

Description

Title of Invention: ANTI-HUMAN INTERLEUKIN-2 ANTIBODIES AND USES THEREOF

Technical Field

- [1] The present invention relates to an antibody that binds to human interleukin-2 (hIL-2), and more particularly to an anti-hIL-2 antibody that binds specifically to a particular epitope of hIL-2, thereby inhibiting the binding of the hIL-2 to CD25.

[2]

Background Art

- [3] Interleukin-2 (IL-2) is a pleiotropic cytokine that plays an essential role in the survival, expansion and function of various lymphocytes including Treg (Foxp3⁺ CD4⁺ regulatory T) cells, natural killer cells (NK cells) and the like, which express IL-2 receptor. Interleukin-2 receptor (IL-2R) is present as high-affinity IL-2 receptor (IL-2R) and low-affinity IL-2 receptor (IL-2R) depending on its affinity. The high-affinity IL-2 receptor consists of three chains, IL-2R γ c (CD132), IL-2R β (CD122) and IL-2R α (CD25), and the low-affinity IL-2 receptor consists only of IL-2R γ c and IL-2R β chains (Boyman, O., et al., Nat Rev Immunol, 2012. 12(3): p. 180-90).
- [4] Since IL-2 stimulates CD8⁺ T cells and NK cells with anti-tumor activity, it was clinically used in the US and Europe in the 1990s for the treatment of metastatic melanoma and metastatic renal cancer (Rosenberg, S.A., J Immunol, 2014. 192(12): p. 5451-8). However, IL-2 therapy was effective in only less than 10% of cancer patients who received the therapy, and involved serious side effects. This is because IL-2 administered has a very short half-life in vivo and CD8 T cells and NK cells with anti-tumor activity express the low-affinity IL-2 receptor, and thus administration of a large amount of IL-2 is required. For this reason, serious diseases of multiple organs are caused by vascular leak syndrome and hypotension (Lotze, M.T., et al., J Immunol, 1985. 134(1): p. 157-66, Schwartz, R.N., et al., Oncology (Williston Park), 2002. 16(11 Suppl 13): p. 11-20). Another problem is that IL-2 administration induces a strong expansion of Treg cells that express the high-affinity IL-2 receptor and that inhibit anti-tumor immunity mediated by CD8⁺ T cells and NK cells (Brandenburg, S., et al., Eur J Immunol, 2008. 38(6): p. 1643-53; Facciabene, A., et al., Cancer Res, 2012. 72(9): p. 2162-71). A method for overcoming these disadvantages of IL-2 therapy is to extend the in vivo half-life of IL-2 and, at the same time, selectively activate the CD8⁺ T cells and NK cells that express the low-affinity IL-2 receptor. There have been many attempts to do this, but there has been little success (Arenas-Ramirez, N., et al., Sci Transl Med, 2016. 8(367): p. 367ra166).

- [5] Recently, modification of the amino acid residues of IL-2 that binds to the high-affinity IL-2 receptor has been proposed as a solution. However, this method has a limitation in that it can provide a modified IL-2 that has immunogenicity or susceptibility to proteases that degrade an artificially introduced amino acid sequence (Levin, A.M., et al., Nature, 2012. 484(7395): p. 529-33).
- [6] Accordingly, the present inventors have made extensive efforts to develop a method that extends the in vivo half-life of IL-2 without causing an unnatural modification of IL-2, and at the same time, selectively activates the CD8⁺ T cells and NK cells that express the low-affinity IL-2 receptor. As a result, the present inventors have found that, when an anti-IL-2 monoclonal antibody (mAb) having a particular specificity is bound to IL-2, it selectively inhibits the binding of IL-2 to the high-affinity IL-2 receptor, thereby completing the present invention.
- [7]
- [8] The information disclosed in the Background Art section is only for the enhancement of understanding of the background of the present invention, and therefore may not contain information that forms a prior art that would already be known to a person of ordinary skill in the art.
- [9]
- [10] **DISCLOSURE OF INVENTION**
- [11] **Technical Problem**
- [12] It is an object of the present invention to provide an anti-hIL-2 antibody or antigen-binding fragment thereof, which binds specifically to human interleukin-2 (hIL-2), and inhibits the binding of the hIL-2 to CD25.
- [13] Another object of the present invention is to provide a nucleic acid encoding the anti-hIL-2 antibody or antigen-binding fragment thereof, a vector comprising the nucleic acid, a cell transformed with the vector, and a method of producing an anti-hIL-2 antibody or antigen-binding fragment thereof using the same.
- [14] Still another object of the present invention is to provide a composition and treatment method for preventing or treating cancer, which comprises the anti-hIL-2 antibody or antigen-binding fragment thereof as an active ingredient.
- [15] Yet another object of the present invention is to provide a bispecific antibody or antibody-drug conjugate comprising the anti-hIL-2 antibody or antigen-binding fragment thereof, and a composition and treatment method for preventing or treating cancer, which comprises the bispecific antibody or antibody-drug conjugate as an active ingredient.
- [16] A further object of the present invention is to provide a co-administration composition and treatment method for cancer treatment, which comprises the anti-hIL-2 antibody or antigen-binding fragment thereof and an immune checkpoint inhibitor.

[17]

[18] **Technical Solution**

[19] To achieve the above object, the present invention provides an anti-hIL-2 antibody or antigen-binding fragment thereof that comprises: a heavy-chain variable region comprising a heavy-chain CDR1 comprising an amino acid sequence of SEQ ID NO: 11, a heavy-chain CDR2 comprising an amino acid sequence of SEQ ID NO: 12, and a heavy-chain CDR3 comprising an amino acid sequence of SEQ ID NO: 13; and a light-chain variable region comprising a light-chain CDR1 comprising an amino acid sequence of SEQ ID NO: 14, a light-chain CDR2 comprising an amino acid sequence of SEQ ID NO: 15, and a light-chain CDR3 comprising an amino acid sequence of SEQ ID NO: 16.

[20] The present invention also provides a nucleic acid encoding the anti-hIL-2 antibody or antigen-binding fragment thereof, a vector comprising the nucleic acid, a cell transformed with the vector, and a method of producing an anti-hIL-2 antibody or antigen-binding fragment thereof using the same.

[21] The present invention also provides a complex in which the anti-hIL-2 antibody or antigen-binding fragment thereof is bound to hIL-2.

[22] The present invention also provides a composition and treatment method for preventing or treating cancer, which comprises the anti-hIL-2 antibody or antigen-binding fragment thereof as an active ingredient.

[23] The present invention also provides a bispecific antibody or antibody-drug conjugate comprising the anti-hIL-2 antibody or antigen-binding fragment thereof, and a composition and treatment method for preventing or treating cancer, which comprises the bispecific antibody or antibody-drug conjugate as an active ingredient.

[24] The present invention also provides a co-administration composition and treatment method for cancer treatment, which comprises the anti-hIL-2 antibody or antigen-binding fragment thereof and an immune checkpoint inhibitor.

[25] The present invention also provides the use of the anti-hIL-2 antibody or antigen-binding fragment thereof for the prevention or treatment of cancer.

[26] The present invention also provides the use of the anti-hIL-2 antibody or antigen-binding fragment thereof for the preparation of a medicine for the prevention or treatment of cancer.

[27] The present invention also provides a composition for enhancing vaccine efficacy, which comprises the anti-hIL-2 antibody or antigen-binding fragment thereof as an active ingredient.

[28]

Brief Description of Drawings

- [29] FIG. 1 shows the results of testing the binding specificity of a TCB2 monoclonal antibody against hIL-2.
- [30] FIG. 2 shows the in vivo immunostimulatory effect of a hIL-2/TCB2 complex. FIG. 2A shows the results of analyzing the frequency of immune cells; FIG. 2B shows the results of analyzing the expression of CD44 and CD62L in CD4 and CD8 T cells; FIG. 2C shows the results of experimental statistical analysis; and FIG. 2D shows the effect of a hIL-2/MAB602 or hIL-2/TCB2 complex on expansion of immune cells and the results of experimental statistical analysis (**p < 0.01, ***p < 0.001 (unpaired t test)).
- [31] FIG. 3 shows surface plasmon resonance curves obtained using Biacore T100 for the affinities of anti-hIL-2 mAbs for hIL-2.
- [32] FIG. 4 shows the effect of a hIL-2/TCB2 complex against a solid tumor (***p < 0.001 (Two way ANOVA for day 12, unpaired t test for day 14)).
- [33] FIG. 5 shows the effect of TCB2 mAb against a metastatic tumor (***p < 0.001 (unpaired t test)).
- [34] FIG. 6 shows the anti-tumor effect of a combination of a hIL-2/TCB2 complex and tumor peptide therapy in B6F10 melanoma models (***p < 0.001 (Two way ANOVA)).
- [35] FIG. 7 shows the anti-tumor effect of a combination of a hIL-2/TCB2 complex and an anti-CTLA-4 antibody in CT26 tumor models (Balb/C colon cancer) (**p < 0.01 (Two way ANOVA for day 17, unpaired t test for day 24)).
- [36] FIG. 8 shows the anti-tumor effect of a combination of a hIL-2/TCB2 complex and an anti-PD-1 antibody in MC38 tumor models (B6 colon cancer) (*p < 0.05, **p < 0.01 (Two way ANOVA for day 19, unpaired t test for day 21)).
- [37] FIG. 9 shows the in vivo immunostimulatory of a hIL-2/hnTCB2 complex and the results of experimental statistical analysis.
- [38] FIG. 10 shows the anti-tumor effect of a combination of a hIL-2/hnTCB2 complex and an anti-PD-1 antibody in MC38 tumor models (B6 colon cancer) (*p < 0.05, **p < 0.01 (Two way ANOVA for day 19 and 22, unpaired t test for day 25)).

[39]

[40] **Best Mode For Carrying Out The Invention**

[41] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present disclosure belongs. In general, the nomenclature used herein is well known and commonly used in the art.

[42]

[43] In the present invention, efforts have been made to develop a method that extends the in vivo half-life of IL-2 without causing an unnatural modification of IL-2, and at the same time, selectively activates the CD8⁺ T cells and NK cells that express the low-

affinity IL-2 receptor. As a result, it has been found that, when an anti-IL-2 monoclonal antibody (mAb) having a particular specificity is bound to IL-2, it selectively inhibits the binding of IL-2 to the high-affinity IL-2 receptor.

[44]

[45] In one aspect, the present invention is directed to an anti-hIL-2 antibody (referred to as “TCB2” in the specification) or antigen-binding fragment thereof, which binds specifically to human interleukin-2 (hIL-2) and inhibits the binding of the hIL-2 to CD25.

[46] As used herein, the term “human interleukin-2 (hIL-2)” refers to a 133-amino-acid protein (15.4 kDa) having no substantial sequence homology with any other factors.

[47] As used herein, the term “CD25” refers to the IL-2R α chain of IL-2 receptor. The IL-2 receptor is present as high-affinity IL-2 receptor (IL-2R) and low-affinity IL-2 receptor (IL-2R) depending on its affinity, and CD25 is a chain that is not present in the low-affinity IL-2 receptor and is present only in the high-affinity IL-2 receptor.

[48] The term “antibody” as used in the invention refers to a substance produced by the stimulus of an antigen in immune system and its kinds are not particularly limited. Lately, the antibodies have been widely used for treating diseases. As the antibodies are very stable in vivo as well as in vitro and have a long half-life, they are favorable for mass expression and production. Also, since the antibody has intrinsically a dimer structure, it has a fairly high avidity. An intact antibody has a structure with two full-length light chains and two full-length heavy chains, and each light chain is linked to each heavy chain via a disulfide bond. The constant region of an antibody is divided into a heavy chain constant region and a light chain constant region, and the heavy chain constant region has gamma (γ), mu (μ), alpha (α), delta (δ) and epsilon (ϵ) types, and has gamma1 (γ 1), gamma2 (γ 2), gamma3 (γ 3), gamma4 (γ 4), alpha1 (α 1) and alpha2 (α 2) as its subclass. The light chain constant region has kappa (κ) and lambda (λ) types.

[49] The antibody in the invention may include an animal-derived antibody, a chimeric antibody, a humanized antibody, or a fully human antibody. An animal-derived antibody which is produced by immunizing an animal with a desired antigen may generally trigger an immune rejection response when administered to humans for treatment purpose, and a chimeric antibody has been developed to suppress such immune rejection response. A chimeric antibody is formed by replacing the constant region of an animal-derived antibody, which is a cause of an anti-isotype response, with the constant region of a human antibody using genetic engineering methods. The chimeric antibody has considerably improved anti-isotype response in comparison with animal-derived antibodies, but animal-derived amino acids are still present in its variable regions and thus it still contains potential side effects resulting from an anti-

idiotypic response. It is a humanized antibody that has been thus developed to improve such side effects. This is manufactured by grafting CDRs (complementarity determining regions) which, of the variable regions of a chimeric antibody, have an important role in antigen binding into a human antibody framework.

[50] A “humanized antibody” as used herein includes a humanized light chain variable domain immunoglobulin and a humanized heavy chain variable domain immunoglobulin. The humanized antibody may include a constant region partially or wholly derived from (including synthetic analogs) one or more human gene sequence. A humanized antibody is expected to bind to the same target antigen as a donor antibody which supplied the CDRs. Typically, all segments or portions of the humanized antibody or immunoglobulin, with the exception of the CDRs, are substantially identical or substantially homologous to corresponding segments or portions of naturally occurring or consensus human immunoglobulin sequences. It is important in CDR grafting technology for manufacturing a humanized antibody to select an optimized human antibody which can receive best the CDR of an animal-derived antibody and for this, utilization of antibody database, analysis of crystal structure, molecule modeling technology, etc. are employed. However, although the CDR of an animal-derived antibody is grafted into an optimized human antibody framework, there are a considerable number of cases where antigen binding affinity is not preserved because there are amino acids which affect antigen binding while being positioned at the framework of the animal-derived antibody. In this regard, it may be necessary to apply an additional antibody engineering technology for restoring antigen binding affinity.

[51] As used herein, the term “monoclonal antibody (mAb)” has the same meaning as commonly used in the technical field to the present invention pertains, and means an antibody that recognizes a single epitope on an antigen to which it binds. This contrasts with a polyclonal antibody which is a collection of different antibodies that bind to the same antigen but bind to different epitopes of the antigen. For this reason, a single antigen molecule can be bound simultaneously by multiple polyclonal antibodies, but a particular monoclonal antibody specific for the antigen can be bound by only one molecule. After being bound by the single monoclonal antibody molecule, the bound epitope is blocked, and thus can no longer be bound by other monoclonal antibodies. The monoclonal nature of antibodies is particularly suitable for use as therapeutic agents. This is because these antibodies are single, homologous molecular species, and thus can be very well characterized, can be produced reproducibly, and purified. These factors make it possible to produce products whose biological activity can be predicted with a very high level of accuracy. These factors are particularly important, because these molecules must obtain permission from authorities for therapeutic administration

to mammals, particularly humans.

- [52] The term “heavy chain” as used herein may be interpreted to include a full-length heavy chain including a variable region domain VH including an amino acid sequence having a variable region sequence sufficient to confer antigen-specificity, three constant region domains CH1, CH2 and CH3, and a hinge, and a fragment thereof. Also, the term “light chain” as used herein may be interpreted to include a full-length light chain including a variable region domain VL including an amino acid sequence having a variable region sequence sufficient to confer antigen-specificity and a constant region domain CL, and a fragment thereof.
- [53] In the present invention, the anti-hIL-2 antibody or antigen-binding fragment thereof may comprise: a heavy-chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 23, 28, 32, and 34; and a light-chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 24, 26, and 30. Preferably, the anti-hIL-2 antibody or antigen-binding fragment thereof may comprise: a heavy-chain variable region of SEQ ID NO: 3 and a light-chain variable region of SEQ ID NO: 4; a heavy-chain variable region of SEQ ID NO: 23 and a light-chain variable region of SEQ ID NO: 24; a heavy-chain variable region of SEQ ID NO: 28 and a light-chain variable region of SEQ ID NO: 26; a heavy-chain variable region of SEQ ID NO: 32 and a light-chain variable region of SEQ ID NO: 30; or a heavy-chain variable region of SEQ ID NO: 34 and a light-chain variable region of SEQ ID NO: 30.
- [54] As used herein, the term “complementarity determining region (CDR)” refers to the amino acid sequence of the hypervariable region of the heavy chain or light chain of immunoglobulin. Each of the heavy and light chains may comprise three CDRs (i.e., a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3; and a light chain CDR1, a light chain CDR2, and a light chain CDR3). The CDR may provide important contact residues for the binding of the antibody to an antigen or an epitope.
- [55] In the present invention, the anti-hIL-2 antibody or antigen-binding fragment thereof may comprise: a heavy-chain variable region comprising a heavy-chain CDR1 comprising a DNA sequence of SEQ ID NO: 5, a heavy-chain CDR2 comprising a DNA sequence of SEQ ID NO: 6, and a heavy-chain CDR3 comprising a DNA sequence of SEQ ID NO: 7; and a light-chain variable region comprising a light-chain CDR1 comprising a DNA sequence of SEQ ID NO: 8, a light-chain CDR2 comprising a DNA sequence of SEQ ID NO: 9, and a light-chain CDR3 comprising a DNA sequence of SEQ ID NO: 10.
- [56] In the present invention, the anti-hIL-2 antibody or antigen-binding fragment thereof may comprise: a heavy-chain variable region comprising a heavy-chain CDR1 comprising an amino acid sequence of SEQ ID NO: 11, a heavy-chain CDR2

comprising an amino acid sequence of SEQ ID NO: 12, and a heavy-chain CDR3 comprising an amino acid sequence of SEQ ID NO: 13; and a light-chain variable region comprising a light-chain CDR1 comprising an amino acid sequence of SEQ ID NO: 14, a light-chain CDR2 comprising an amino acid sequence of SEQ ID NO: 15, and a light-chain CDR3 comprising an amino acid sequence of SEQ ID NO: 16.

[57] As used herein, the term “specifically binding” has the same meaning as generally known to a person of ordinary skill in the art, indicating that an antigen and an antibody specifically interact with each other to lead to an immunological response. In the present invention, the human monoclonal antibody or its fragment has the ability to discriminate human IL-2 (hIL-2) from several other potential antigens. The discrimination is achieved such that the monoclonal antibody or its fragment binds only or to a significant extent to hIL-2 as a potential binding partner in a pool of multiple different antigens. In this regard, “bind to a significant extent to hIL-2” means that hIL-2 as a potential binding partner in a pool of a plurality of equally accessible different antigens binds with an affinity at least 10-fold, preferably 50-fold, preferably 100-fold higher than antigens other than hIL-2.

[58] As used herein, the term “antigen-binding fragment,” which is a fragment of the full structure of an immunoglobulin, refers to some of a polypeptide including a portion to which an antigen can bind. For example, it may be a scFv, a (scFv)₂, a Fab, a Fab' or a F(ab')₂, but is not limited thereto. Among the above antigen-binding fragments, a Fab, which is a structure having the light chain and heavy chain variable regions, the light chain constant region, and the heavy chain first constant region (C_{H1}), has one antigen binding site. A Fab' differs from the Fab in that the Fab' has a hinge region including at least one cysteine residue at the C-terminal of the heavy chain CH1 domain. A F(ab')₂ is produced when cysteine residues at the hinge region of Fab' are joined by a disulfide bond. A Fv is a minimal antibody fragment, having only heavy chain variable regions and light chain variable regions, and a recombinant technique for producing the Fv fragment is well known in the art. A two-chain Fv may have a structure in which heavy chain variable regions are linked to light chain variable regions by a non-covalent bond, and a single-chain Fv may generally form a dimer structure as in the two-chain Fv, wherein heavy chain variable regions are covalently bound to light chain variable regions via a peptide linker or the heavy and light chain variable regions are directly linked to each other at the C-terminals thereof. The linker may be a peptide linker including 1 to 100 or 2 to 50 any amino acids, and proper sequences thereof have been known in the art. The antigen-binding fragment may be obtained using a protease (for example, a whole antibody can be digested with papain to obtain Fab fragments, or can be digested with pepsin to obtain F(ab')₂ fragments), or may be prepared by a genetic recombinant technique. The antigen-binding fragment of the antibody of the present

invention may be a fragment including one or more CRDs.

[59] In the present invention, the anti-hIL-2 antibody or antigen-binding fragment thereof may induce expansion of CD8⁺ T cells and NK cells. In an example of the present invention, it was found that the anti-hIL-2 antibody according to the present invention induced activation of CD8⁺ T cells and NK cells and induced little expansion of Treg cells.

[60]

[61] In another aspect, the present invention is directed to a nucleic acid encoding the anti-hIL-2 antibody or the antigen-binding fragment thereof.

[62] In the present invention, the nucleic acid encoding the anti-hIL-2 antibody or antigen-binding fragment thereof may comprise a sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31 or SEQ ID NO: 33. Specifically, the nucleic acid encoding the heavy chain of the antibody according to the present invention may comprise a sequence of SEQ ID NO: 27, 31 or 33, and/or the nucleic acid encoding the light chain of the antibody according to the present invention may comprise a sequence of SEQ ID NO: 2, 25 or 29.

[63] The antibody or antigen-binding fragment thereof of the present invention may be recombinantly produced by isolating the nucleic acid encoding an antibody or antigen-binding fragment thereof. The nucleic acid is isolated and inserted into a replicable vector to result in further cloning (amplification of DNA) or further expression.

[64] As used herein, the term "Nucleic acid" has a broad meaning including DNA (gDNA and cDNA) and RNA molecules. Nucleotides, basic elements of nucleic acids, include natural nucleotides as well as analogues in which sugar or base sites are modified. The sequence of the nucleic acid encoding the heavy and light chain variable regions of the present invention may be modified. Such modifications include the addition, deletion, or non-conservative substitution or conservative substitution of nucleotides.

[65] The nucleic acid of the present invention is interpreted to include a nucleotide sequence that exhibits substantial identity to the nucleotide sequence. The substantial identity means a nucleotide sequence showing at least 80% homology, more preferably at least 90% homology, and most preferably at least 95% homology by aligning the nucleotide sequence of the present invention with any other sequence as much as possible and analyzing the aligned sequence using algorithms commonly used in the art.

[66] The DNA encoding the antibody can be easily separated or synthesized using conventional procedures (for example, using an oligonucleotide probe capable of specifically binding to DNA encoding the heavy chain and the light chain of the antibody).

[67] In still another aspect, the present invention is directed to a recombinant vector including the nucleic acid.

- [68] Many vectors are available. Vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.
- [69] The term “vector” as used herein, includes a plasmid vector; a cosmid vector; a bacteriophage vector; and a viral vector, e.g., an adenovirus vector, retroviral vectors, and adeno-associated viral vectors as a mean for expressing a target gene in a host cell. The nucleic acid encoding the antibody in the vector is operably linked to a promoter.
- [70] As used herein, the term “operably linked” refers to a functional linkage between a nucleic acid expression control sequence (e.g., an array of promoter, signal sequence, or transcription regulation factor binding site) and another nucleic acid sequence, and thus the control sequence controls the transcription and/or translation of the other nucleic acid sequence.
- [71] When a prokaryotic cell is used as a host, a strong promoter capable of promoting transcription (such as a tac promoter, lac promoter, lacUV5 promoter, lpp promoter, pL λ promoter, pR λ promoter, rac5 promoter, amp promoter, recA promoter, SP6 promoter, trp promoter, and T7 promoter), a ribosome binding site for initiation of translation, and a transcription/translation termination sequence are generally included. Further, for example, when a eukaryotic cell is used as a host, a promoter derived from a genome of a mammalian cell (e.g., a metallothionein promoter, a β -actin promoter, a human hemoglobin promoter and a human muscle creatine promoter) or a promoter derived from an mammalian virus (e.g., adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, cytomegalovirus (CMV) promoter, HSV tk promoter, mouse mammary tumor virus (MMTV) promoter, HIV LTR promoter, epstein barr virus (EBV) promoter of moloney virus and Rous sarcoma virus (RSV) promoter) can be used, and generally have a polyadenylation sequence as a transcription termination sequence.
- [72] Optionally, the vector may be fused with another sequence in order to facilitate purification of an antibody expressed therefrom. Fused sequences include, for example, glutathione S-transferase (Pharmacia, USA), maltose binding protein (NEB, USA), FLAG (IBI, USA), and 6 $\times$ His (hexahistidine; Quiagen, USA).
- [73] The vector includes an antibiotic resistance gene commonly used in the art as a selective marker, and may include, for example, genes having resistance to ampicillin, gentamicin, carbenicillin, chloramphenicol, streptomycin, kanamycin, geneticin, neomycin, and tetracycline.
- [74] In yet another aspect, the present invention is directed to a cell transformed with the recombinant vector. Cells used to produce the antibody of the present invention may be prokaryotic cells, yeasts, or other higher eukaryotic cells, but are not limited thereto.

- [75] In the present invention, as the transformed cell, the prokaryotic host cell can be used, for example, a strain belonging to the genus *Bacillus* such as *Escherichia coli*, *Bacillus subtilis*, and *Bacillus thuringiensis*, *Streptomyces*, *Pseudomonas* (for example, *Pseudomonas putida*), *Proteus mirabilis*, and *Staphylococcus* (for example, *Staphylococcus carnosus*).
- [76] Meanwhile, interest in animal cells is greatest, and an example of a useful host cell line may be, but is not limited thereto, COS-7, BHK, CHO, CHOK1, DXB-11, DG-44, CHO/-DHFR, CV1, COS-7, HEK293, BHK, TM4, VERO, HELA, MDCK, BRL 3A, W138, Hep G2, SK-Hep, MMT, TRI, MRC 5, FS4, 3T3, RIN, A549, PC12, K562, PER.C6, SP2/0, NS-0, U205, or HT1080.
- [77]
- [78] In a further aspect, the present invention is directed to a method of producing an anti-hIL-2 antibody or antigen-binding fragment thereof, comprising culturing the cell, thereby expressing the anti-hIL-2 antibody or antigen-binding fragment thereof according to the present invention.
- [79] The cells can be cultured in various media. Commercially available media can be used as a culture medium without limitation. All other essential supplements known to those skilled in the art may be included in the appropriate concentrations. Culturing conditions, e.g., temperature and pH have already been used with the selected host cells for expression, which will be apparent to those skilled in the art.
- [80] When the antibody or antigen-binding fragment thereof is recovered, impurities can be removed, e.g., by centrifugation or ultrafiltration, and the resultant can be purified, for example, by affinity chromatography. Additional purification techniques may be used, such as anion or cation exchange chromatography, hydrophobic interaction chromatography, and hydroxyl apatite chromatography.
- [81]
- [82] In a still further aspect, the present invention is directed to a complex in which an anti-hIL-2 antibody or antigen-binding fragment thereof is bound to hIL-2.
- [83]
- [84] In a yet further aspect, the present invention is directed to an antibody-drug conjugate (ADC) comprising a drug conjugated to the anti-hIL-2 antibody or antigen-binding fragment thereof.
- [85] An antibody-drug conjugate (ADC) requires that the anticancer drug should be stably bound to the antibody before the anticancer drug is delivered to target cancer cells. The drug delivered to the target should be released from the antibody and should induce death of the target cells. To this end, the drug should be stably bound to the antibody and, at the same time, should have enough cytotoxicity to induce death of the target cells when being released from the antibody.

- [86] In the present invention, the anti-hIL-2 antibody or antigen-binding fragment thereof and cytotoxic substances including drugs such as anticancer drugs may be linked to each other by, for example, a covalent bond, a peptide bond or the like, so that they may be used as conjugates or fusion proteins (where cytotoxic substances and/or labeling substances are proteins). The cytotoxic substance may be any substance having toxicity against cancer cells, particularly solid cancer cells, and may be one or more selected from the group consisting of, but not limited to, radioisotopes, cytotoxic compounds (small molecules), cytotoxic proteins, anticancer agents, and the like. The cytotoxic proteins may be one or more selected from the group consisting of, but not limited to, ricin, saporin, gelonin, momordin, debouganin, diphtheria toxin, and pseudomonas toxin. The radioisotopes may be one or more selected from the group consisting of, but not limited to, ^{131}I , ^{188}Rh , and ^{90}Y . The cytotoxic compounds may be one or more selected from the group consisting of, but not limited to, duocarmycin, monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl)maytansine (DM1), and PBD(Pyrrolobenzodiazepine) dimer.
- [87] In the present invention, the antibody-drug conjugate may be obtained according to a technique well known in the technical field to which the present invention pertains.
- [88] In the present invention, the antibody-drug conjugate may be one in which the antibody or antigen-binding fragment thereof is bound to the drug by a linker.
- [89] In the present invention, the linker may be a cleavable linker or a non-cleavable linker.
- [90] The linker is a region that connects between anti-hIL-2 antibody and the drug. For example, the linker is configured such that it is cleavable under intracellular conditions, that is, the drug can be released from the antibody through cleavage of the linker in an intracellular environment.
- [91] The linker can be cleaved by a cleaving agent present in an intracellular environment, for example, lysosome or endosome. The linker may be a peptide linker that can be cleaved by intracellular peptidase or protease enzyme, for example, lysosome or endosome protease. Generally, the peptide linker has a length of at least two amino acids. The cleaving agents may include cathepsin B, cathepsin D, and plasmin, and are capable of hydrolyzing the peptide to enable the drug to be released into target cells. The peptide linker can be cleaved by thiol-dependent protease cathepsin B which is highly expressed in cancer tissue. For example, the linker that is used in the present invention may be a Phe-Leu or Gly-Phe-Leu-Gly linker. In addition, the peptide linker may also be a Val-Cit or Phe-Lys linker which is cleavable by, for example, intracellular protease.
- [92] In the present invention, the cleavable linker is pH-sensitive, i.e., sensitive to hy-

drololysis at certain pH values. Typically, the pH-sensitive linker is hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (e.g., a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) can be used.

- [93] The linker is cleavable under reducing conditions (e.g., a disulfide linker). A variety of disulfide linkers can be formed using SATA (N-succinimidyl-S-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxy-carbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene).
- [94] In the present invention, the drug and/or the drug-linker may be conjugated randomly through the lysine of the antibody or may be conjugated through a cysteine which is exposed when a disulfide bond chain is reduced. In some cases, the linker-drug may be bound through a cysteine present in a genetically engineered tag, for example, a peptide or a protein. The genetically engineered tag, for example, a peptide or a protein, may include an amino acid motif that may be recognized by, for example, isoprenoid transferase. The above-described peptide or protein has a deletion at the carboxy terminus of the peptide or protein, or has an addition at the carboxy (C) terminus of the peptide or protein through covalent bonding to a spacer unit. The peptide or the protein may be covalently bonded directly to the amino acid motif or may be linked to the amino acid motif by covalent bonding to a spacer unit. The amino acid spacer unit is composed of 1 to 20 amino acids, and is preferably a glycine unit.
- [95] The linker may include a beta-glucuronide linker which is recognized and hydrolyzed by β -glucuronidase which is present in lysosomes or is highly expressed in some tumor cells. Unlike a peptide linker, the beta-glucuronide linker has an advantage in that it has high hydrophilicity, and thus can increase the solubility of an antibody-drug conjugate when it is bound to a highly hydrophobic drug.
- [96] In addition, the linker may be a non-cleavable linker. In this case, the drug may be released through only a single step (antibody hydrolysis), thus producing, for example, an amino acid-linker-drug conjugate. This type of linker may be thioether or maleimide-caproyl, and may maintain its stability in blood.
- [97] In the present invention, the drug may be a chemotherapeutic agent, toxin, micro RNA (miRNA), siRNA, shRNA, or radioisotope. The drug that is a formulation exhibiting a pharmacological effect may be conjugated to the antibody.
- [98] The chemotherapeutic agent may be a cytotoxic agent or an immune checkpoint inhibitor. Specifically, the chemotherapeutic agent may include a chemotherapeutic agent capable of functioning as a microtubulin inhibitor, a mitotic inhibitor, a topoisomerase inhibitor, or a DNA intercalator. In addition, the chemotherapeutic agent may include an immunomodulatory compound, an anticancer agent, an antiviral agent, an

antibacterial agent, an antifungal agent, an antiparasitic agent, or a combination thereof.

- [99] The drug may be one or more selected from the group consisting of, but not limited to, for example, maytansinoid, auristatin, aminopterin, actinomycin, bleomycin, talisomycin, camptothecin, N8-acetyl spermidine, 1-(2-chloroethyl)-1,2-methylsulfonyl hydrazide, esperamycin, etoposide, 6-mercaptopurine, dolastatin, tricotecene, calicheamycin, taxol, taxane, paclitaxel, docetaxel, methotrexate, vincristine, vinblastine, doxorubicin, melphalan, mitomycin A, mitomycin C, chlorambucil, duocarmycin, L-asparaginase, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosourea, cisplatin, carboplatin, mitomycin, dacarbazine, procarbazine, topotecan, nitrogen mustard, cytoxan, etoposide, 5-fluorouracil, bischloroethylnitrosourea (BCNU), irinotecan, camptothecin, bleomycin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinorelbine, chlorambucil, melphalan, carmustine, lomustine, busulfan, treosulfan, decarbazine, etoposide, teniposide, topotecan, 9-aminocamptothecin, crisnatol, mitomycin C, trimetrexate, mycophenolic acid, tiazofurin, ribavirin, 5-ethynyl-1-beta-dribofuranosylimidazole-4-carboxamide (EICAR), hydroxyurea, deferoxamine, floxuridine, doxifluridine, raltitrexed, cytarabine (ara C), cytosine arabinoside, fludarabine, tamoxifen, raloxifene, megestrol, goserelin, leuprolide acetate, flutamide, bicalutamide, EB1089, CB1093, KH1060, verteporfin, phthalocyanine, photosensitizer Pe4, demethoxy-hypocrellin A, interferon- α , interferon- γ , tumor necrosis factor, gemcitabine, velcade, revamid, thalamid, lovastatin, 1-methyl-4-phenylpyridiniumion, staurosporine, actinomycin D, dactinomycin, bleomycin A2, bleomycin B2, peplomycin, epirubicin, pirarubicin, zorubicin, mitoxantrone, verapamil and thapsigargin, nuclease, and toxins derived from bacteria or animals/plants.
- [100] In the present invention, the drug may include one or more nucleophilic groups selected from the group consisting of amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate and aryl hydrazide groups, which can react to form covalent bonds with the linker and the electrophilic group on a linker reagent.
- [101]
- [102] In another further aspect, the present invention is directed to a bispecific antibody comprising the anti-hIL-2 antibody or antigen-binding fragment thereof.
- [103] In the present invention, the bispecific antibody means an antibody form in which one of the two arms of the antibody comprises the anti-hIL-2 antibody or antigen-binding fragment thereof according to the present invention, and the other arm comprises either an antibody specific for an antigen other than hIL-2, preferably a

cancer-related antigen or an immune checkpoint protein antigen, or an antibody or antigen-binding fragment thereof which binds specifically to an immune effector cell-related antigen.

[104] The antigen to which the antibody other than the anti-hIL-2 antibody included in the bispecific antibody binds is a cancer-related antigen or an immune checkpoint protein antigen, which may be selected from among Her2, EGFR, VEGF, VEGF-R, CD-20, MUC16, CD30, CD33, CD52, 4-1BB, TIM3, PD-1, PD-L1, CTLA4, BTLA4, EphB2, E-selectin, EpCam, CEA, PSMA, PSA, ERB3, c-MET, and the like, and the immune effector cell-related antigen may be selected from among, but not limited to, TCR/CD3, CD16 (FcγRIIIa), CD28, CD28, CD44, CD56, CD69, CD64 (FcγRI), CD89, CD11b/CD18 (CR3), and the like.

[105]

[106] In another still further aspect, the present invention is directed to a composition for preventing or treating cancer, which comprises the anti-hIL-2 antibody or antigen-binding fragment thereof as an active ingredient.

[107] In another yet further aspect, the present invention is directed to a composition for preventing or treating cancer, which comprises the bispecific antibody or antibody-drug conjugate as an active ingredient.

[108] “Cancer” refers to a condition in which cells proliferate abnormally and excessively due to a problem in the function of regulating the normal division, differentiation and death of the cells, and invade the surrounding tissues and organs, thereby forming a mass and destroying or deforming the existing structures. “Solid cancer” refers to a cancer which has features distinguishable from those of blood cancer and which is composed of a mass caused by abnormal growth of cells in various solid organs, including bladder, breast, intestines, kidneys, lungs, brain, esophagus, gallbladder, ovary, pancreas, stomach, cervix, thyroid, prostate, skin and the like. “Metastatic cancer” is caused by the metastasis of cancer cells, separated from a primary cancer site, to another site through blood, lymphatic vessels or the like, and proliferation of the metastasized cancer cells. The composition of the present invention can be used for the prevention or treatment of solid cancers and/or metastatic cancers. The composition of the present invention may be used for the prevention or treatment of, but not limited to, for example, skin cancer, breast cancer, colorectal cancer, kidney cancer, lung cancer, liver cancer, brain cancer, esophageal cancer, gallbladder cancer, ovarian cancer, pancreatic cancer, stomach cancer, uterine cervical cancer, thyroid cancer, prostate cancer, and bladder cancer, but is not limited thereto.

[109] As used herein, the term "preventing/prevention" refers to all actions that inhibit the metastasis, growth, and the like of cancers or delay the onset of cancers by administering the composition. As used herein, the term “treating/treatment” refers to any

action resulting in improvements in symptoms of cancers or the beneficial alteration of cancers owing to the administration of the composition.

- [110] The composition of the present invention may further comprise a pharmaceutically acceptable carrier. The carrier that is typically used in the formulation of drugs may be one or more selected from the group consisting of, but not limited to, lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium phosphate, alginate, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrups, methyl cellulose, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate, and mineral oil. In addition, the composition may further comprise one or more selected from the group consisting of excipients, lubricants, wetting agents, sweeteners, aromatics, emulsifiers, suspensions, and preservatives.
- [111] The composition or pharmaceutical composition of the antibody may be administered orally or parenterally. Such a parenteral administration includes intravenous injection, subcutaneous injection, intramuscular injection, intraperitoneal injection, endothelial administration, topical administration, nasal administration, intrapulmonary administration, intrarectal administration, etc. Because a protein or peptide is digested when administered orally, it is preferred that a composition for oral administration may be formulated to coat an active substance or to be protected against degradation in stomach. Also, the composition may be administered by any device which can transport active substances to target cells.
- [112] The content of the anti-hIL-2 antibody (TCB2 mAb) in the composition may vary depending on various factors such as formulation method, administration method, age, body weight, sex or pathological condition of the patient, diet, administration time, administration interval, administration route, excretion rate and reaction sensitivity. For example, a daily administration dosage of the anti-hIL-2 antibody (TCB2 mAb) may be in the range from 0.001 to 1,000 mg/kg, specifically 0.01 to 100 mg/kg, more specifically 0.1 to 50 mg/kg, but is not limited thereto. The effective dose for single administration of the anti-hIL-2 antibody (TCB2 mAb) may be formulated as one formulation in a unit-dose form or formulated in an appropriate amount, or prepared by injecting into a multiple-dose vial. The "pharmaceutically effective dose" as used herein may refer to the content or the dose of an active ingredient capable of exhibiting a desired a pharmacological effect, and can be determined variously depending on various factors such as formulation method, administration method, age, body weight, sex or pathological condition of the patient, diet, administration time, administration interval, administration route, excretion rate and reaction sensitivity.
- [113] The composition may be formulated with pharmaceutically acceptable carriers and/or excipients according to a method that can be easily carried out by a person having an ordinary skill in the art to which the present invention pertains, and may be provided in

a unit-dose form or enclosed in a multiple-dose vial. Here, the formulation of the composition may be in the form of a solution, a suspension, syrup or an emulsion in oily or aqueous medium, or may be extracts, powders, granules, tablets or capsules, and may further include a dispersion agent or a stabilizer. Also, the composition may be administered individually or in combination with other therapeutic agents.

[114] In particular, the composition including the anti-hIL-2 antibody (TCB2 mAb) includes an antibody, and thus may be formulated into immuno liposome. Liposome including an antibody may be prepared according to a method well known in the pertinent art. The immuno liposome is a lipid composition including phosphatidylcholine, cholesterol and polyethyleneglycol-derived phosphatidylethanolamine, and may be prepared by reverse phase evaporation method. For example, a Fab' fragment of the antibody may be conjugated to liposome through disulfide exchange reaction.

[115]

[116] In another yet further aspect, the present invention is directed to a co-administration composition for cancer treatment, which comprises the anti-hIL-2 antibody or antigen-binding fragment thereof and an immune checkpoint inhibitor.

[117] In the present invention, the immune checkpoint inhibitor (also, called "checkpoint inhibitor") may be an anti-CTLA-4 antibody or an anti-PD-1 antibody, but is not limited thereto.

[118] As used herein, the term "co-administration" (also, called "combination") means that the anti-hIL-2 antibody or antigen-binding fragment thereof and the immune checkpoint inhibitor may be administered simultaneously, sequentially, or in reverse order, and the anti-hIL-2 antibody or antigen-binding fragment thereof and the immune checkpoint inhibitor may be administered in a combination of appropriate effective amounts of the active ingredients within the range determined by those skilled in the art.

[119] In an example of the present invention, it was found that when the anti-CTLA-4 or anti-PD-1 antibody and the anti-hIL-2 antibody according to the present invention are treated sequentially, the growth of tumor cells is further suppressed.

[120] The co-administration composition includes the anti-hIL-2 antibody, and the components related thereto are the same as the components included in the above-described composition for preventing or treating cancer. Thus, the description of each constitution applies equally to the co-administration composition.

[121]

[122] In another yet further aspect, the present invention is directed to a method for prevention and/or treatment of cancer, which comprises a step of administering to a patient a therapeutically effective amount of the anti-hIL-2 antibody or antigen-binding

fragment thereof, the bispecific antibody or the antibody-drug conjugate.

[123] The composition of the present invention may be administered as an individual therapeutic agent or in combination with other therapeutic agents, and may be administered sequentially or simultaneously with conventional therapeutic agents.

[124] Any anticancer drug, for example, cisplatin, has side effects such as cachexia, sarcopenia, muscle wasting, bone wasting or involuntary body weight loss. Thus, the present invention may include a composition or a cancer treatment method, which treats cancer while preventing, minimizing or lowering the severity, frequency or occurrence of cachexia, sarcopenia, muscle wasting, bone wasting or involuntary body weight loss.

[125] The method comprises a step of administering a pharmaceutical composition comprising an effective amount of the anti-hIL-2 antibody of the present invention in combination with at least one anticancer agent. In particular embodiments, the present invention includes a method which treats cancer while preventing, minimizing or lowering the severity, frequency or occurrence of cachexia, sarcopenia, muscle wasting, bone wasting or involuntary body weight loss, the method comprising a step of administering to a patient a pharmaceutical composition comprising an effective amount of the anti-hIL-2 antibody of the present invention in combination with one or more anticancer agents known to induce or increase the severity, frequency or occurrence of cachexia, sarcopenia, muscle wasting, bone wasting or involuntary body weight loss.

[126] In another yet further aspect, the present invention is directed to a method for treating cancer, which comprises a step of co-administering a composition comprising the anti-hIL-2 antibody or antigen-binding fragment thereof with an immune checkpoint inhibitor.

[127] In the method for treating cancer according to the present invention, the anti-hIL-2 antibody or antigen-binding fragment thereof and the immune checkpoint inhibitor may be administered simultaneously, sequentially, or in reverse order. Preferably, the method may comprise the steps of: (A) treating with an immune checkpoint inhibitor; and (B) treating with the anti-hIL-2 antibody or antigen-binding fragment thereof, but is not limited thereto.

[128] The immune checkpoint inhibitor may be an anti-CTLA-4 antibody or an anti-PD-1 antibody, but is not limited thereto. The method for treating cancer includes the composition comprising the anti-hIL-2 antibody, and the components related thereto are the same as the components included in the above-described composition. Thus, the description of each constitution applies equally to the method of treating cancer by co-administration.

[129]

[130] In another yet further aspect, the present invention is directed to the use of the anti-hIL-2 antibody or antigen-binding fragment thereof for the prevention or treatment of cancer.

[131] In another yet further aspect, the present invention is directed to the use of the anti-hIL-2 antibody or antigen-binding fragment thereof for the preparation of a medicine for the prevention or treatment of cancer.

[132]

[133] In another yet further aspect, the present invention is directed to a composition for enhancing vaccine efficacy, which comprises the anti-hIL-2 antibody or antigen-binding fragment thereof as an active ingredient.

[134] As used herein, the term “vaccine” refers to a biological agent containing an antigen that immunizes a living body, and means an immunogenic or antigenic substance that produces immunity *in vivo* by its administration to humans or animals in order to prevent infection.

[135]

[136] Examples

[137] Hereinafter, the present invention will be described in further detail with reference to examples. It will be obvious to a person having ordinary skill in the art that these examples are for illustrative purposes only and are not to be construed to limit the scope of the present invention.

[138]

[139] Example 1: Experiment on the Binding Specificity of TCB2 Monoclonal Antibody against hIL-2

[140] *In vivo* mouse models were used to evaluate the therapeutic efficacy of a hIL-2/TCB2 mAb complex. For this reason, in order to examine whether TCB2 mAb shows cross-reactivity with mouse IL-2 (mIL-2), the binding specificity TCB2 mAb against hIL-2 was tested. First, the splenocytes of BALB/c mice immunized 3-4 times with hIL-2 over several weeks were fused with SP/2 myeloma cells. When the hybridoma colony was visualized, the culture supernatant was subjected to ELISA. 5 µg/ml of hIL-2 or mIL-2 was added to and mixed with PBS, and a total of 50 µl of the mixture was coated on an ELISA plate. Next, 200 µl of 10% FBS was added to the PBS and incubated at room temperature for 30 minutes in order to prevent non-specific binding, and a titrated dose of the monoclonal antibody was incubated for 30 minutes. The binding of the monoclonal antibody to the coated hIL-2 or mIL-2 was detected with anti-mouse IgG HRP or anti-rat IgG HRP. In each step, the plate was washed 3-5 times with 200 µl of PBS. As positive controls, commercially available monoclonal antibodies were used. As a positive control for hIL-2, Mab602 was used, and as positive controls for mIL-2, JES6-1 and S4B6 were used.

[141] As a result, Mab602 used as the positive control for hIL-2 showed low cross-reactivity, whereas TCB2 mAb showed no cross-reactivity with mIL-2 (FIG. 1). Thus, it could be seen that TCB2 mAb did specifically bind only to hIL-2.

[142]

[143] Example 2: *In Vivo* Immunostimulatory Effect of hIL-2/TCB2 Complex

[144] MAB602, a previously reported mouse anti-hIL-2 mAb, stimulated human CD8⁺ T cells in humanized mice, thus demonstrating the efficacy of a hIL-2/mAb complex for anticancer immunotherapy in clinical applications. However, the sequence of the CDR region of MAB602 was not published, and it is unclear whether MAB602 is an antibody which has a maximum anticancer effect when used as a hIL-2/anti-hIL-2 mAb complex. Thus, it was attempted to develop an excellent hIL-2 mAb that induces the maximum activation of CD8⁺ T cells and NK cells and the minimum expansion of Treg cells.

[145] On days 0, 1, 2 and 3, a hIL-2/TCB2 mAb (0.8μg/8 μg) complex was injected into B6 mice, and on day 5, the extent of cell expansion of splenic CD8⁺ T cells and Treg cells was analyzed. The hIL-2/TCB2 complex minimized expansion of Treg cells and CD4 T cells, but induced a strong expansion of CD8⁺ T cells and NK cells (FIG. 2). Specifically, when the hIL-2/TCB2 mAb complex were injected, memory phenotype (MP) CD8⁺ T cells were about 59-fold expanded, and the expanded MP CD8⁺ T cells constituted the majority of CD8⁺ T cells. NK cells were also 18-fold expanded, but Treg cells were only about 5-fold expanded, which was lower than the extent of expansion of CD8⁺ T cells and NK cells. The effective ratio of MP CD8⁺ T cells to expanded Treg cells was 970% for the hIL-2/TCB2 mAb complex. Therefore, it can be seen that TCB2 mAb is a monoclonal antibody that selectively stimulates CD8⁺ T cells and NK cells, not Treg cells.

[146] In addition, the effective ratio of MP CD8⁺ T cells to expanded Treg cells was 970% for the hIL-2/TCB2 mAb complex, but 530% for the hIL-2/MAB602 complex (FIG. 2D). Thus, TCB2 is a monoclonal antibody superior to MAB602.

[147]

[148] Example 3: Analysis of the Affinity of TCB2 for hIL-2

[149] The selective stimulation of CD8⁺ T cells and NK cells by the TCB2 antibody requires that the antibody be bound to the epitope of hIL-2. Since the epitope of hIL-2 is also recognized by high-affinity IL-2R (CD25), TCB2 is likely to bind to hIL-2 near a site to which the IL-2Rα chain binds. Since MAB602 is also likely to bind to hIL-2 near a site to which the IL-2Rα chain binds, TCB2 was analyzed competitively with MAB602 in order to observe the specificity of TCB2 which is an anti-hIL-2 mAb. Another anti-hIL-2 mAb (5344.111), which is available commercially and known to bind to an epitope different from an epitope to which MAB602 binds, was used as a

control.

[150] For detection of hIL-2, sandwich ELISA was used. 900 RU ($R_{max}=90$) of anti-hIL-2 clones were immobilized on a CM5 chip by amine coupling. A 2-fold dilution (100 nM) of hIL-2 was allowed to flow on the chip at a rate of 10 μ l/min for 3 minutes, and then dissociation of the hIL-2 was monitored for 10 minutes.

[151] From the competitive analysis, it was found that TCB2 competed with MAB602. It was shown that, due to its specificity, TCB2 mAb did not compete with 5344.111, but completed with MAB602. As a result, it was confirmed that TCB2 had a higher affinity for human IL-2 than other anti-hIL-2 mAbs (FIG. 3).

[152]

[153] Example 4: Anti-tumor Effect of hIL-2/TCB2 Complex

[154] Example 4-1: Effect of TCB2 mAb against Solid Tumor

[155] In order to demonstrate the clinical usefulness of TCB2 mAb against a solid tumor, 1×10^6 B16F10 melanoma cells were injected subcutaneously into B6 mice, and then PBS, hIL-2 (0.8 μ g) alone or the hIL-2/TCB2 (0.8 μ g/8 μ g) complex was injected on days 4 to 7. Next, tumor progression was monitored for 7 days.

[156] As a result, inhibition of solid tumor growth had a correlation with the magnitude of cytokine-induced expansion of CD8⁺ T cells and NK cells (FIG. 4). The hIL-2/TCB2 mAb complex inhibited tumor growth better than hIL-2 alone.

[157]

[158] Example 4-2: Effect of TCB2 mAb against Metastatic Tumor

[159] In order to demonstrate the clinical usefulness of TCB2 mAb against a metastatic tumor, 3×10^5 B16F10 melanoma cells were injected intravenously into B6 mice. 7 Days after tumor injection, hIL-2 alone (0.8 μ g) or the hIL-2/TCB2 (0.8 μ g/8 μ g) complex was injected from day 7 to day 10. On day 18, the number of pulmonary tumor nodules was measured.

[160] As a result, inhibition of the hIL-2/TCB2 well inhibited of pulmonary tumor nodules, unlike hIL-2 (FIG. 5). Thus, it can be see that TCB2 mAb has a potent anticancer effect when used as the hIL-2/TCB2 mAb complex.

[161]

[162] Example 5: Analysis of the Effect of Combination of hIL-2/TCB2 Complex and Other Anticancer Therapies

[163] Anticancer therapies, which are currently developed worldwide, include a method that immunizes patients with a tumor neo-antigen, and a method that uses checkpoint inhibitors such as anti-CTLA-4 antibodies or anti-PD-1 antibodies. In this Example, whether the hIL-2/TCB2 complex can be used in combination with these anticancer therapies was analyzed.

[164]

- [165] Example 5-1: Effect of Combination of hIL-2/TCB2 Complex and Anticancer Therapy Based on Neo-Antigen
- [166] In order to test the compatibility of the hIL-2/TCB2 complex with neo-antigen-based therapy, 1×10^6 B16F10 cells were injected subcutaneously into B6 mice on day 0. Next, PBS or a mixture of TRP2 peptide (100 μ g) and Poly I:C (100 μ g) was injected on days 3 and 7. The hIL-2/TCB2 complex (0.8 μ g/8 μ g) was injected in two rounds of four daily injections on days 4 to 7 and days 11 to 14. Next, tumor progression was monitored for 5 days.
- [167] As a result, injection of the hIL-2/TCB2 complex and the neo-antigen-based therapy inhibited the growth of the B16F10 tumor to similar extents. However, when the mice were co-treated with the hIL-2/TCB2 complex and the neo-antigen-based therapy, tumor growth was more inhibited (FIG. 6). Thus, it can be seen that the hIL-2/TCB2 complex can be used in combination with the neo-antigen-based therapy.
- [168]
- [169] Example 5-2: Effect of Combination of hIL-2/TCB2 Complex and Checkpoint Inhibitor
- [170] To test whether the hIL-2/TCB2 complex can be used in combination with checkpoint inhibitors, CT26 (Balb/C colon cancer) and MC38 (B6 colon cancer) models were used. After treatment with the hIL-2/TCB2 complex in combination with anti-CTLA-4 antibody or anti-PD-1 antibody or treatment with each of these antibodies, tumor growth was observed.
- [171] For an experiment in which mice were treated with the hIL-2/TCB2 complex in combination with the anti-CTLA-4 antibody, 5×10^5 CT26 cells were injected subcutaneously into Balb/C mice (day 0), and the anti-CTLA-4 antibody (100 μ g) was injected three times at 3-day intervals from day 7. The hIL-2/TCB2 complex (0.8 μ g/8 μ g) was injected once a day from day 8 to day 11 (four times). As a result, the anti-CTLA-4 antibody strongly inhibited growth of the CT26 tumor, and the tumor was rejected in 33% of the mice. In the mice injected with the hIL-2/TCB2 complex, tumor growth was less inhibited than that in the mice injected with the anti-CTLA-4 antibody. However, when the mice were treated with the anti-CTLA-4 antibody in combination with the hIL-2/TCB2 complex, tumor growth was more inhibited than treatment with the anti-CTLA-4 antibody, and the tumor was rejected in 63% of the mice (FIG. 7).
- [172] For an experiment in which mice were treated with the hIL-2/TCB2 complex in combination with the anti-PD-1 antibody, 5×10^5 MC38 cells were injected subcutaneously into B6 mice (day 0). Then, the anti-PD-1 antibody (100 μ g) was injected three times at 3-day intervals from day 7, and the hIL-2/TCB2 complex (1.5 μ g/15 μ g) was injected once a day from day 8 to day 11 (four times). As a result, treatment with the

anti-PD-1 antibody was not effective in delaying tumor growth (the anti-PD-1 antibody was used at a dose lower than the optimum dose), but treatment with the hIL-2/TCB2 complex strongly inhibited the growth of the MC38 tumor and rejected the tumor in 37% of the mice. When the hIL-2/TCB2 complex and the anti-PD-1 antibody were injected together, the tumor was rejected in 100% of the mice (FIG. 8).

[173]

[174] Example 5-3: Effect on Memory Response Acquisition in Immune Anticancer Therapy with hIL-2/TCB2 Complex

[175] In order to examine whether mice that rejected a tumor would acquire a memory response to the same tumor, 5×10^5 MC38 cells were injected into naive B6 mice (that have never been inoculated with a tumor) or the mice that rejected the tumor by hIL-2/TCB2 in Example 5-2 (day 25). The MC38 tumor grew rapidly in the naive B6 mice injected with it, but it did not grow in the mice that rejected the tumor (FIG. 8). This suggests that immunotherapy with the hIL-2/TCB2 complex is particularly helpful in preventing cancer recurrence in patients.

[176]

[177] Taking these results together, it can be seen that the hIL-2/TCB2 complex may be used in combination with checkpoint inhibitors such as anti-CTLA-4 antibody or anti-PD-1 antibody and is more effective when used in combination with these checkpoint inhibitors.

[178]

[179] Example 6: Sequencing of TCB2 Monoclonal Antibody

[180] The complementarity determining region (CDR) of TCB2 mAb was sequenced (Tables 1 to 3).

[181]

[182] [Table 1]

DNA sequence and amino Acid Sequence of variable region of TCB2 antibody

	Heavy chain	Light chain
DNA sequence of variable region of TCB2	GAGGTGCAACTGCAGCAGTCTGGGG CTGAGCTGGCAAGACCTGGGGCTTC AGTGAAGTTGTCTGCAAGGCTTCT GGCTACACCTTTACTACCTACTGGA TTCAGTGGGTGAAACAGAGGCCTGG ACAGGGTCTGGAATGGATTGGGGCT ATTTATCCTGGAGATGGTGATACTA GGTACATTGAGAATTTCAAGGGCAA GGCCACATTGACTGCAGATAAATCC TCCAGCACAGCCTACATGCAACTCA GCAGCTTGGCATCTGAGGACTCTGC GGTCTATTACTGTGCAAGATCCCTG GCAACTCGGGGCTTCTATGCTATGG ACTACTGGGGTCAAGGAACCTCAGT CACCGTCTCCTCA (SEQ ID NO: 1)	GACATTGTGATGACCCAGTCTCC AGCATCCCTGTCCATGGCTATAG GAGAAAAAGTCACCATCAGATGC ATAACCAGCACTGATATTGATGA TGATATGAACTGGTACCAGCAGA AGCCAGGGGAACCTCCTAAGCTC CTTATTTTCTAGAAGGCAATACTCT TCGTCTGGAGTCCCATCCCGAT TCTCCAGCAGTGGCTATGGTACA GATTTTGT'TTTTACAATTGAAAA CATGCTCTCAGAAGATGTTGCAG ATTACTACTGTTTGCAAAGTGAT AACTTGCCGTACACGTTCTGGAGG GGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 2)
Amino Acid Sequence of variable region of TCB2	EVQLQQSGAELARPGASVKLSCKAS GYTFTTYWIQWVKQRPQGGEWIGAI YPGDGDTRYIQNFKGKATLTADKSS STAYMQLSSLASEDSAVYYCARSLA TRGFYAMDYWGQGTSTVTVSS (SEQ ID NO: 3)	DIVMTQSPASLSMAIGEKVTIRC ITSTDIDDDMNWYQQKPGEPKPL LISEGNTLRPGVPSRFSSSGYGT DFVFTIENMLSEDVADYYCLQSD NLPYTFGGGTKLEIK (SEQ ID NO: 4)

[183]

[184] [Table 2]

CDR DNA sequence of TCB2 antibody

Variable region	CDR	DNA sequence	SEQ ID NO:
Heavy chain	CDR1	ACCTACTGGATTCAG	5
	CDR2	GCTATTTATCCTGGAGATGGTGATACTAGGTAC ATTCAGAAATTTCAAGGGC	6
	CDR3	TCCCTGGCAACTCGGGGCTTCTATGCTATGGAC TAC	7
Light chain	CDR1	ATAACCAGCACTGATATTGATGATGATATGAAC	8
	CDR2	GAAGGCAATACTCTTCGTCCT	9
	CDR3	TTGCAAAGTGATAACTTGCCGTACACG	10

[185]

[186] [Table 3]

CDR amino acid sequence of TCB2 antibody

Variable region	CDR	Amino acid sequence	SEQ ID NO:
Heavy chain	CDR1	TYWIQ	11
	CDR2	AIYPGDGDTRYIQNFKG	12
	CDR3	SLATRGFYAMDY	13
Light chain	CDR1	ITSTDIDDDMN	14
	CDR2	EGNTLRP	15
	CDR3	LQSDNLPYT	16

[187]

[188] It can be seen that the amino acid sequence of TCB2 differs from that of Nara1 (Table 4) which is an anti-hIL-2 mAb antibody recently developed by Onur Boyman and Natalia Ramirez (WO 2016005950 A1). The CDR similarities between TCB2 and Nara1 are 40%, 52.94% and 8.33% for heavy-chain CDRs 1 to 3, respectively, and 33.33%, 14.28% and 55.55% for light-chain CDRs 1 to 3 (Table 5).

[189]

[190] [Table 4]

CDR amino acid sequence of Naral antibody

Variable region	CDR	Amino acid sequence	SEQ ID NO:
Heavy chain	CDR1	NYLIE	17
	CDR2	VINPGSGGTNYNEKFKG	18
	CDR3	WRGDGYAYFDV	19
Light chain	CDR1	KASQSVDYDGDSYMN	20
	CDR2	AASNLES	21
	CDR3	QQSNEDPYT	22

[191]

[192] [Table 5]

Comparison of CDR amino acid sequence between TCB2 and Naral antibodies

Variable region	CDR	Number of the same residues of Naral and TCB2	Length of amino acid of Naral	Similarity (%)
TCB2	CDR1	2	5	40
Heavy chain	CDR2	9	17	52.94
	CDR3	1	12	8.33
TCB2	CDR1	5	15	33.33
Light chain	CDR2	1	7	14.28
	CDR3	5	9	55.55

[193]

[194] Based on the sequencing data, the Fab region of TCB2 mAb was cloned into an IgG2 expression vector. The amino acid sequence of the cloned vector is shown in Table 6 below.

[195]

[196] [Table 6]

Amino acid sequence of human chimeric TCB2

	Amino acid sequence
Heavy chain	EVQLQQSGAELARPGASVKLSCKASGYTFTTYWIQWVKQRPGQGLEWIGAIYP GDGDTRYIQNFKGKATLTADKSSSTAYMQLSSLASEDSAVYYCARSLATRGFY AMDYWGQGTSTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDPKPSN TKVDKTKVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVSVLTVVHQDWLNGKE YKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 23)
Light chain	DIVMTQSPASLSMAIGEKVTIRCITSTDDIDDDMNWYQQKPGEPKLLISEGNT LRPGVPSRFSSSGYGTDVFVTIENMLSEADVADYYCLQSDNLPYTFGGGTGLEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 24)

[197]

[198] Example 7: Humanized TCB2 Antibody

[199] In order to reduce the host immune response to mouse IgG, TCB2 mAb was humanized and expressed with human IgG1 Fc (Table 7). The CDR of mouse TCB2 (mTCB2) was introduced into the variable region of human IgG. Then, for an in vivo experiment, three humanized TCB2 (hnTCB2) mAb clones (VH1 + VL2, VH2 + VL2, and AH03463 (VL03463 + VH03463)) having the highest affinity were selected (Table 8).

[200]

[201] [Table 7]

DNA sequence and amino acid sequence of
variable region of humanized TCB2

		DNA sequence	Amino acid sequence
VL03463	Light Chain	GACATTCAGATGACCCAGAGCCCTTCCAGCC TGAGCGCCAGCGTCGGGGACAGAGTGACCAT TACCTGCATTACCTCCACAGACATTGACGAT GACATGAACTGGTACCAGCAGAAGCCAGGGA AAGCCCCCAAGCTGCTGATCTATGAGGGAAA TACTCTGCGGCCCCGGCGTGCCTAGCAGATTC AGCTCCTCTGGCTCTGGGACCGATTTCACCT TTACAATCAGTTCAGTGCAGCCCCGAAGACAT TGCTACATACTATTGCCTGCAGAGCGACAAC CTGCCTTACACCTTCGGGGGAGGGACCAAAC TGGAATCAAA (SEQ ID NO: 25)	DI <u>Q</u> MTQSPSSLSA SVGDRV <u>T</u> ITCITS TDIDDDMNWYQQK PGKAPKLLI <u>Y</u> EGN TLRPGVPSRFSS <u>S</u> GS <u>G</u> TDFTFTIS <u>S</u> L QPEDIAT <u>Y</u> YCLQS DNLPTYTFGGGTKL EIK (SEQ ID NO: 26)

[202]

VH03463	Heavy	GAAGTGCAGCTGGTGCAGAGCGGAGCAGAAG	EVQLVQSGAEVKK
	Chain	TGAAAAAGCCTGGGGCAAGCGTGAAGGTGTC	PGASVKV_SCKASG
		CTGTAAAGCAAGCGGATATACATTCACCACA	YTFTTYWIQWVKQ
		TACTGGATCCAGTGGGTGAAGCAGGCACCAG	APGQGLEWMGAIY
		GACAGGGACTGGAGTGGATGGGAGCAATCTA	PGDGDTRYIQNFK
		CCCTGGAGACGGCGATACACGATATATTTCAG	GRVTMTTRDTSTST
		AACTTCAAAGGCCGGGTGACTATGACCAGAG	VYME_LSSLRSED
		ACACATCTACTAGTACCGTCTATATGGAGCT	AVYYCARSLATRG
		GAGCTCCCTGAGGAGCGAAGATACCGCTGTC	FYAMDYWGQGTIV
		TACTATTGCGCCCGCTCTCTGGCTACAAGAG	TVSS
		GGTTCTACGCTATGGATTATTGGGGACAGGG	(SEQ ID NO:
		GACACTGGTCACCGTCAGCAGC	28)
		(SEQ ID NO: 27)	

[203]

VL2	Light	GACATCGTGATGACCCAGAGCCCCAGTTCCC	DIVMTQSPSSLSA
	Chain	TGAGCGCCAGCGTCGGAGACAGAGTGACTAT	SVGDRVITIRCITS
		TAGGTGTATTACTTCCACAGATATTGACGAT	TDIDDDMNWYQQK
		GACATGAACTGGTACCAGCAGAAGCCAGGCA	PGKAPKLLISEGN
		AAGCCCCCAAGCTGCTGATCAGCGAGGGAAA	TLRPGVPSRFSGS
		TACTCTGCGACCAGGAGTGCCTTCTAGATTC	GYGTDFTFTISL
		TCTGGCAGTGGGTATGGAACCGATTTACCT	QPEDIADYYCLQS
		TTACAATCAGCTCCCTGCAGCCCGAAGATAT	DNLPYTFGGGTKL
		TGCTGACTACTATTGCCTGCAGAGCGATAAC	EIK
		CTGCCATACACCTTCGGCGGGGGGACCAAAC	(SEQ ID NO:
		TGGAAATCAAA	30)
		(SEQ ID NO: 29)	

[204]

VH1	Heavy Chain	CAGGTGCAGCTGGTCCAGTCAGGAGCAGAAG	QVQLVQSGAEVKK
		TCAAGAAGCCCGGAGCAAGCGTCAAAGTGTC	PGASVKVSCKASG
		ATGCAAAGCAAGCGGATATACATTTACCACA	YTFTTYWIQWVRQ
		TACTGGATCCAGTGGGTGCGACAGGCACCAG	APGQGLEWMGAIY
		GACAGGGACTGGAGTGGATGGGAGCAATCTA	PGDGDTRYIQNFK
		CCCTGGAGACGGCGATACAAGATATATTTCAG	GRVTMTTRDTSTST
		AACTTCAAGGGCCGGGTGACTATGACCAGAG	VYMESSLRSED
		ACACATCTACTAGTACCGTCTATATGGAGCT	AVYYCARSLATRG
		GAGCTCCCTGAGGAGCGAAGATACCGCTGTC	FYAMDYWGQGT
		TACTATTGCGCCCGCTCTCTGGCTACAAGGG	TVSS
		GGTTCTACGCAATGGATTACTGGGGGCAGGG	(SEQ ID NO:
		GACACTGGTCAACCGTCTCATCA	32)
		(SEQ ID NO: 31)	

[205]

VH2	Heavy Chain	CAGGTCCAGCTGGTCCAGAGCGGAGCCGAGG	QVQLVQSGAEVKK
		TGAAGAAGCCCGGAGCAAGCGTCAAAGTGTC	PGASVK <u>L</u> SCKASG
		ATGCAAGGCAAGCGGATACACTTTTACCACA	YTFTTYWIQWV <u>KQ</u>
		TACTGGATCCAGTGGGTGAAGCAGGCACCAG	APGQGLEW <u>I</u> GAIY
		GACAGGGACTGGAGTGGATCGGAGCAATCTA	PGDGDTRYIQNFK
		CCCTGGAGACGGCGATACACGGTATATTTCAG	GRVTMT <u>A</u> DTSTST
		AACTTCAAAGGCAGAGTGACTATGACCGCTG	VYMESSLRSED
		ACACATCTACTAGTACCGTCTATATGGAGCT	AVYYCARSLATRG
		GAGCTCCCTGAGGAGCGAAGATACCGCCGTC	FYAMDYWGQGT
		TACTATTGCGCCCGGTCTCTGGCTACAAGGG	TVSS
		GCTTTTATGCTATGGATTATTGGGGACAGGG	(SEQ ID NO:
		CACACTGGTCAACCGTCTCATCT	34)
		(SEQ ID NO: 33)	

[206]

[207] Residues in the amino acid sequence of VL03463, which were different from those in VL2, were underlined. For comparison of the sequence of the heavy-chain region,

residues in VH2 and VH03463, which were different from those in VH1, were underlined. VL2 was used together with VH1 or VH2 to express two different humanized TCB2 antibodies (VL2 + VH1 or VL2 + VH2).

[208]

[209] [Table 8]

Affinity of humanized TCB2 for hIL-2

	mAbs	Ka (1/Ms)	Kd (1/s)	KD (M)	Relative similarity of humanized TCB2 to chimeric TCB2
Set 1	Chimeric	2.27E+07	1.63E-03	7.17E-11	
	VH1 + VL2	1.89E+07	1.97E-03	1.04E-10	68.9%
	VH2 + VL2	1.68E+07	4.63E-03	2.75E-10	26%
Set 2	Chimeric	2.29E+07	1.41E-03	6.16E-11	
	AH03463	2.11E+07	4.18E-03	1.98E-10	31%

[210]

[211] To compare the immune cell activation function between original mouse TCB2, human chimeric TCB2 (hcTCB2) and humanized TCB2 (hnTCB2), hIL-2 was allowed to form complexes with different TCB2s (mouse TCB2 (mTCB2), hcTCB2, and hnTCB2). Each of the complexes was injected into B6 mice once a day from day 0 to day 3 (four times), and on day 5, the splenic immune cells were analyzed by flow cytometry. As a result, it was shown that the affinity of hnTCB2 was slightly lower than that of mTCB2 or hcTCB2 (Table 8), but the function of hnTCB2 to activate immune cells was similar to that of mTCB2 (FIG. 9; VL2+VH2 was not indicated due to its low functionality). Thus, it was demonstrated that mTCB2 was successfully humanized.

[212]

[213] In order to examine whether hnTCB2 has anticancer activity in addition to the function of activating immune cells, 5x10⁵ MC38 cells were injected subcutaneously into B6 mice on day 0, and anti-PD-1 antibody (200 µg) was injected three times at 3-day intervals from day 7. Next, the hIL-2/hnTCB2 (VL2+ VH1, 1.5 µg/15 µ)

complex was injected once a day from day 8 to day 11 (four times), and then growth of the MC38 tumor was observed. As a result, growth of the tumor was delayed even by treatment with a high concentration of the anti-PD-1 antibody alone, but when the mice were treated with the hIL-2/hnTCB2 complex, growth of the MC38 tumor was strongly inhibited to a level similar to a level shown in treatment with the hIL-2/mTCB2 complex, and the tumor was rejected in 40% of the mice. When the hIL-2/hnTCB2 or hIL-2/mTCB2 complex was injected together with the anti-PD-1 antibody, the tumor was rejected in 85% of the mice (FIG. 10). Thus, it was confirmed that the function of original mTCB2 was conserved in humanized TCB2.

[214]

Industrial Applicability

[215] The anti-hIL-2 antibody of the present invention binds specifically to a particular epitope of hIL-2, thereby inhibiting the binding of the hIL-2 to CD25, thereby minimizing expansion of Treg cells. In addition, it stimulates the CD8⁺ T cells and NK cells that exhibit anti-tumor activity. Thus, the anti-hIL-2 antibody of the present invention is useful as a new anticancer therapeutic agent.

[216]

[217] Although the present invention has been described in detail with reference to the specific features, it will be apparent to those skilled in the art that this description is only for a preferred embodiment and does not limit the scope of the present invention. Thus, the substantial scope of the present invention will be defined by the appended claims and equivalents thereof.

[218]

Sequence Listing Free Text

[219] Attached the electric file.

【CLAIMS】**【Claim 1】**

The anti-hIL-2 antibody or antigen-binding fragment thereof, which binds specifically to human interleukin-2 (hIL-2), and inhibits the binding of the hIL-2 to CD25,

wherein the anti-hIL-2 antibody or antigen-binding fragment thereof comprises:

a heavy-chain variable region comprising a heavy-chain CDR1 consisting of an amino acid sequence of SEQ ID NO: 11, a heavy-chain CDR2 consisting of an amino acid sequence of SEQ ID NO: 12, and a heavy-chain CDR3 consisting of an amino acid sequence of SEQ ID NO: 13; and

a light-chain variable region comprising a light-chain CDR1 consisting of an amino acid sequence of SEQ ID NO: 14, a light-chain CDR2 consisting of an amino acid sequence of SEQ ID NO: 15, and a light-chain CDR3 consisting of an amino acid sequence of SEQ ID NO: 16.

【Claim 2】

The anti-hIL-2 antibody or antigen-binding fragment thereof of claim 1, wherein the anti-hIL-2 antibody or antigen-binding fragment thereof comprises:

a heavy-chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 23, 28, 32, and 34; and

a light-chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 24, 26, and 30.

【Claim 3】

The anti-hIL-2 antibody or antigen-binding fragment thereof of claim 1, wherein the anti-hIL-2 antibody or antigen-binding fragment thereof comprises:

a heavy-chain variable region of SEQ ID NO: 3 and a light-chain variable region of SEQ ID NO: 4;

a heavy-chain variable region of SEQ ID NO: 23 and a light-chain variable region of SEQ ID NO: 24;

a heavy-chain variable region of SEQ ID NO: 28 and a light-chain variable region of SEQ ID NO: 26;

a heavy-chain variable region of SEQ ID NO: 32 and a light-chain variable region of SEQ ID NO: 30; or

a heavy-chain variable region of SEQ ID NO: 34 and a light-chain variable region of SEQ ID NO: 30.

【Claim 4】

The anti-hIL-2 antibody or antigen-binding fragment thereof of claim 1, wherein the anti-hIL-2 antibody or antigen-binding fragment thereof induces expansion of CD8⁺ T cells and NK cells.

【Claim 5】

A nucleic acid encoding the anti-hIL-2 antibody or antigen-binding fragment thereof of any one of claims 1, 2 to 4.

【Claim 6】

A recombinant vector comprising the nucleic acid of claim 5.

【Claim 7】

A cell transformed with the recombinant vector of claim 6.

【Claim 8】

A method of producing an anti-hIL-2 antibody or antigen-binding fragment thereof, comprising culturing the cell of claim 7.

【Claim 9】

A complex in which the anti-hIL-2 antibody or antigen-binding fragment thereof of any one of claims 1, 2 to 4 is bound to hIL-2.

【Claim 10】

A composition suitable for preventing or treating cancer, which comprises the anti-hIL-2 antibody or antigen-binding fragment thereof of any one of claims 1, 2 to 4 as an active ingredient.

【Claim 11】

The composition of claim 10, wherein the cancer is selected from the group consisting of skin cancer, breast cancer, colorectal cancer, kidney cancer, lung cancer, liver cancer, brain cancer, esophageal cancer, gallbladder cancer, ovarian cancer, pancreatic cancer, stomach cancer, uterine cervical cancer, thyroid cancer, prostate cancer, and bladder cancer.

【Claim 12】

A bispecific antibody comprising the anti-hIL-2 antibody or antigen-binding fragment thereof of any one of claim 1, 2 to 4.

【Claim 13】

A composition suitable for preventing or treating cancer, which comprises the bispecific antibody or antibody-drug conjugate of claim 12 as an active ingredient.

【Claim 14】

The composition of claim 13, wherein the cancer is selected from the group consisting of skin cancer, breast cancer, colorectal cancer, kidney cancer, lung cancer, liver cancer, brain cancer, esophageal cancer, gallbladder cancer, ovarian cancer, pancreatic cancer, stomach cancer, uterine cervical cancer, thyroid cancer, prostate cancer, and bladder cancer.

【Claim 15】

A co-administration composition suitable for cancer treatment, which comprises the anti-hIL-2 antibody or antigen-binding fragment thereof of any one of claims 1, 2 to 4 and an immune checkpoint inhibitor.

【Claim 16】

The composition of claim 15, wherein the immune checkpoint inhibitor is an anti-CTLA-4 antibody or an anti-PD-1 antibody.

【Claim 17】

A composition suitable for enhancing vaccine efficacy, which comprises the anti-hIL-2 antibody or antigen-binding fragment thereof any one of claims 1, 2 to 4 as an active ingredient.

【Claim 18】

A antibody-drug conjugate comprising the anti-hIL-2 antibody or antigen-binding fragment thereof of any one of claims 1, 2 to 4.

【Claim 19】

A composition suitable for preventing or treating cancer, which comprises the antibody-drug conjugate of claim 18 as an active ingredient.

【Claim 20】

The composition of claim 19, wherein the cancer is selected from the group consisting of skin cancer, breast cancer, colorectal cancer, kidney cancer, lung cancer, liver cancer, brain cancer, esophageal cancer, gallbladder cancer, ovarian cancer, pancreatic cancer, stomach cancer, uterine cervical cancer, thyroid cancer, prostate cancer, and bladder cancer.