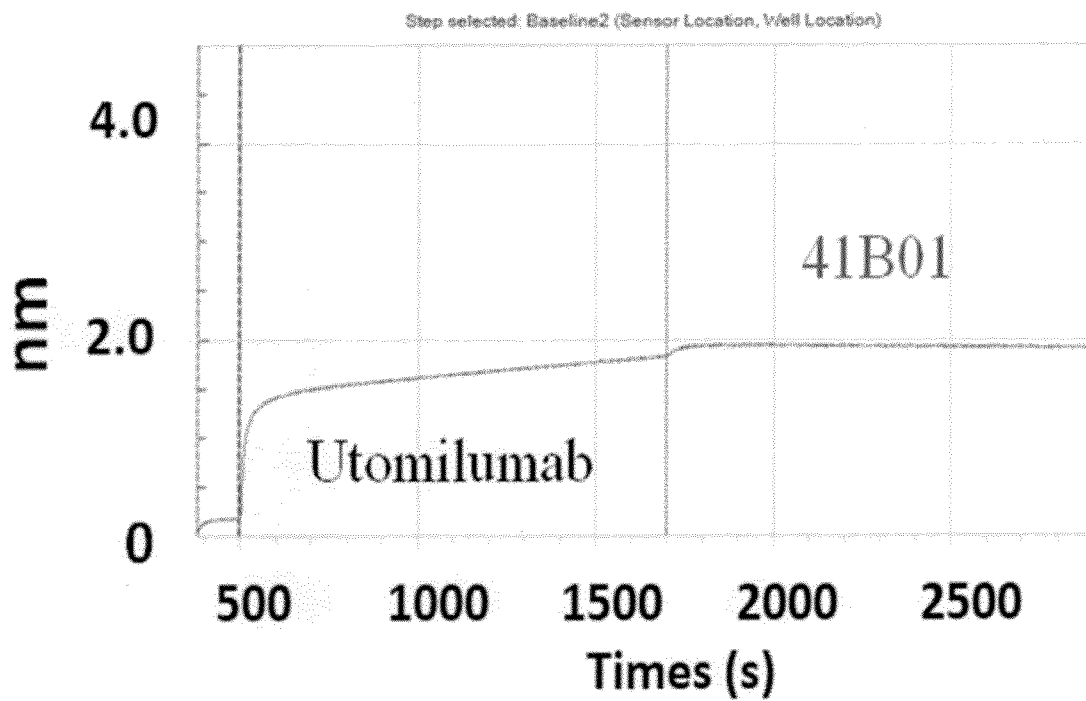


FIG. 10C

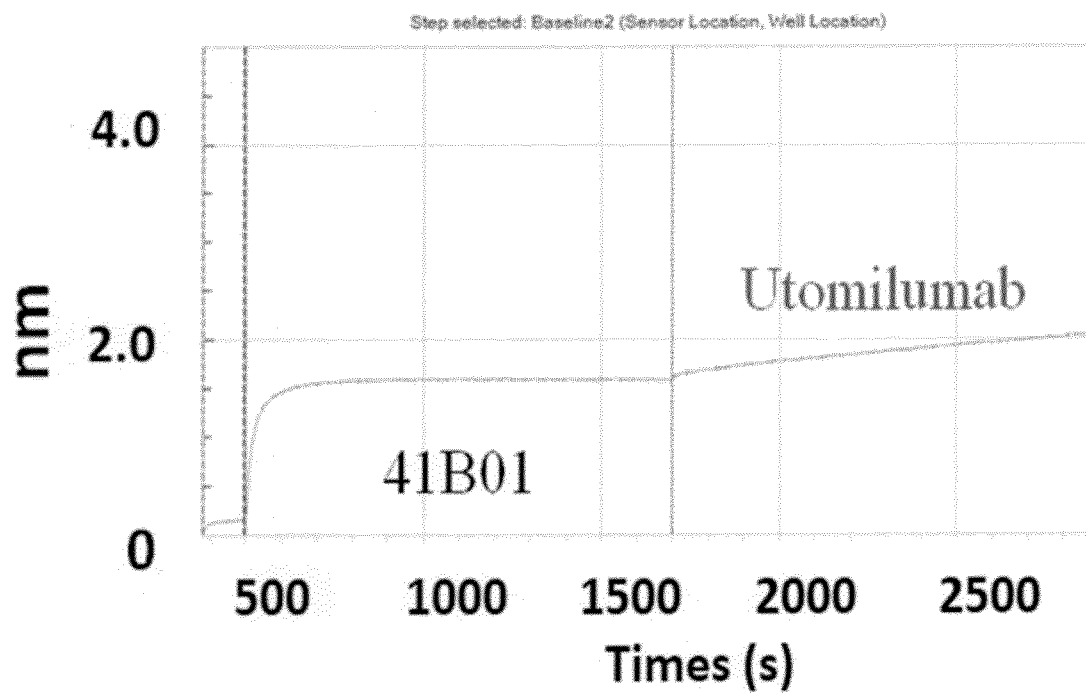


FIG. 10D

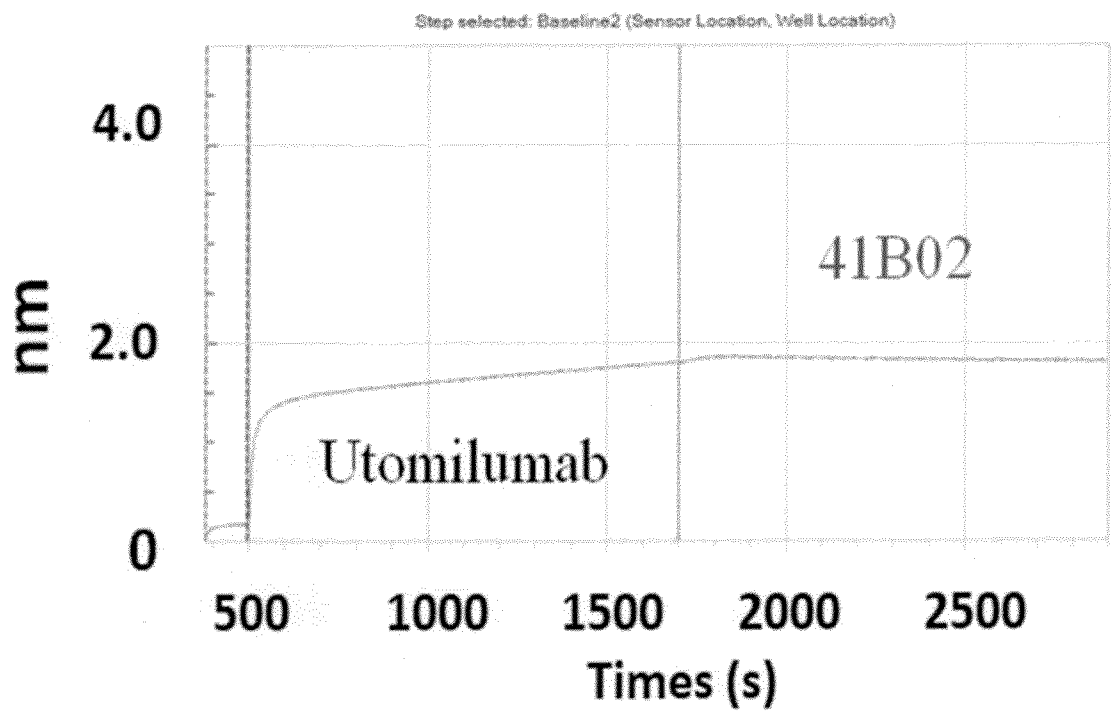


FIG. 10E

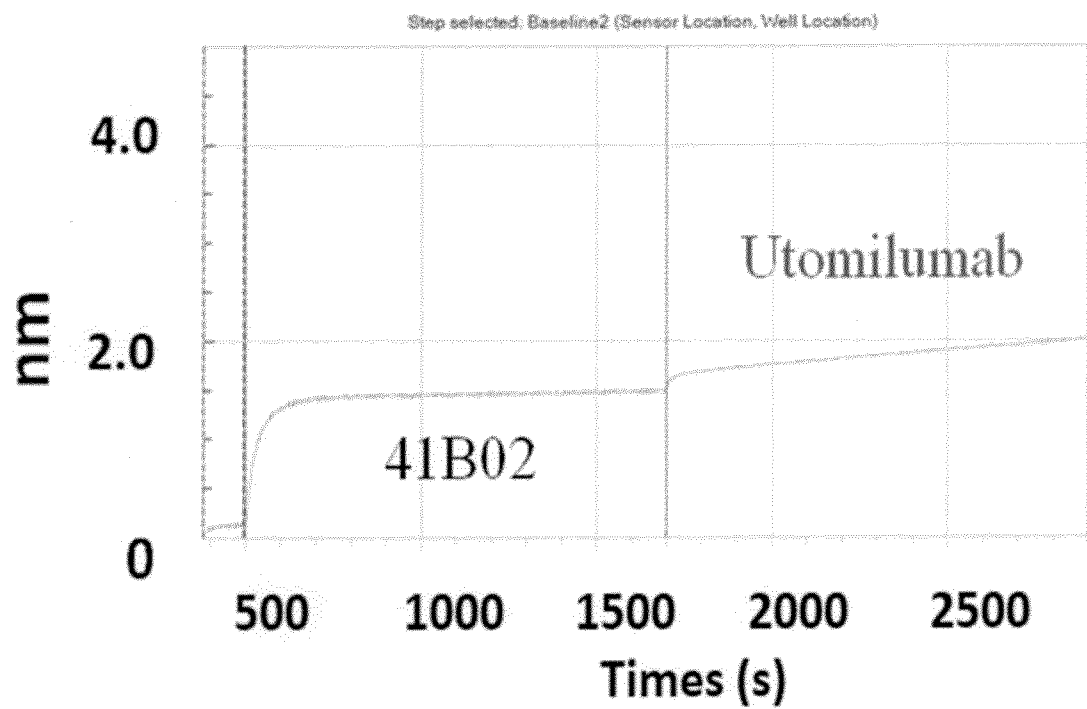


FIG. 11A

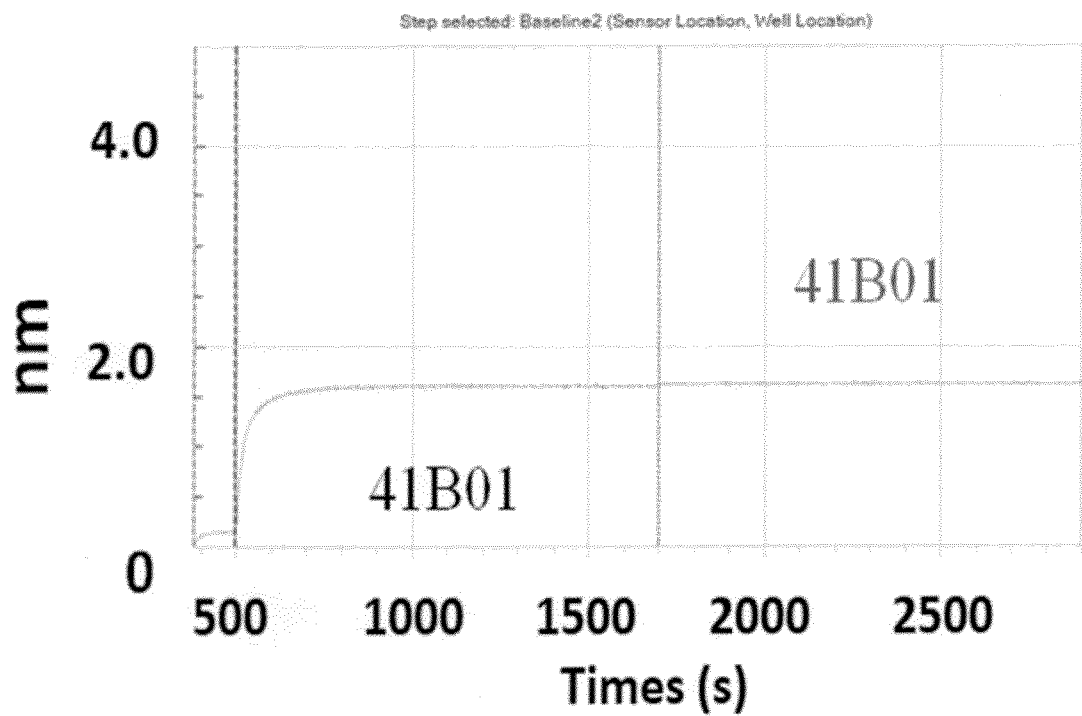


FIG. 11B

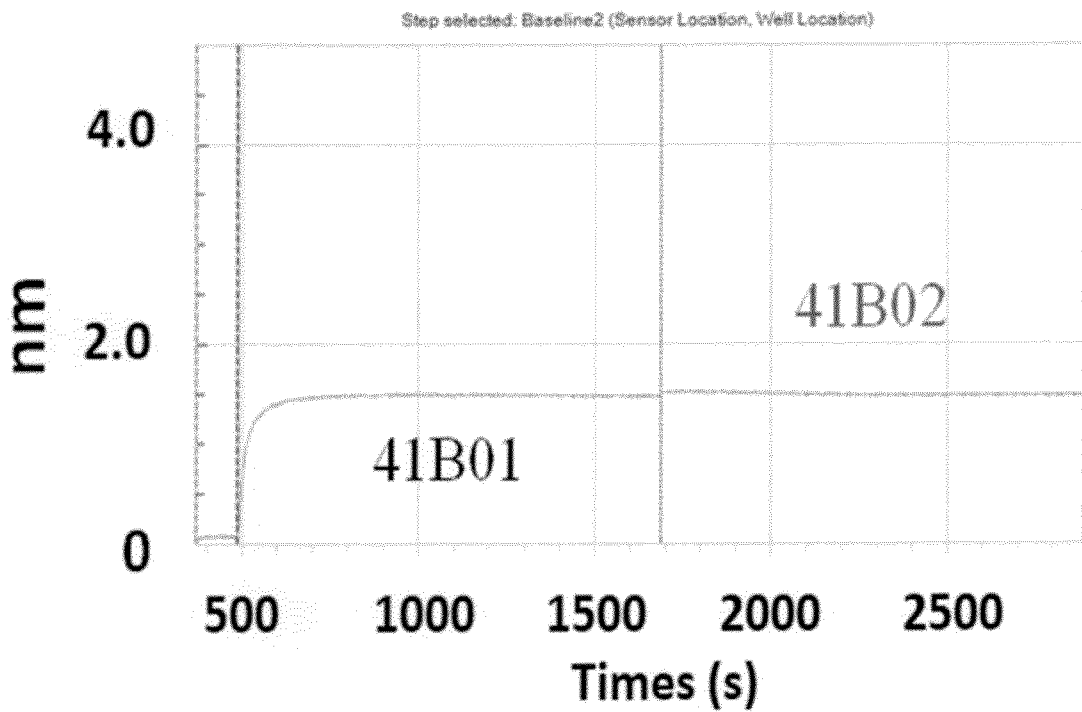


FIG. 11C

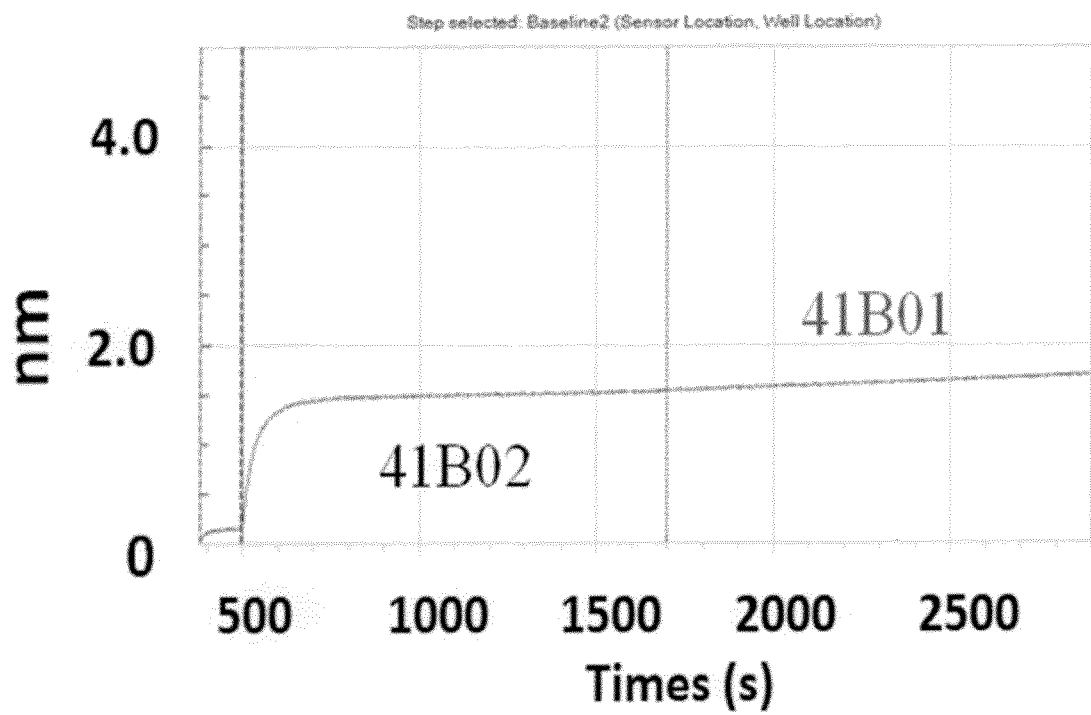


FIG. 11D

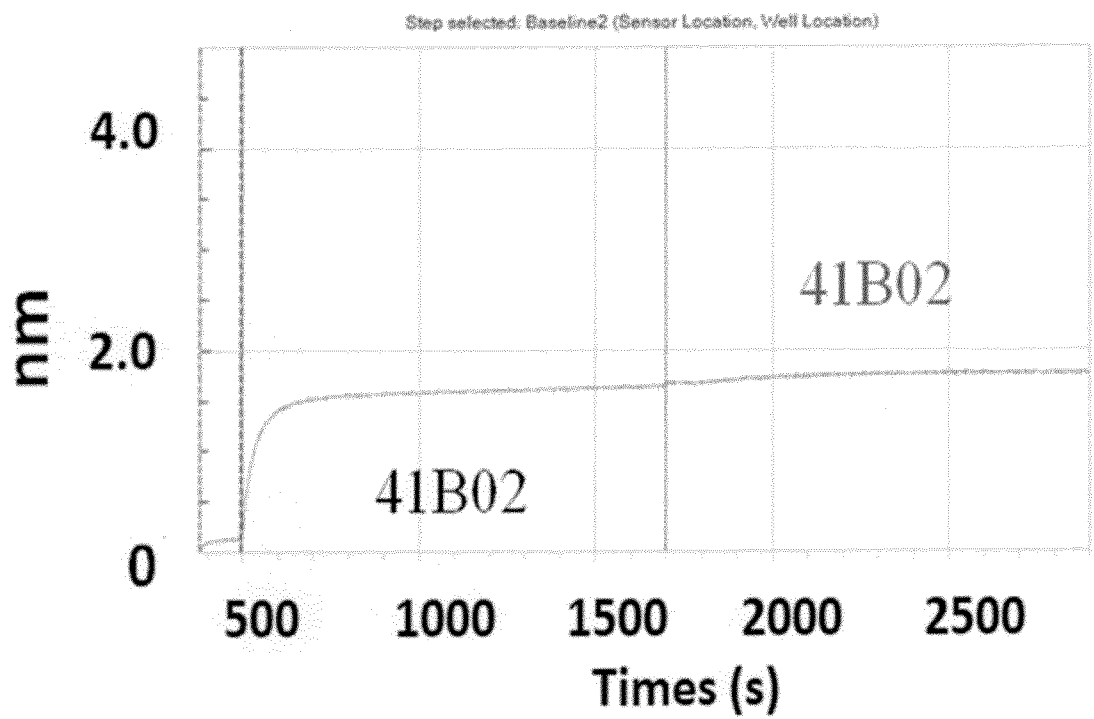


FIG. 12A

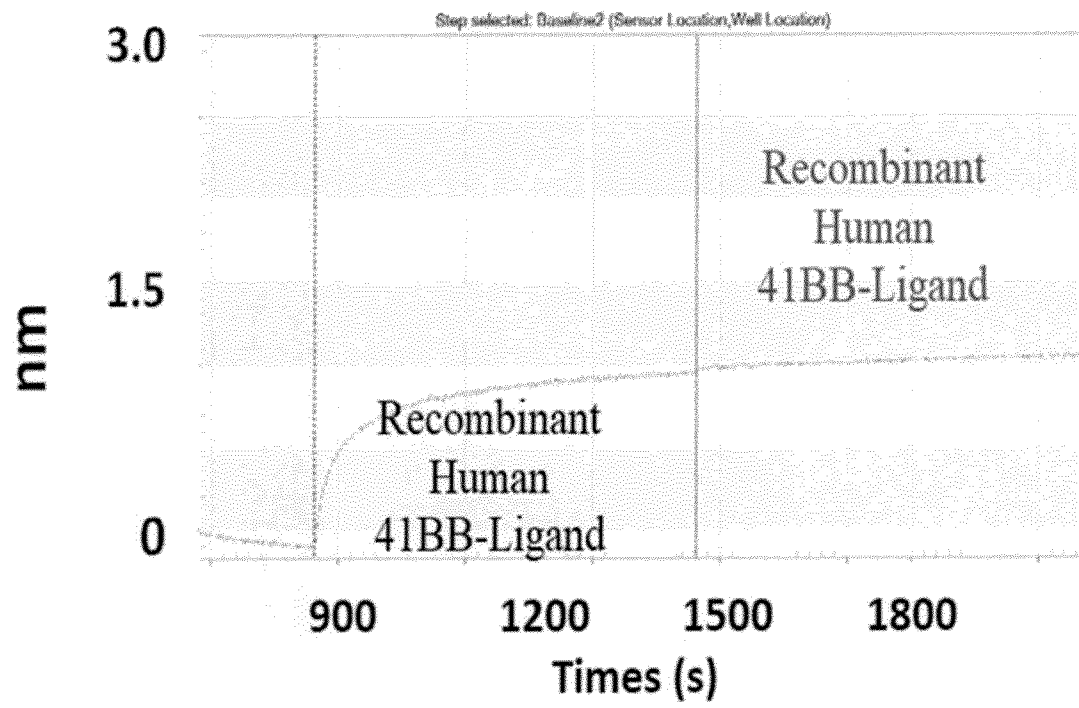


FIG. 12B

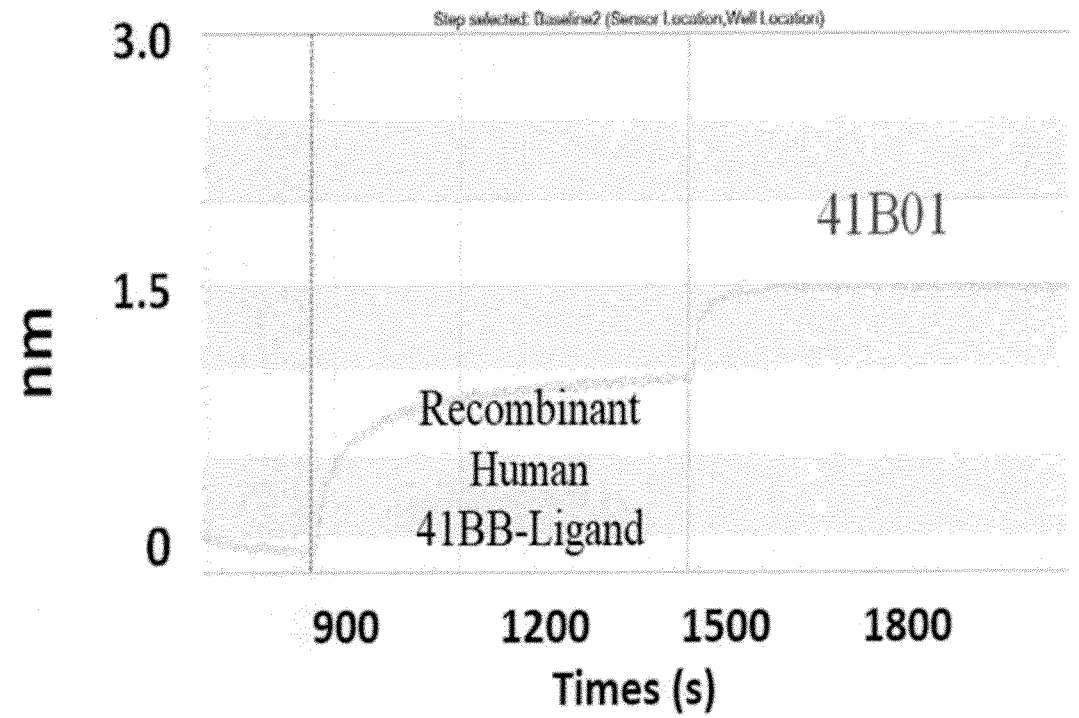
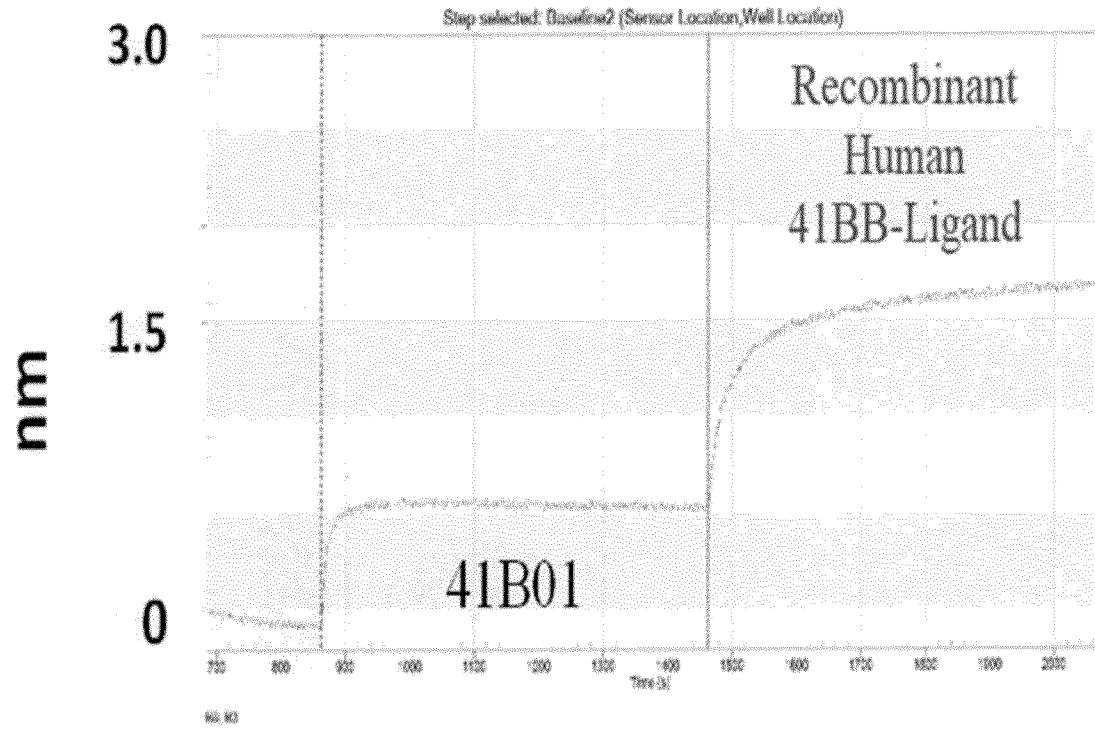


FIG. 12C



A. CLASSIFICATION OF SUBJECT MATTER**C07K 16/28(2006.01)i, A61P 35/00(2006.01)i, A61K 39/00(2006.01)i**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K 16/28; A61K 39/395; C07K 16/30; A61P 35/00; A61K 39/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & keywords: anti-4-1BB antibody, CDR, heavy chain, light chain, variable region

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2017-205745 A1 (ABBVIE BIOTHERAPEUTICS INC.) 30 November 2017 abstract; claims 1-22	1-3
A	US 2018-0258177 A1 (EUTILEX CO., LTD.) 13 September 2018 abstract; claims 1-25	1-3
A	US 2014-0178368 A1 (SHARP, L. L. et al.) 26 June 2014 the whole document	1-3
A	US 2016-0244528 A1 (SORRENTO THERAPEUTICS, INC.) 25 August 2016 the whole document	1-3
A	WO 2017-181034 A1 (BRISTOL-MYERS SQUIBB COMPANY) 19 October 2017 the whole document	1-3



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

24 March 2020 (24.03.2020)

Date of mailing of the international search report

24 March 2020 (24.03.2020)

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea

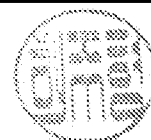


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Authorized officer

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

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

1. ☒ Claims Nos.: 12-14
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 12-14 pertain to a method for treatment of the human body by therapy or surgery, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.
2. ☒ Claims Nos.: 7,9-11,14
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Each of claims 7, 9-11, 14 refers to a claim which is not drafted in accordance with Rule 6.4(a).
3. ☒ Claims Nos.: 4-6,8,12-13
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2019/016863

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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