# Isolated bispecific antibody that specifically binds to CD3 and tumor antigen, and use thereof

## Field of the invention

The present invention relates to the field of biotechnology and medicine, in particular to a bispecific antibody that specifically binds to CD3 and a tumor antigen. The invention further relates to a nucleic acid encoding the subject bispecific antibody, an expression vector, a host cell for producing the subject bispecific antibody and a method for producing said cell, pharmaceutical compositions comprising the bispecific antibody according to the invention, pharmaceutical compositions comprising the bispecific antibody according to the invention and other therapeutically active compounds, methods for treating diseases or disorders mediated by a tumor antigen, use of the bispecific antibody or pharmaceutical compositions thereof for treating diseases or disorders mediated by a tumor antigen, and the use of the bispecific antibody according to the invention and other therapeutically active compounds for treating diseases or disorders mediated by a tumor antigen.

## Background of the invention

Monoclonal antibodies in the form of chimeric, humanized or fully human molecules have proven to be useful as effective medicine for treating a number of disorders and diseases.

Naturally occurring human antibody molecules consist of two heavy chain homodimers, each of which forms a heterodimer in partnership with two identical light chain molecules. Conventional monoclonal antibodies in the form of whole molecules consist of bivalent ("two-armed") heterodimers of heavy and light chains.

Diseases are often caused as a result of multiple pathologies and are accompanied by many concomitant diseases. Bispecific antibodies are capable of binding and thereby neutralizing two different antigens per antibody molecule. The potential for a significant improvement in the therapeutic properties (and value) of medicinal products as compared to monoclonal antibodies has made bispecific antibodies an active area of research. Over the past twenty years, the literature has described many solutions regarding engineered versions of bispecific antibodies, as described in Brinkmann, U and RE Kontermann, 2017, The Making of Bispecific Antibodies, MAbs; 209 Feb/Mar; 9(2):182–212, doi: 10.1080/19420862.2016.1268307.

CD3 (cluster of differentiation 3) is a molecule present on the surface of T lymphocytes.

A tumor antigen is present either only on cancer cells or is upregulated on tumor cells but is also expressed at lower levels on healthy cells.

The bispecific antibody that specifically binds to CD3 and a tumor antigen provides for the recruitment of T cells (including cytotoxic CD8 lymphocytes that kill tumors) to tumor antigen-expressing cells.

Patent documents WO2016166629, WO2017031104, WO2020018820, WO1999054440, WO2017210485, WO2016020309, WO2016110576 disclose various bispecific antibodies that specifically bind to CD3 and a tumor antigen.

To date, only two bispecific antibodies in the world that specifically bind to CD3 and a tumor antigen have been approved for therapeutic use, in particular antibody blinatumomab that specifically binds to CD3 and CD19, and antibody katumaxomab that specifically binds to CD3 and EpCAM. In connection with the above, there is a need to create novel bispecific antibodies that specifically bind to CD3 and a tumor antigen which is different from CD19 and EpCAM.

**Disclosure of the invention**

The authors of the present group of inventions have developed bispecific antibodies that specifically bind to CD3 and a tumor antigen and have high affinity parameters for binding to the CD3 antigen.

*Definitions and general methods*

Unless defined otherwise herein, all technical and scientific terms used in connection with the present invention will have the same meaning as is commonly understood by those skilled in the art.

Furthermore, unless otherwise required by context, singular terms shall include plural terms, and the plural terms shall include the singular terms. Typically, the present classification and methods of cell culture, molecular biology, immunology, microbiology, genetics, analytical chemistry, organic synthesis chemistry, medical and pharmaceutical chemistry, as well as hybridization and chemistry of protein and nucleic acids described herein are well known by those skilled and widely used in the art. Enzyme reactions and purification methods are performed according to the manufacturer's guidelines, as is common in the art, or as described herein.

The term "KD" in this description refers to the affinity constant (or equilibrium constant) which is calculated from the ratio of Kd to Ka (i.e. Kd/Ka), and it is expressed as a molar concentration (M).

"Binding affinity" typically refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g. an antibody) and its binding partner (e.g. an antigen). Unless indicated otherwise, "binding affinity" refers to intrinsic (characteristic, true) binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g. antibody and antigen). The affinity of a molecule X for its binding partner Y can generally be represented by the affinity constant (KD). The preferred Kd value is about 200 nM, 150 nM, 100 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 8 nM, 6 nM, 4 nM, 2 nM, 1 nM, or less. Affinity can be measured by common methods known in the art, including those described in the present description. Low-affinity antibodies typically bind an antigen slowly and tend to dissociate

readily, whereas high-affinity antibodies typically bind an antigen faster and tend to remain bound longer. A variety of methods for measuring binding affinity are known in the art, any one of these methods may be used for the purposes of the present invention.

The term "Kd", "koff" or "kdis" refers to the off rate constant of a particular interaction between a binding molecule and antigen. The off rate constant koff can be measured using bio-layer interferometry, for example, using the Octet™ system.

The term "Ka", "kon" or "on-rate" refers to the association rate constant.

The term "$R^2$" refers to the coefficient of determination.

The term "Response" refers to an antibody-antigen binding signal.

The term "in vitro" refers to a biological entity, a biological process, or a biological reaction outside the body under artificial conditions. For example, a cell grown in vitro is to be understood as a cell grown in an environment outside the body, e.g. in a test tube, a culture vial, or a microtiter plate.

The term "$ED_{50}$" ($EC_{50}$) (50% effective dose/concentration) refers to concentrations of a formulation producing 50% biological effect (which may include cytoxicity).

As used in the present description and claims that follow, unless otherwise dictated by the context, the words "include" and "comprise", or variations thereof such as "includes", "including", "comprises", or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

*Antibody*

The present invention relates to a bispecific antibody that specifically binds to CD3 and a tumor antigen.

The bispecific antibody according to the present invention is a monoclonal antibody.

The term "monoclonal antibody" or "mAb" refers to an antibody that is synthesized and secreted by an individual clonal population of cells.

The bispecific antibody according to the invention is a recombinant antibody.

The term "recombinant antibody" refers to an antibody that is expressed in a cell or cell line comprising nucleotide sequence(s) encoding the antibody, wherein said nucleotide sequence(s) is (are) not associated with the cell in nature.

In one aspect, the present invention relates to an isolated bispecific antibody that specifically binds to CD3 and a tumor antigen and comprises:

1) a first antigen-binding fragment that specifically binds to CD3 and comprises:

(a) a light chain variable domain comprising:

(i) CDR1 with the amino acid sequence RASQS$X_1$SSNLA,

$X_1$=I or V;

(ii) CDR2 with the amino acid sequence of SEQ ID NO: 3, and

(iii) CDR3 with the amino acid sequence $QX_2YX_3NWPLT$, where

$X_2$=H or Q;

$X_3$=N or I; and

(b) a heavy chain variable domain comprising:

(i) CDR1 with the amino acid sequence of SEQ ID NO: 6,

(ii) CDR2 with the amino acid sequence of SEQ ID NO: 7, and

(iii) CDR3 with the amino acid sequence $DNSGYGHYYYGX_4DI$, wherein

$X_4$=M or L;

and

2) a second antigen-binding fragment that specifically binds to a tumor antigen.

The term "isolated" used to describe various antibodies according to the present description refers to an antibody which has been identified and isolated and/or regenerated from a cell or cell culture, in which the antibody is expressed. Impurities (contaminant components) from natural environment are materials which typically interfere with diagnostic or therapeutic uses of the polypeptide, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. The isolated polypeptide is typically prepared by at least one purification step.

The term "antibody" or "immunoglobulin" (Ig) as used in the present description includes whole antibodies. The term "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds. Each heavy chain comprises a heavy chain variable region (abbreviated referred to in the present description as VH) and a heavy chain constant region. Each light chain consists of a light chain variable region (abbreviated referred to in the present description as VL) and light chain constant region. Preferably the light chain is a kappa (κ) light chain, and the constant domain CL is preferably C kappa (κ).

The term "bispecific antibody" refers to an antibody having antigen-binding fragments that are capable of specific binding to two distinct epitopes on a single biological molecule or capable of specific binding to epitopes on two distinct biological molecules. The bispecific antibody is also referred to herein as having "dual specificity" or as being a "dual specificity" antibody.

The fragment crystallizable region ("Fc region, Fc") of an immunoglobulin is the terminal region of an immunoglobulin molecule that interacts with cell surface Fc receptor as well as with some proteins of the complement system. This property allows antibodies to activate the immune system. In IgG, IgA and IgD isotypes, the Fc region is composed of two identical protein fragments, derived from the second and third constant domains, respectively, of the two heavy chains.

The "Fc fragment monomer" refers to the Fc region from the second and third constant domains of any one of the two heavy chains (for IgG, IgA and IgD isotypes).

Antibodies according to the invention may be of any class (e.g., IgA, IgD, IgE, IgG, and IgM, preferably IgG), or subclass (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2, preferably IgG1).

VL and VH regions may be further subdivided into hyper-variability regions called complementarity determining regions (CDRs), interspersed between regions that are more conserved, termed framework regions (FRs). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of heavy and light chains contain a binding domain that interacts with an antigen.

The constant regions of antibodies may mediate the binding of immunoglobulin to host tissues or factors, including various cells of the immune system (e.g. effector cells) and the first component (C1q) of the classical complement system.

The term "antigen-binding portion" of antibody or "antigen-binding fragment", as used in the present description, refers to one or more antibody fragments that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of antibody can be performed by fragments of a full-length antibody. Examples of binding fragments which are included within the term "antigen-binding portion" of an antibody include (i) Fab-fragment, monovalent fragment, consisting of VL, VH, CL and CH1 domains; (ii) F(ab')2 fragment, a bivalent fragment comprising two Fab-fragments linked by a disulfide bridge at the hinge region; (iii) Fd-fragment consisting of VH and CH1 domains; (iv) Fv-fragment consisting of VL and VH domains of a single arm of an antibody; (v) dAb-fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH/VHH domain. In addition, two regions of the Fv-fragment, VL and VH, are encoded by different genes, they can be joined using recombinant methods using a synthetic linker that enables to receive them as a single protein chain in which the VL and VH regions are paired to form monovalent molecules (known as a single-chain Fv (scFv); see e.g. Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). It is assumed that such single-stranded molecules are also included within the term "antigen-binding portion" of antibody. Such antibody fragments are produced using conventional techniques known to those skilled in the art, and these fragments are screened in the same manner as intact antibodies are.

"Kabat numbering scheme" or "numbering according to Kabat" as used in the present application refers to the system for numbering of amino acid residues that are more variable (i.e. hypervariable) than other amino acid residues in variable regions of heavy and light chains of antibody (Kabat et al. Ann. N.Y. Acad. Sci., 190:382-93 (1971); Kabat et al. Sequences of Proteins

of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991)).

The antibody of the present invention "which specifically binds" a target antigen refers to an antibody that binds an antigen with sufficient affinity such that the antibody can be used as a diagnostic and/or therapeutic agent targeting a protein or cell or tissue expressing the antigen, and slightly cross-reacts with other proteins.

The term "specifically binds to" a particular polypeptide or an epitope on a particular target polypeptide may be described by example of a molecule having a Kd for the target of at least about 200 nM, or at least about 150 nM, or at least about 100 nM, or at least about 60 nM, or at least about 50 nM, or at least about 40 nM, or at least about 30 nM, or at least about 20 nM, or at least about 10 nM, or at least about 8 nM, or at least about 6 nM, or at least about 4 nM, or at least about 2 nM, or at least about 1 nM, or lower.

In one embodiment, the term "specific binding" refers to binding where a molecule binds to a particular polypeptide or epitope on a particular polypeptide without substantially binding to any other polypeptide or epitope on a polypeptide.

Tumor antigens (TA) are understood to mean both tumor-specific antigens (TSA) found only on cancer cells, not on healthy cells, and tumor-associated antigens (TAA), which are highly expressed on tumor cells, but are also expressed at lower levels on healthy cells.

In some embodiments of the invention, the isolated bispecific antibody comprises the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprises CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 1 or SEQ ID NO: 2.

In some embodiments of the invention, the isolated bispecific antibody comprises the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprises CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 4 or SEQ ID NO: 5.

In some embodiments of the invention, the isolated bispecific antibody comprises the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprises CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 8 or SEQ ID NO: 9.

In some embodiments of the invention, the isolated bispecific antibody comprises the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 1 or SEQ ID NO: 2;

CDR2 with the amino acid sequence of SEQ ID NO: 3;

and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 4 or SEQ ID NO: 5.

In some embodiments of the invention, the isolated bispecific antibody comprises the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6;

CDR2 with the amino acid sequence of SEQ ID NO: 7;

and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 8 or SEQ ID NO: 9.

In some embodiments of the invention, the isolated bispecific antibody comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 1 or SEQ ID NO: 2;

CDR2 with the amino acid sequence of SEQ ID NO: 3;

and

CDR3 with the amino acid sequence $QX_1YX_2NWPLT$, wherein

$X_1$=H or Q;

$X_2$=N or I; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6;

CDR2 with the amino acid sequence of SEQ ID NO: 7;

and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 8 or SEQ ID NO: 9.

In some embodiments of the invention, the isolated bispecific antibody comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 1 or SEQ ID NO: 2;

CDR2 with the amino acid sequence of SEQ ID NO: 3;

and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 4 or SEQ ID NO: 5; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6;

CDR2 with the amino acid sequence of SEQ ID NO: 7;

and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 8 or SEQ ID NO: 9.

In some embodiments of the invention, the isolated bispecific antibody comprises:

(i) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8; or

(ii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 1,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8; or

(iii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 5; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 9; or

(iv) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 1,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 5; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 9.

In some embodiments of the invention, the isolated bispecific antibody comprises the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, wherein the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 or SEQ ID NO: 156.

In some embodiments of the invention, the isolated bispecific antibody comprises the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, wherein the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 or SEQ ID NO: 157.

In some embodiments of the invention, the isolated bispecific antibody comprises variable domains of light and heavy chains of the first antigen-binding fragment that specifically binds to CD3, wherein:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 or SEQ ID NO: 156; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 or SEQ ID NO: 157.

In some embodiments of the invention, the isolated bispecific antibody comprises a first antigen-binding fragment that specifically binds to CD3, is designed in scFv, Fab, scFab or CrossMab format.

In some embodiments of the invention, the isolated bispecific antibody comprises a second antigen-binding fragment that specifically binds to a tumor antigen, is designed in scFv, Fab, scFab or CrossMab format.

In some embodiments of the invention, the isolated bispecific antibody comprises variable domains of light and heavy chains of the first antigen-binding fragment that specifically binds to CD3, wherein:

(i) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 10 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 16; or

(ii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 16; or

(iii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 10 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 17; or

(iv) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 10 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 18; or

(v) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 10 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19; or

(vi) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19; or

(vii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 12 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 18; or

(viii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 13 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 20; or

(ix) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 14 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 20; or

(x) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 13 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 21; or

(xi) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 13 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 22; or

(xii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 13 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 23; or

(xiii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 14 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 23; or

(xiv) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 15 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 22;

or

(xv) (a) the light chain variable domain of the first antigen-binding fragment, designed in CrossMab format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 156 and

(b) the heavy chain variable domain of the first antigen-binding fragment, designed in CrossMab format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 157.

In some embodiments of the invention, the isolated bispecific antibody is a full-length IgG antibody.

In some embodiments of the invention, the isolated bispecific antibody is a full-length IgG antibody that is of human IgG1, IgG2, IgG3 or IgG4 isotype.

In some embodiments of the invention, the isolated bispecific antibody is a full-length IgG antibody that is of human IgG1 isotype.

In some embodiments of the invention, the isolated bispecific antibody comprises the mutations L234A and L235A according to the EU numbering scheme for amino acids of antibodies (Edelman G.M. et al., Proc. Natl. Acad. Sci. USA 63 (1969) pp. 78-85; Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD, (1991)) in the CH2 region.

In some embodiments of the invention, the isolated bispecific antibody comprises the mutations M252Y, S254T, T256E according to the EU numbering scheme for amino acids of antibodies in the CH2 region.

In some embodiments of the invention, the isolated bispecific antibody comprises the mutations S354C and T366W according to the EU numbering scheme for amino acids of antibodies in the CH3 region.

In some embodiments of the invention, the isolated bispecific antibody comprises the mutations Y349C, T366S, L368A and Y407V according to the EU numbering scheme for amino acids of antibodies in the CH3 region.

In some embodiments of the invention, the isolated bispecific antibody comprises the light chain of the first antigen-binding fragment that specifically binds to CD3, and comprises an amino acid sequence that is selected from the group: SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 158, SEQ ID NO: 160 or SEQ ID NO: 162.

In some embodiments of the invention, the isolated bispecific antibody comprises the heavy chain of the first antigen-binding fragment that specifically binds to CD3, and comprises an amino acid sequence that is selected from the group: SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID

NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 159, SEQ ID NO: 161 or SEQ ID NO: 163.

In some embodiments of the invention, the isolated bispecific antibody comprises a light and heavy chain, wherein

a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 158, SEQ ID NO: 160 or SEQ ID NO: 162; and

b) wherein the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 159, SEQ ID NO: 161 or SEQ ID NO: 163.

In some embodiments of the invention, the isolated bispecific antibody comprises the light and heavy chain of the first antigen-binding fragment that specifically binds to CD3, wherein:

(i) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 24 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 30; or

(ii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 25 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 30; or

(iii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 24 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, of the first antigen-binding fragment that specifically binds to CD3, comprises the amino acid sequence of SEQ ID NO: 31; or

(iv) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 24 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 32; or

(v) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 24 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 33; or

(vi) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 25 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 33; or

(vii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 26 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 32; or

(viii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 27 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 34; or

(ix) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 28 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 34; or

(x) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 27 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 35; or

(xi) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 27 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 36; or

(xii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 27 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 37; or

(xiii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 28 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 37; or

(xiv) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 29 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 36; or

(xv) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 25 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 38; or

(xvi) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159; or

(xvii) (a) the light chain of the first antigen-binding fragment, designed in CrossMab format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 160 and

(b) the heavy chain of the first antigen-binding fragment, designed in CrossMab format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 161;

or

(xviii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 162 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 163.

In some embodiments of the invention, the isolated bispecific antibody comprises a second antigen-binding fragment that specifically binds to a tumor antigen that is selected from the group: GUCY2C, AXL, GD2, CD19, EpCAM, EGFR, CD20, CEA, HER2/neu, PSMA, BCMA, MUC17, CD38, FLT3, ENPP3, TPBG, FAP, CD70, Cadherin-19, Mesothelin, CD33, ROR1, IL-3Rα, Cadherin-17, P-cadherin, MUC1, B7-H6, DLL3, B7-H3, CD28, CD155, IRTA2, GPRC5D, RNF43, IL-1RAP, Globo H, Glypican 3, CLDN18, TPBG, LYPD1, PD-L1, GPNMB, NY-ESO-1, MAGE-A4, TACSTD-2, DR5, CD8, TMEFF2, PRLR, CD22, HLA-G, Robo1, EphA2, Prominin-1, HMW-MAA, A33, TAG-72, HER3, IL-23R, CD2, MIF, PD-1, Epha10, GCC, ALP, LFL2, MAGE-A4, STEAP2, CA125, TYRP1, 4-1BB, FRA, CD44v6, Robo4, TIM-3, CLEC12A, B7-H4, FCRL5, SSTR2, c-Kit, MOSPD2, PSA or CD26.

In one aspect, the present invention relates to an isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA.

In one aspect, the present invention relates to an isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA and comprises:

1) a first antigen-binding fragment that specifically binds to CD3 and comprises:

(a) a light chain variable domain comprising:

(i) CDR1 with the amino acid sequence RASQS$X_1$SSNLA,

$X_1$=I or V;

(ii) CDR2 with the amino acid sequence of SEQ ID NO: 3, and

(iii) CDR3 with the amino acid sequence Q$X_2$Y$X_3$NWPLT, where

$X_2$=H or Q;

$X_3$=N or I; and

(b) a heavy chain variable domain comprising:

(i) CDR1 with the amino acid sequence of SEQ ID NO: 6,

(ii) CDR2 with the amino acid sequence of SEQ ID NO: 7, and

(iii) CDR3 with the amino acid sequence DNSGYGHYYYG$X_4$DI, wherein

$X_4$=M or L;

and

2) a second antigen-binding fragment that specifically binds to the tumor antigen BCMA and comprises:

(a) a light chain variable domain comprising:

(i) CDR1 with the amino acid sequence $X_7$G$X_8$$X_9$SNIG$X_{10}$$X_{11}$$X_{12}$$X_{13}$V$X_{14}$, wherein

$X_7$=S or T;

$X_8$=S, G or T;

$X_9$=S, T, I or R;

$X_{10}$=0 or A;

$X_{11}$=S, H, N, G or T;

$X_{12}$=N, R or Y;

$X_{13}$=T, A, I or D;

$X_{14}$=N or H;

(ii) CDR2 with the amino acid sequence $X_{15}$$X_{16}$$X_{17}$$X_{18}$RPS, wherein

$X_{15}$=N, R, K, S or G;

$X_{16}$=D, N, G, T or H;

$X_{17}$=S, N or T;

$X_{18}$=Q or N;

and

(iii) CDR3 with the amino acid sequence $X_{19}$$X_{20}$WD$X_{21}$$X_{22}$$X_{23}$$X_{24}$$X_{25}$W$X_{26}$, wherein

$X_{19}$=A or S;

$X_{20}$=A, S, T or V;

$X_{21}$=G, D, H or S;

$X_{22}$=S, D or R;

$X_{23}$=L or V;

$X_{24}$=N, T, R or S;

$X_{25}$=V, A, G or N;

$X_{26}$=M, V or L;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 with the amino acid sequence $SX_1X_2MS$, wherein

$X_1$=S or G;

$X_2$=A, L or V;

(ii) CDR2 with the amino acid sequence $X_3YNGGSX_0RAGX_4X_5DSVX_6G$, wherein

$X_0$=D or T;

$X_3$=G or C;

$X_4$=F or Y;

$X_5$=A or T;

$X_6$=E or K; and

(iii) CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 83 or SEQ ID NO: 130.

The embodiments of the first antigen-binding fragment that specifically binds to CD3 for the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA are analogous to the above embodiments of the first antigen-binding fragment that specifically binds to CD3 for the isolated bispecific antibody that specifically binds to CD3 and a tumor antigen.

In some embodiments of the invention, the isolated bispecific antibody comprises:

a first antigen-binding fragment that specifically binds to CD3 and is designed in scFv, Fab, scFab or CrossMab format;

and

a second antigen-binding fragment that specifically binds to the tumor antigen BCMA and is designed in scFv, Fab, scFab or CrossMab format.

In some embodiments of the invention, the isolated bispecific antibody, which comprises the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprises CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56 or SEQ ID NO: 122.

In some embodiments of the invention, the isolated bispecific antibody, which comprises the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprises CDR2 with an amino acid sequence that is selected from the group: SEQ ID

NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 123.

In some embodiments of the invention, the isolated bispecific antibody, which comprises the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprises CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO:73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76 or SEQ ID NO: 124.

In some embodiments of the invention, the isolated bispecific antibody, which comprises the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprises CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79 or SEQ ID NO: 125.

In some embodiments of the invention, the isolated bispecific antibody, which comprises the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprises CDR2 with an amino acid sequence that is selected from the group: SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128 or SEQ ID NO: 129.

In some embodiments of the invention, the isolated bispecific antibody that comprises the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56 or SEQ ID NO: 122;

CDR2 with an amino acid sequence that is selected from the group: SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 123;

and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO:73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76 or SEQ ID NO: 124.

In some embodiments of the invention, the isolated bispecific antibody that comprises the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79 or SEQ ID NO: 125;

CDR2 with an amino acid sequence that is selected from the group: SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128 or SEQ ID NO: 129;

and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 83 or SEQ ID NO: 130.

In some embodiments of the invention, the isolated bispecific antibody comprises:

(a) CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56 or SEQ ID NO: 122;

CDR2 with an amino acid sequence that is selected from the group: SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 123;

and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO:73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76 or SEQ ID NO: 124; and

(b) CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79 or SEQ ID NO: 125;

CDR2 with an amino acid sequence that is selected from the group: SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128 or SEQ ID NO: 129;

and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 83 or SEQ ID NO: 130.

In some embodiments of the invention, the isolated bispecific antibody comprises:

(i) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 49,

CDR2 with the amino acid sequence of SEQ ID NO: 57 and

CDR3 with the amino acid sequence of SEQ ID NO: 66; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 77,

CDR2 with the amino acid sequence of SEQ ID NO: 80 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(ii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 50,

CDR2 with the amino acid sequence of SEQ ID NO: 58 and

CDR3 with the amino acid sequence of SEQ ID NO: 67; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 81 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(iii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 51,

CDR2 with the amino acid sequence of SEQ ID NO: 59 and

CDR3 with the amino acid sequence of SEQ ID NO: 68; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 79,

CDR2 with the amino acid sequence of SEQ ID NO: 80 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(iv) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 52,

CDR2 with the amino acid sequence of SEQ ID NO: 60 and

CDR3 with the amino acid sequence of SEQ ID NO: 69; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 80 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(v) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 53,

CDR2 with the amino acid sequence of SEQ ID NO: 61 and

CDR3 with the amino acid sequence of SEQ ID NO: 70; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 81 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(vi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 53,

CDR2 with the amino acid sequence of SEQ ID NO: 61 and

CDR3 with the amino acid sequence of SEQ ID NO: 71; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 82 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(vii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 54,

CDR2 with the amino acid sequence of SEQ ID NO: 63 and

CDR3 with the amino acid sequence of SEQ ID NO: 73; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 81 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(viii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 53,

CDR2 with the amino acid sequence of SEQ ID NO: 61 and

CDR3 with the amino acid sequence of SEQ ID NO: 71; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 81 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(ix) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 55,

CDR2 with the amino acid sequence of SEQ ID NO: 64 and

CDR3 with the amino acid sequence of SEQ ID NO: 74; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 80 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(x) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 49,

CDR2 with the amino acid sequence of SEQ ID NO: 65 and

CDR3 with the amino acid sequence of SEQ ID NO: 75; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 80 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(xi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 56,

CDR2 with the amino acid sequence of SEQ ID NO: 58 and

CDR3 with the amino acid sequence of SEQ ID NO: 76; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 81 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(xii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 51,

CDR2 with the amino acid sequence of SEQ ID NO: 62 and

CDR3 with the amino acid sequence of SEQ ID NO: 72; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 81 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(xiii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 126 and

CDR3 with the amino acid sequence of SEQ ID NO: 130; or

(xiv) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 127 and

CDR3 with the amino acid sequence of SEQ ID NO: 130; or

(xv) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 128 and

CDR3 with the amino acid sequence of SEQ ID NO: 130;

or

(xvi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 129 and

CDR3 with the amino acid sequence of SEQ ID NO: 130.

In some embodiments of the invention, the isolated bispecific antibody comprises the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprises an amino acid sequence that is selected from the group: SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94 or SEQ ID NO: 131.

In some embodiments of the invention, the isolated bispecific antibody comprises the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprises an amino acid sequence that is selected from the group: SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141 or SEQ ID NO: 142.

In some embodiments of the invention, the isolated bispecific antibody comprises:

(a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises an amino acid sequence that is selected from the group: SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94 or SEQ ID NO: 131;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises an amino acid sequence that is selected from the group: SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO:

134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141 or SEQ ID NO: 142.

In some embodiments of the invention, the isolated bispecific antibody comprises:

(i) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 84;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 95; or

(ii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 85;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 96; or

(iii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 86;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 97; or

(iv) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 87;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 98; or

(v) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 88;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 99; or

(vi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 89;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 100; or

(vii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 91;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 99; or

(viii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 89;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 99; or

(ix) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 92;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 101; or

(x) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 93;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 102; or

(xi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 94;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 99; or

(xii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 90;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 99; or

(xiii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 132; or

(xiv) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 133; or

(xv) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 134; or

(xvi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 135; or

(xvii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO:136; or

(xviii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO:137; or

(xix) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 138; or

(xx) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 139; or

(xxi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 140; or

(xxii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 141;

or

(xxiii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 142.

In some embodiments of the invention, the isolated bispecific antibody comprises the light chain of the second antigen-binding fragment that specifically binds to BCMA, and comprises an amino acid sequence that is selected from the group: SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 143 or SEQ ID NO: 144.

In some embodiments of the invention, the isolated bispecific antibody comprises the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, and comprises an amino acid sequence that is selected from the group: SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154 or SEQ ID NO: 155.

In some embodiments of the invention, the isolated bispecific antibody comprises:

(a) the light chain of the second antigen-binding fragment that specifically binds to BCMA, and comprises an amino acid sequence that is selected from the group: SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 143 or SEQ ID NO: 144;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, and comprises an amino acid sequence that is selected from the group: SEQ ID NO: 114,

SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154 or SEQ ID NO: 155.

In some embodiments of the invention, the isolated bispecific antibody comprises light and heavy chains of the second antigen-binding fragment that specifically binds to BCMA, wherein:

(i) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 103;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 114; or

(ii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 104;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 115; or

(iii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 105;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 116; or

(iv) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 106;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 117; or

(v) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 107;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 118; or

(vi) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 108;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 119; or

(vii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 110;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 118; or

(viii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 108;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 118; or

(ix) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 111;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 120; or

(x) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 112;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 121; or

(xi) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 113;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 118; or

(xii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 109;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 118; or

(xiii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 145; or

(xiv) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 146; or

(xv) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 147; or

(xvi) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 148; or

(xvii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 149; or

(xviii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 150; or

(xix) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 151; or

(xx) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 152; or

(xxi) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 153; or

(xxii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 154;

or

(xxiii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 144;

and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 155.

In some embodiments of the invention, the isolated bispecific antibody comprises:

(i) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 126 and

CDR3 with the amino acid sequence of SEQ ID NO: 130; or

(ii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 127 and

CDR3 with the amino acid sequence of SEQ ID NO: 130; or

(iii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 128 and

CDR3 with the amino acid sequence of SEQ ID NO: 130; or

(iv) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 129 and

CDR3 with the amino acid sequence of SEQ ID NO: 130;

or

(v) (a) the light chain variable domain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8;

(b) the heavy chain variable domain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 126 and

CDR3 with the amino acid sequence of SEQ ID NO: 130.

In some embodiments of the invention, the isolated bispecific antibody comprises:

(i) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 132; or

(ii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 133; or

(iii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 134; or

(iv) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 135; or

(v) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(b) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(c) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO:136; or

(vi) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO:137; or

(vii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 138; or

(viii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 139; or

(ix) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 140; or

(x) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 141; or

(xi) (a) the light chain variable domain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 156;

(b) the heavy chain variable domain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 157;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 142;

or

(xii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 142.

In some embodiments of the invention, the isolated bispecific antibody comprises the light and heavy chains of the first antigen-binding fragment that specifically binds to CD3, as well as the light and heavy chains of the second antigen-binding fragment that specifically binds to BCMA, wherein:

(i) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 145; or

(ii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 146; or

(iii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 147; or

(iv) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 148; or

(v) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 149; or

(vi) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 150; or

(vii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 151; or

(viii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 152; or

(ix) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 153; or

(x) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 154; or

(xi) (a) the light chain of the first antigen-binding fragment, designed in CrossMab format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 160;

(b) the heavy chain of the first antigen-binding fragment, designed in CrossMab format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 161;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 144;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 155;

or

(xii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 162;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 163;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 144;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 155.

In one aspect, the present invention relates to an isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA and comprises:

1) a first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3, and comprises:

(a) a light chain variable domain comprising:

(i) CDR1 with the amino acid sequence of SEQ ID NO: 6;

(ii) CDR2 with the amino acid sequence of SEQ ID NO: 7, and

(iii) CDR3 with the amino acid sequence of SEQ ID NO: 8; and

(b) a heavy chain variable domain comprising:

(i) CDR1 with the amino acid sequence of SEQ ID NO: 2,

(ii) CDR2 with the amino acid sequence of SEQ ID NO: 3, and

(iii) CDR3 with the amino acid sequence of SEQ ID NO: 4;

and

2) a second antigen-binding fragment that specifically binds to the tumor antigen BCMA and comprises:

(a) a light chain variable domain comprising:

(i) CDR1 with the amino acid sequence of SEQ ID NO: 122;

(ii) CDR2 with the amino acid sequence of SEQ ID NO: 123;

and

(iii) CDR3 with the amino acid sequence of SEQ ID NO: 124;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 with the amino acid sequence of SEQ ID NO: 125;

i) CDR1 with the amino acid sequence of SEQ ID NO: 126;

and

(iii) CDR3 with the amino acid sequence of SEQ ID NO: 130.

In some embodiments of the invention, the isolated bispecific antibody comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 156;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 157;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 142.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is a full-length IgG antibody.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is a full-length IgG antibody that is of human IgG1, IgG2, IgG3 or IgG4 isotype.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is a full-length IgG antibody that is of human IgG1 isotype.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA comprises the mutations L234A and L235A according to the EU numbering scheme for amino acids of antibodies in the CH2 region.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA comprises the mutations M252Y, S254T, T256E according to the EU numbering scheme for amino acids of antibodies in the CH2 region.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA comprises the mutations S354C and T366W according to the EU numbering scheme for amino acids of antibodies in the CH3 region.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA comprises the mutations Y349C, T366S, L368A and Y407V according to the EU numbering scheme for amino acids of antibodies in the CH3 region.

In some embodiments of the invention, the isolated bispecific antibody comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, and comprises the amino acid sequence of SEQ ID NO: 160;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, and comprises the amino acid sequence of SEQ ID NO: 161;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, and comprises the amino acid sequence of SEQ ID NO: 144;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, and comprises the amino acid sequence of SEQ ID NO: 155.

In some embodiments of the invention, the isolated bispecific antibody is a bispecific antibody that specifically binds to CD3 and the tumor antigen CD20.

In some embodiments of the invention, the bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 may be selected from a group comprising 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014 or 019.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 001.

Antibody 001 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 001 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 10;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 16;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 001 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 24;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 30;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 002.

Antibody 002 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 002 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 16;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 002 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 25;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 30;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 003.

Antibody 003 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 003 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 10;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 17;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 003 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 24;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 31;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 004.

Antibody 004 includes:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 004 includes:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 10;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 18;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 004 includes:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 24;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 32;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 005.

Antibody 005 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 005 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 10;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 005 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 24;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 33;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 006.

Antibody 006 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 006 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 006 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 25;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 33;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 007.

Antibody 007 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 1,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 007 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 12;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 18;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 007 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 26;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 32;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 008.

Antibody 008 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 5;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 9;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 008 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 13;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 20;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 008 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 27;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 34;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 009.

Antibody 009 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 5;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 9;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 009 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 14;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 20;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 009 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 28;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 34;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 010.

Antibody 010 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 5;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 9;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 010 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 13;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 21;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 010 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 27;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 35;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 011.

Antibody 011 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 5;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 9;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 011 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 13;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 22;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 011 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 27;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 36;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 012.

Antibody 012 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 5;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 9;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 012 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 13;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 23;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 012 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 27;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 37;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 013.

Antibody 013 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 5;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 9;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 013 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 14;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 23;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 013 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 28;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 37;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 014.

Antibody 014 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 1,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 5;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 9;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 014 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 15;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 22;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 014 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 29;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 36;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 is antibody 019.

Antibody 019 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 39,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 40 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 41; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 42,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 43 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 44.

Antibody 019 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 45;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 46.

Antibody 019 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 25;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 38;

(c) the light chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 47;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to CD20, comprising the amino acid sequence of SEQ ID NO: 48.

The examples describe antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033 and 01-037 that specifically bind to BCMA and are characterized by the following amino acid sequences.

Antibody 01-001 that specifically binds to BCMA comprises:

(a) a light chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 49,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 57,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 66;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 77,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 80,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 83.

Antibody 01-001 that specifically binds to BCMA comprises:

(a) a light chain variable domain that comprises the amino acid sequence of SEQ ID NO: 84;

and

(b) a heavy chain variable domain that comprises the amino acid sequence of SEQ ID NO: 95.

Antibody 01-001 that specifically binds to BCMA comprises:

(a) a light chain comprising the amino acid sequence of SEQ ID NO: 103;

and

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 114.

Antibody 01-006 that specifically binds to BCMA comprises:

(a) a light chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 50,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 58,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 67;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 78,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 81,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 83.

Antibody 01-006 that specifically binds to BCMA comprises:

(a) a light chain variable domain that comprises the amino acid sequence of SEQ ID NO: 85;

and

(b) a heavy chain variable domain that comprises the amino acid sequence of SEQ ID NO: 96.

Antibody 01-006 that specifically binds to BCMA comprises:

(a) a light chain comprising the amino acid sequence of SEQ ID NO: 104;

and

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 115.

Antibody 01-016 that specifically binds to BCMA comprises:

(a) a light chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 51,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 59,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 68;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 79,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 80,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 83.

Antibody 01-016 that specifically binds to BCMA comprises:

(a) a light chain variable domain that comprises the amino acid sequence of SEQ ID NO: 86;

and

(b) a heavy chain variable domain that comprises the amino acid sequence of SEQ ID NO: 97.

Antibody 01-016 that specifically binds to BCMA comprises:

(a) a light chain comprising the amino acid sequence of SEQ ID NO: 105;

and

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 116.

Antibody 01-021 that specifically binds to BCMA comprises:

(a) a light chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 52,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 60,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 69;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 78,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 80,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 83.

Antibody 01-021 that specifically binds to BCMA comprises:

(a) a light chain variable domain that comprises the amino acid sequence of SEQ ID NO: 87;

and

(b) a heavy chain variable domain that comprises the amino acid sequence of SEQ ID NO: 98.

Antibody 01-021 that specifically binds to BCMA comprises:

(a) a light chain comprising the amino acid sequence of SEQ ID NO: 106;

and

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 117.

Antibody 01-022 that specifically binds to BCMA comprises:

(a) a light chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 53,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 61,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 70;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 78,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 81,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 83.

Antibody 01-022 that specifically binds to BCMA comprises:

(a) a light chain variable domain that comprises the amino acid sequence of SEQ ID NO: 88;

and

(b) a heavy chain variable domain that comprises the amino acid sequence of SEQ ID NO: 99.

Antibody 01-022 that specifically binds to BCMA comprises:

(a) a light chain comprising the amino acid sequence of SEQ ID NO: 107;

and

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 118.

Antibody 01-024 that specifically binds to BCMA comprises:

(a) a light chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 53,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 61,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 71;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 78,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 82,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 83.

Antibody 01-024 that specifically binds to BCMA comprises:

(a) a light chain variable domain that comprises the amino acid sequence of SEQ ID NO: 89;

and

(b) a heavy chain variable domain that comprises the amino acid sequence of SEQ ID NO: 100.

Antibody 01-024 that specifically binds to BCMA comprises:

(a) a light chain comprising the amino acid sequence of SEQ ID NO: 108;

and

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 119.

Antibody 01-025 that specifically binds to BCMA comprises:

(a) a light chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 51,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 62,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 72;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 78,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 81,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 83.

Antibody 01-025 that specifically binds to BCMA comprises:

(a) a light chain variable domain that comprises the amino acid sequence of SEQ ID NO: 90;

and

(b) a heavy chain variable domain that comprises the amino acid sequence of SEQ ID NO: 99.

Antibody 01-025 that specifically binds to BCMA comprises:

(a) a light chain comprising the amino acid sequence of SEQ ID NO: 109;

and

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 118.

Antibody 01-026 that specifically binds to BCMA comprises:

(a) a light chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 54,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 63,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 73;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 78,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 81,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 83.

Antibody 01-026 that specifically binds to BCMA comprises:

(a) a light chain variable domain that comprises the amino acid sequence of SEQ ID NO: 91;

and

(b) a heavy chain variable domain that comprises the amino acid sequence of SEQ ID NO: 99.

Antibody 01-026 that specifically binds to BCMA comprises:

(a) a light chain comprising the amino acid sequence of SEQ ID NO: 110;

and

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 118.

Antibody 01-027 that specifically binds to BCMA comprises:

(a) a light chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 53,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 61,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 71;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 78,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 81,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 83.

Antibody 01-027 that specifically binds to BCMA comprises:

(a) a light chain variable domain that comprises the amino acid sequence of SEQ ID NO: 89;

and

(b) a heavy chain variable domain that comprises the amino acid sequence of SEQ ID NO: 99.

Antibody 01-027 that specifically binds to BCMA comprises:

(a) a light chain comprising the amino acid sequence of SEQ ID NO: 108;

and

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 118.

Antibody 01-030 that specifically binds to BCMA comprises:

(a) a light chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 55,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 64,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 74;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 78,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 80,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 83.

Antibody 01-030 that specifically binds to BCMA comprises:

(a) a light chain variable domain that comprises the amino acid sequence of SEQ ID NO: 92;

and

(b) a heavy chain variable domain that comprises the amino acid sequence of SEQ ID NO: 101.

Antibody 01-030 that specifically binds to BCMA comprises:

(a) a light chain comprising the amino acid sequence of SEQ ID NO: 111;

and

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 120.

Antibody 01-033 that specifically binds to BCMA comprises:

(a) a light chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 49,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 65,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 75;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 78,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 80,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 83.

Antibody 01-033 that specifically binds to BCMA comprises:

(a) a light chain variable domain that comprises the amino acid sequence of SEQ ID NO: 93;

and

(b) a heavy chain variable domain that comprises the amino acid sequence of SEQ ID NO: 102.

Antibody 01-033 that specifically binds to BCMA comprises:

(a) a light chain comprising the amino acid sequence of SEQ ID NO: 112;

and

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 121.

Antibody 01-037 that specifically binds to BCMA comprises:

(a) a light chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 56,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 58,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 76;

and

(b) a heavy chain variable domain comprising:

(i) CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 78,

(ii) CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 81,

(iii) CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 83.

Antibody 01-037 that specifically binds to BCMA comprises:

(a) a light chain variable domain that comprises the amino acid sequence of SEQ ID NO: 94;

and

(b) a heavy chain variable domain that comprises the amino acid sequence of SEQ ID NO: 99.

Antibody 01-037 that specifically binds to BCMA comprises:

(a) a light chain comprising the amino acid sequence of SEQ ID NO: 113;

and

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 118.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is an antibody that is selected from the group: 02-001, 02-002, 02-003, 02-004, 02-005, 02-006, 02-007, 02-008, 02-009, 02-010, 02-011 or 02-012.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is antibody 02-001.

Antibody 02-001 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 122,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 123 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 125,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 127 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 130.

Antibody 02-001 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 132.

Antibody 02-001 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 145.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is antibody 02-002.

Antibody 02-002 comprises:

(iii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 122,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 123 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 125,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 128 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 130.

Antibody 02-002 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 133.

Antibody 02-002 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 146.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is antibody 02-003.

Antibody 02-003 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 122,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 123 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 125,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 127 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 130.

Antibody 02-003 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 134.

Antibody 02-003 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 147.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is antibody 02-004.

Antibody 02-004 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 122,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 123 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 125,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 128 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 130.

Antibody 02-004 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 135.

Antibody 02-004 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 148.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is antibody 02-005.

Antibody 02-005 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 122,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 123 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 125,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 126 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 130.

Antibody 02-005 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(b) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 131;

and

(c) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO:136.

Antibody 02-005 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 149.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is antibody 02-006.

Antibody 02-006 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 122,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 123 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 125,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 127 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 130.

Antibody 02-006 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO:137.

Antibody 02-006 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 150.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is antibody 02-007.

Antibody 02-007 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 122,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 123 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 125,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 127 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 130.

Antibody 02-007 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 138.

Antibody 02-007 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 151.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is antibody 02-008.

Antibody 02-008 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 122,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 123 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 125,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 128 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 130.

Antibody 02-008 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 139.

Antibody 02-008 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 152.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is antibody 02-009.

Antibody 02-009 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 122,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 123 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 125,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 129 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 130.

Antibody 02-009 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 140.

Antibody 02-009 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 153.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is antibody 02-010.

Antibody 02-010 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 122,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 123 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 125,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 128 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 130.

Antibody 02-010 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 141.

Antibody 02-010 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 143;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 154.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is antibody 02-011. Antibody 02-011 comprises the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3.

As used in this description, CrossMAb format means a replacement of the light chain variable domain and the heavy chain variable domain by one another.

Antibody 02-011 comprises:

(a) the light chain variable domain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(b) the heavy chain variable domain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 122,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 123 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 125,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 126 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 130.

Antibody 02-011 comprises:

(a) the light chain variable domain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 156;

(b) the heavy chain variable domain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 157;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 142.

Antibody 02-011 comprises:

(a) the light chain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 160;

(b) the heavy chain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 161;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 144;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 155.

In some embodiments of the invention, the isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA is antibody 02-012.

Antibody 02-012 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 2,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 3 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 6,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 7 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 122,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 123 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 (Kabat) with the amino acid sequence of SEQ ID NO: 125,

CDR2 (Kabat) with the amino acid sequence of SEQ ID NO: 126 and

CDR3 (Kabat) with the amino acid sequence of SEQ ID NO: 130.

Antibody 02-012 comprises:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 131;

and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 142.

Antibody 02-012 comprises:

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 162;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, comprising the amino acid sequence of SEQ ID NO: 163;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 144;

and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA, comprising the amino acid sequence of SEQ ID NO: 155.

The hypervariable regions of variable domains of light and heavy chains (LCDR1, 2, 3 and HCDR1, 2, 3) of all the above antibodies are provided in accordance with the Kabat nomenclature. Those skilled will appreciate that the hypervariable regions of variable domains of light and heavy chains (LCDR1, 2, 3 and HCDR1, 2, 3) may also be represented in accordance with other commonly known numbering scheme, for example, IMGT, Chothia or AbM. Thus, all of the above antibodies which are characterized by means of hypervariable regions of variable domains of light and heavy chains (LCDR1, 2, 3 and HCDR1, 2, 3) using the IMGT, Chothia or AbM numbering scheme are also encompassed by the present invention.

*Nucleic acid molecule*

In one aspect, the present invention relates to a nucleic acid that encodes any above bispecific antibody that specifically binds to CD3 and a tumor antigen.

In one aspect, the present invention relates to a nucleic acid that encodes any above bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA.

In any one of said embodiments, the nucleic acid molecules may be isolated.

The terms "nucleic acid", "nucleic sequence" or "nucleic acid sequence", "polynucleotide", "oligonucleotide", "polynucleotide sequence" and "nucleotide sequence", used interchangeably in the present description, mean a precise sequence of nucleotides, modified or not, determining a fragment or a region of a nucleic acid, containing unnatural nucleotides or not, and being either a double-strand DNA or RNA, a single-strand DNA or RNA, or transcription products of said DNAs.

Unless otherwise indicated, the term nucleotide sequence encompasses its complement. Thus, a nucleic acid having a particular sequence should be understood as one which encompasses the complementary strand thereof with the complementary sequence thereof.

An "isolated" nucleic acid molecule is one which is identified and separated from at least one nucleic acid molecule-impurity. An isolated nucleic acid molecule is different from the form or set in which it is found under natural conditions. Thus, an isolated nucleic acid molecule is different from a nucleic acid molecule that exists in cells under natural conditions.

In one aspect, the present invention relates to a nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence selected from SEQ ID NO: 1-165. A nucleic acid molecule may also comprise any combination of said nucleotide sequences.

As would be appreciated by those skilled in the art, because of the redundancy of the genetic code, a variety of different DNA sequences can encode the amino acid sequence of the light chain or heavy chain of the bispecific antibody according to the invention or fragments thereof (VH, VL, CDR, and so forth). It is well within the skill of those trained in the art to create these alternative DNA sequences encoding one and the same amino acid sequences. Such variant DNA sequences are within the scope of the present invention.

In some embodiments of the invention, the isolated nucleic acid is DNA.

The nucleic acid molecule according to the present invention may be isolated from any source that produces the monoclonal bispecific antibody according to the present invention or antigen-binding fragment thereof. In certain embodiments of the invention, the nucleic acid molecule of the invention may be synthesized by way of chemical synthesis, rather than isolated.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 001, 003, 004 and 005 and comprises a nucleotide sequence with SEQ ID NO: 166.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 002, 006, 019, 02-001, 02-002, 02-003, 02-004, 02-

005, 02-006, 02-007, 02-008, 02-009, 02-010, 02-012 and comprises a nucleotide sequence with SEQ ID NO: 167.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibody 007 and comprises a nucleotide sequence with SEQ ID NO: 168.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 008, 010, 011 and 012 and comprises a nucleotide sequence with SEQ ID NO: 169.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 009 and 013 and comprises a nucleotide sequence with SEQ ID NO: 170.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibody 014 and comprises a nucleotide sequence with SEQ ID NO: 171.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 001, 003, 004 and 005 and comprises a nucleotide sequence with SEQ ID NO: 172.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 002, 006, 019, 02-001, 02-002, 02-003, 02-004, 02-005, 02-006, 02-007, 02-008, 02-009, 02-010, 02-012 and comprises a nucleotide sequence with SEQ ID NO: 173.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of the first antigen-binding fragment, which specifically binds to CD3, of antibody 007 and comprises a nucleotide sequence with SEQ ID NO: 174.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 008, 010, 011 and 012, and comprises a nucleotide sequence with SEQ ID NO: 175.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of the first antigen-binding fragment, which specifically

binds to CD3, of antibodies 009 and 013, and comprises a nucleotide sequence with SEQ ID NO: 176.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of the first antigen-binding fragment, which specifically binds to CD3, of antibody 014, and comprises a nucleotide sequence with SEQ ID NO: 177.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 001, 002, and comprises a nucleotide sequence with SEQ ID NO: 178.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibody 003, and comprises a nucleotide sequence with SEQ ID NO: 179.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 004, 007, and comprises a nucleotide sequence with SEQ ID NO: 180.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 005, 006, 019, 02-001, 02-002, 02-003, 02-004, 02-005, 02-006, 02-007, 02-008, 02-009, 02-010 and 02-012, and comprises a nucleotide sequence with SEQ ID NO: 181.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 008, 009, and comprises a nucleotide sequence with SEQ ID NO: 182.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibody 010, and comprises a nucleotide sequence with SEQ ID NO: 183.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 011, 014, and comprises a nucleotide sequence with SEQ ID NO: 184.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 012, 013, and comprises a nucleotide sequence with SEQ ID NO: 185.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 001, 002, and comprises a nucleotide sequence with SEQ ID NO: 186.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the first antigen-binding fragment, which specifically binds to CD3, of antibody 003, and comprises a nucleotide sequence with SEQ ID NO: 187.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 004, 007, and comprises a nucleotide sequence with SEQ ID NO: 188.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 005, 006, 019, 02-001, 02-002, 02-003, 02-004, 02-005, 02-006, 02-007, 02-008, 02-009, 02-010 and 02-012, and comprises a nucleotide sequence with SEQ ID NO: 189.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 008, 009, and comprises a nucleotide sequence with SEQ ID NO: 190.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the first antigen-binding fragment, which specifically binds to CD3, of antibody 010, and comprises a nucleotide sequence with SEQ ID NO: 191.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 011, 014, and comprises a nucleotide sequence with SEQ ID NO: 192.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 012, 013, and comprises a nucleotide sequence with SEQ ID NO: 193.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the first antigen-binding fragment, which specifically binds to CD3, of antibody 019, and comprises a nucleotide sequence with SEQ ID NO: 194.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of the second antigen-binding fragment, which specifically

binds to CD20, of antibodies 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 019, and comprises a nucleotide sequence with SEQ ID NO: 195.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the second antigen-binding fragment, which specifically binds to CD20, of antibodies 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 019, and comprises a nucleotide sequence with SEQ ID NO: 196.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of antibody 01-001, and comprises a nucleotide sequence with SEQ ID NO: 197.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of antibody 01-006, and comprises a nucleotide sequence with SEQ ID NO: 198.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of antibody 01-016, and comprises a nucleotide sequence with SEQ ID NO: 199.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of antibody 01-021, and comprises a nucleotide sequence with SEQ ID NO: 200.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of antibody 01-022, and comprises a nucleotide sequence with SEQ ID NO: 201.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of antibodies 01-024 and 01-027, and comprises a nucleotide sequence with SEQ ID NO: 202.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of antibody 01-025, and comprises a nucleotide sequence with SEQ ID NO: 203.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of antibody 01-026, and comprises a nucleotide sequence with SEQ ID NO: 204.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of antibody 01-030, and comprises a nucleotide sequence with SEQ ID NO: 205.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of antibody 01-033, and comprises a nucleotide sequence with SEQ ID NO: 206.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of antibody 01-037, and comprises a nucleotide sequence with SEQ ID NO: 207.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of antibody 01-001, and comprises a nucleotide sequence with SEQ ID NO: 208.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of antibody 01-006, and comprises a nucleotide sequence with SEQ ID NO: 209.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of antibody 01-016, and comprises a nucleotide sequence with SEQ ID NO: 210.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of antibody 01-021, and comprises a nucleotide sequence with SEQ ID NO: 211.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of antibodies 01-022, 01-025, 01-026, 01-027 and 01-037, and comprises a nucleotide sequence with SEQ ID NO: 212.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of antibody 01-024, and comprises a nucleotide sequence with SEQ ID NO: 213.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of antibody 01-030, and comprises a nucleotide sequence with SEQ ID NO: 214.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of antibody 01-033, and comprises a nucleotide sequence with SEQ ID NO: 215.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of antibody 01-001, and comprises a nucleotide sequence with SEQ ID NO: 216.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of antibody 01-006, and comprises a nucleotide sequence with SEQ ID NO: 217.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of antibody 01-016, and comprises a nucleotide sequence with SEQ ID NO: 218.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of antibody 01-021, and comprises a nucleotide sequence with SEQ ID NO: 219.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of antibody 01-022, and comprises a nucleotide sequence with SEQ ID NO: 220.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of antibodies 01-024 and 01-027, and comprises a nucleotide sequence with SEQ ID NO: 221.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of antibody 01-025, and comprises a nucleotide sequence with SEQ ID NO: 222.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of antibody 01-026, and comprises a nucleotide sequence with SEQ ID NO: 223.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of antibody 01-030, and comprises a nucleotide sequence with SEQ ID NO: 224.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of antibody 01-033, and comprises a nucleotide sequence with SEQ ID NO: 225.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of antibody 01-037, and comprises a nucleotide sequence with SEQ ID NO: 226.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of antibody 01-001, and comprises a nucleotide sequence with SEQ ID NO: 227.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of antibody 01-006, and comprises a nucleotide sequence with SEQ ID NO: 228.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of antibody 01-016, and comprises a nucleotide sequence with SEQ ID NO: 229.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of antibody 01-021, and comprises a nucleotide sequence with SEQ ID NO: 230.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of antibodies 01-022, 01-025, 01-026, 01-027 and 01-037, and comprises a nucleotide sequence with SEQ ID NO: 231.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of antibody 01-024, and comprises a nucleotide sequence with SEQ ID NO: 232.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of antibody 01-030, and comprises a nucleotide sequence with SEQ ID NO: 233.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of antibody 01-033, and comprises a nucleotide sequence with SEQ ID NO: 234.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of the second antigen-binding fragment, which specifically binds to BCMA, of antibodies 02-001, 02-002, 02-003, 02-004, 02-005, 02-006, 02-007, 02-008, 02-009, 02-010, 02-011, 02-012, and comprises a nucleotide sequence with SEQ ID NO: 235.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-001, and comprises a nucleotide sequence with SEQ ID NO: 236.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-002, and comprises a nucleotide sequence with SEQ ID NO: 237.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-003, and comprises a nucleotide sequence with SEQ ID NO: 238.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-004, and comprises a nucleotide sequence with SEQ ID NO: 239.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-005, and comprises a nucleotide sequence with SEQ ID NO: 240.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-006, and comprises a nucleotide sequence with SEQ ID NO: 241.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-007, and comprises a nucleotide sequence with SEQ ID NO: 242.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-008, and comprises a nucleotide sequence with SEQ ID NO: 243.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-009, and comprises a nucleotide sequence with SEQ ID NO: 244.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-010, and comprises a nucleotide sequence with SEQ ID NO: 245.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the second antigen-binding fragment,

which specifically binds to BCMA, of antibodies 02-011, 02-012, and comprises a nucleotide sequence with SEQ ID NO: 246.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibodies 02-001, 02-002, 02-003, 02-004, 02-005, 02-006, 02-007, 02-008, 02-009, 02-010, and comprises a nucleotide sequence with SEQ ID NO: 247.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibodies 02-011, 02-012, and comprises a nucleotide sequence with SEQ ID NO: 248.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-001, and comprises a nucleotide sequence with SEQ ID NO: 249.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-002, and comprises a nucleotide sequence with SEQ ID NO: 250.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-003, and comprises a nucleotide sequence with SEQ ID NO: 251.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-004, and comprises a nucleotide sequence with SEQ ID NO: 252.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-005, and comprises a nucleotide sequence with SEQ ID NO: 253.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-006, and comprises a nucleotide sequence with SEQ ID NO: 254.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-007, and comprises a nucleotide sequence with SEQ ID NO: 255.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-008, and comprises a nucleotide sequence with SEQ ID NO: 256.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-009, and comprises a nucleotide sequence with SEQ ID NO: 257.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibody 02-010, and comprises a nucleotide sequence with SEQ ID NO: 258.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the second antigen-binding fragment, which specifically binds to BCMA, of antibodies 02-011, 02-012, and comprises a nucleotide sequence with SEQ ID NO: 259.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain variable domain of the first antigen-binding fragment, designed in CrossMab format, which specifically binds to CD3, of antibody 02-011, and comprises a nucleotide sequence with SEQ ID NO: 260.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain variable domain of the first antigen-binding fragment, designed in CrossMab format, which specifically binds to CD3, of antibody 02-011, and comprises a nucleotide sequence with SEQ ID NO: 261.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 02-001, 02-002, 02-003, 02-004, 02-005, 02-006, 02-007, 02-008, 02-009, 02-010, and comprises a nucleotide sequence with SEQ ID NO: 262.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the first antigen-binding fragment, which specifically binds to CD3, of antibodies 02-001, 02-002, 02-003, 02-004, 02-005, 02-006, 02-007, 02-008, 02-009, 02-010, and comprises a nucleotide sequence with SEQ ID NO: 263.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of the first antigen-binding fragment, designed in CrossMab format, which specifically binds to CD3, of antibody 02-011, and comprises a nucleotide sequence with SEQ ID NO: 264.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the first antigen-binding fragment, designed in CrossMab format, which specifically binds to CD3, of antibody 02-011, and comprises a nucleotide sequence with SEQ ID NO: 265.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the light chain of the first antigen-binding fragment, designed in scFv format, which specifically binds to CD3, of antibody 02-012, and comprises a nucleotide sequence with SEQ ID NO: 266.

In some embodiments of the invention, the nucleic acid is a nucleic acid that encodes the amino acid sequence of the heavy chain of the first antigen-binding fragment, designed in scFv format, which specifically binds to CD3, of antibody 02-012, and comprises a nucleotide sequence with SEQ ID NO: 267.

Nucleic acid molecules may be used to express the recombinant monoclonal bispecific antibody of the present invention or an antigen-binding fragment thereof.

*Vector*

In one aspect, the present invention relates to an expression vector comprising any one of the above nucleic acid molecules that encode the corresponding amino acid sequences of the bispecific antibody that specifically binds to CD3 and a tumor antigen, or portions thereof (for example, the sequences of the binding domain of the heavy chain and/or light chain). The present invention relates to a vector suitable for the expression of any one of the nucleotide sequences described herein.

In one aspect, the present invention relates to an expression vector comprising any one of the above nucleic acid molecules that encode the corresponding amino acid sequences of the bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA, or portions thereof (for example, the sequences of the binding domain of the heavy chain and/or light chain). The present invention relates to a vector suitable for the expression of any one of the nucleotide sequences described herein.

The term "vector" as used herein means a nucleic acid molecule capable of transporting other nucleic acid to which it has been linked.

As used in the present description, the term “expression” is defined as the transcription and/or translation of a particular nucleotide sequence driven by its promoter.

In some embodiments of the invention, the vector is a plasmid, i.e. a circular double stranded piece of DNA into which additional DNA segments may be inserted.

In some embodiments of the invention, the vector is a viral (expression) vector, wherein additional DNA segments may be inserted into the viral genome.

In some embodiments of the invention, the vectors are capable of autonomous replication in a host cell into which they have been introduced (e.g. bacterial vectors having a bacterial site of replication origin and episomal vectors). In further embodiments of the invention, vectors (e.g. non-episomal vectors) may be integrated into the genome of a host cell upon introduction into a

host cell, and thereby are replicated along with the host gene. Moreover, certain vectors are capable of directing the expression of genes to which they are operably linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors").

In some embodiments of the invention, expression vectors include plasmids, retroviruses, adenoviruses, adeno-associated viruses (AAVs), plant viruses, such as cauliflower mosaic virus, tobacco mosaic virus, cosmids, YACs, and the like. DNA molecules may be inserted into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of DNA. An expression vector and expression control sequences may be chosen to be compatible with the expression host cell used.

In one embodiment of the invention, DNA molecules partially or fully encoding the sequences of first and second binding domains (for example, heavy and light chain sequences where a binding domain comprises a heavy and light chain sequence) may be introduced into individual vectors.

In one embodiment, any combination of the above DNA molecules is introduced into the same expression vector.

In one embodiment of the invention, DNA molecules may be introduced into an expression vector by standard methods (e.g. ligation of complementary restriction sites on a gene fragment of antibody and vector, or blunt end ligation if no restriction sites are present).

In some embodiments of the invention, a suitable vector is one that includes restriction sites such that any VH or VL sequence can easily be inserted and expressed, as described above. A recombinant expression vector can also encode a signal peptide that facilitates secretion of an antibody chain from a host cell. An antibody chain gene may be cloned into a vector such that the signal peptide is linked in-frame to the amino terminus of an immunoglobulin chain. A signal peptide may be an immunoglobulin signal peptide or a heterologous signal peptide (i.e. a signal peptide from a non-immunoglobulin protein).

In some embodiments of the invention, the vector may include an expression control sequence. The term "expression control sequence" as used in the present description refers to polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are inserted. It will be understood by those skilled in the art that the design of an expression vector, including the selection of expression control sequences, may depend on such factors as the choice of the type of a host cell to be transformed, the required level of expression of antibody, and so forth. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that

enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such expression control sequences differs depending upon the host organism; in prokaryotes, such expression control sequences typically include a promoter, a ribosome binding site, as well as transcription termination sequences; in eukaryotes, such expression control sequences typically include promoters and transcription termination sequences. Preferred expression control sequences for an expression host cell in a mammal include viral elements that ensure high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from a retroviral LTR, cytomegalovirus (CMV) (such as a CMV promoter/enhancer), simian virus 40 (SV40) (such as a SV40 promoter/enhancer), adenovirus, (e.g. the major late promoter adenovirus (AdMLP)), polyomavirus and strong mammalian promoters such as TTR promoter, native immunoglobulin promoter or actin promoter. Expression control sequences encompass at least all components whose presence is important for expression and processing.

In some embodiments of the invention, in addition to antibody chain genes and expression control sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of a vector in host cells (e.g. origins of replication) and selectable marker genes. The selectable marker gene facilitates the selection of host cells into which a vector has been introduced.

*Host cell*

In one aspect, the present invention relates to a method for producing a host cell to produce any above bispecific antibody that specifically binds to CD3 and a tumor antigen, and comprises transforming the cell with the above vector.

In one aspect, the present invention relates to a host cell for producing any above bispecific antibody that specifically binds to CD3 and a tumor antigen, which comprises any one of the above nucleic acids.

In one aspect, the present invention relates to a method for producing a host cell to produce any above bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA, and comprises transforming the cell with the above vector.

In one aspect, the present invention relates to a host cell for producing any above bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA, which comprises any one of the above nucleic acids.

The term "host cell" as used herein refers to a cell into which a recombinant expression vector has been introduced. The present invention relates to host cells, which may include, for example, the above-described vector according to the invention. The present invention also relates to host cells that comprise, for example, a nucleotide sequence encoding the heavy chain of the first antigen-binding fragment or antigen-binding portions thereof, a nucleotide sequence encoding

the light chain of the first antigen-binding fragment or antigen-binding portions thereof, a nucleotide sequence encoding the heavy chain of the second antigen-binding fragment or antigen-binding portions thereof, a nucleotide sequence, encoding the light chain of the second antigen-binding fragment or antigen-binding portions thereof, or all four of them. It should be understood that "host cell" refers not only to a particular subject cell but to the progeny of such cell as well. Since modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to a parental cell; however, such cells are still included within the scope of the term "host cell" as used herein.

Nucleic acid molecules encoding the monoclonal bispecific antibody or antigen-binding fragment thereof according to the invention and vectors comprising these nucleic acid molecules may be used for transfection of a mammalian cell, plant cell, bacterial cell, or yeast cell. Transfection may be carried out by any known method for introducing polynucleotides into a host cell. Methods for introducing heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, cationic polymer-nucleic acid complex transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, encapsulation of the polynucleotides in liposomes, and direct microinjection of DNA into nuclei. In addition, the nucleic acid molecules may be introduced into mammalian cells by viral (expression) vectors.

Mammalian cell lines used as hosts for transformation are well known in the art and include a plurality of immortalized cell lines available. These include, e.g., Chinese hamster ovary (CHO) cells, NS0 cells, SP2 cells, HEK-293T cells, FreeStyle 293 cells (Invitrogen), NIH-3T3 cells, HeLa cells, baby hamster kidney (BHK) cells, African green monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), A549, SK-HEP1, HUH7, Hep-RG cells and a number of other cell lines. Cell lines are selected by way of determining which cell lines have high expression levels and provide for necessary characteristics of the protein being produced. Other cell lines that may be used are insect cell lines, such as Sf9 or Sf21 cells. When recombinant expression vectors encoding the bispecific antibody of the invention are introduced into mammalian host cells, the antibodies or fragments thereof are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibodies or fragments thereof in host cells or, more preferably, secretion of the antibodies or fragments thereof into a culture medium in which the host cells are grown. The bispecific antibody of the invention can be isolated from a culture medium using standard protein purification techniques. Plant host cells include e.g. Nicotiana, Arabidopsis, duckweed, corn, wheat, potato, etc. Bacterial host cells include Escherichia and Streptomyces species. Yeast host cells include Schizosaccharomyces pombe, Saccharomyces cerevisiae and Pichia pastoris.

Furthermore, level of production of the bispecific antibody of the invention from a producing cell line may be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain conditions.

It is likely that the bispecific antibodies of the invention in different cell lines will have different glycosylation patterns from one another. However, the bispecific antibody of the invention encoded by nucleic acid molecules described herein, or comprising amino acid sequences provided herein are part of the present invention, regardless of the glycosylation of the binding molecules, and, in general, regardless of the presence or absence of post-translational modifications.

The above host cell does not relate to a host cell produced using human embryos.

The above host cell does not relate to a host cell produced by modifying the genetic integrity of human germline cells.

*Method for producing antibody*

In one aspect, the present invention relates to a method for producing a bispecific antibody that specifically binds to CD3 and a tumor antigen, comprising culturing the above host cell in a growth medium under conditions sufficient to produce said antibody or fragment thereof, followed by isolation and purification of the resulting antibody.

In one aspect, the present invention relates to a method for producing a bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA, comprising culturing the above host cell in a growth medium under conditions sufficient to produce said antibody or fragment thereof, followed by isolation and purification of the resulting antibody.

*Pharmaceutical compositions*

In one aspect, the present invention relates to a pharmaceutical composition comprising, as an active ingredient (or as the only active ingredient), the bispecific antibody of the present invention that specifically binds to CD3 and a tumor antigen.

In one aspect, the present invention relates to a pharmaceutical composition comprising, as an active ingredient (or as the only active ingredient), the bispecific antibody of the present invention that specifically binds to CD3 and a tumor antigen in combination with one or more pharmaceutically acceptable excipients.

In one aspect, the present invention relates to a pharmaceutical composition comprising, as an active ingredient (or as the only active ingredient), the bispecific antibody of the present invention that specifically binds to CD3 and a tumor antigen in a therapeutically effective amount in combination with one or more pharmaceutically acceptable excipients.

In one aspect, the present invention relates to a pharmaceutical composition for treating a disease or disorder mediated by a tumor antigen, which comprises any bispecific antibody of the present invention that specifically binds to CD3 and a tumor antigen in a therapeutically effective amount in combination with one or more pharmaceutically acceptable excipients.

In another aspect, the present invention relates to a pharmaceutical composition comprising, as an active ingredient (or as the only active ingredient), the bispecific antibody of the present invention that specifically binds to CD3 and the tumor antigen BCMA.

In one aspect, the present invention relates to a pharmaceutical composition comprising, as an active ingredient (or as the only active ingredient), the bispecific antibody of the present invention that specifically binds to CD3 and the tumor antigen BCMA in combination with one or more pharmaceutically acceptable excipients.

In one aspect, the present invention relates to a pharmaceutical composition comprising, as an active ingredient (or as the only active ingredient), the bispecific antibody of the present invention that specifically binds to CD3 and the tumor antigen BCMA in a therapeutically effective amount in combination with one or more pharmaceutically acceptable excipients.

In one aspect, the present invention relates to a pharmaceutical composition for treating a disease or disorder mediated by a tumor antigen, which comprises any bispecific antibody of the present invention that specifically binds to CD3 and the tumor antigen BCMA in a therapeutically effective amount in combination with one or more pharmaceutically acceptable excipients.

"Pharmaceutical composition" means a composition comprising the antibody according to the invention and at least one of components selected from the group consisting of pharmaceutically acceptable and pharmacologically compatible fillers, solvents, diluents, carriers, auxiliary, distributing and sensing agents, delivery agents.

The term "pharmaceutically acceptable" refers to one or more compatible liquid or solid components that are suitable for administration in a mammal, preferably in a human.

The term "excipient" is used herein to describe any ingredient other than the antibody according to the present invention. These are substances of inorganic or organic nature which are used in the pharmaceutical production/manufacturing in order to give drug products the necessary physicochemical properties.

In some embodiments, the compositions are intended to improve, prevent, or treat diseases or disorders that may be mediated by a tumor antigen.

The term "disease or disorder mediated by a tumor antigen" refers to any disease or disorder that is either directly, or indirectly associated with tumor antigen, including etiology, development, progression, persistence or pathology of a disease or disorder.

"Treat", "treatment" and "therapy" refer to a method of alleviating or abrogating a biological disorder and/or at least one of attendant symptoms thereof. Further, references herein to "treatment" include references to curative, palliative and prophylactic treatment.

The term "disorder" means any condition that would benefit from treatment according to the present invention. The definition of the term includes chronic and acute disorders or diseases including those pathological conditions that predispose the mammal to the disorder in question.

"Therapeutically effective amount" refers to that amount of the therapeutic agent being administered during treatment which will relieve to some extent one or more of the symptoms of the disease being treated. A therapeutically effective amount may vary according to factors such as the specific condition to be treated, age, sex, and weight of a patient, and whether the bispecific antibody according to the present invention is administered alone or in combination with one or more additional drugs or treatment techniques.

In one aspect, the subject of treatment, or patient, is a mammal, preferably a human subject. Said subject may be either male or female, of any age.

The pharmaceutical compositions of the present invention and methods of preparation thereof will be undoubtedly apparent to those skilled in the art. The pharmaceutical compositions should preferably be manufactured in compliance with the GMP (Good Manufacturing Practice) requirements.

In some embodiments of the pharmaceutical composition, it may include a buffer composition, tonicity agents (osmolyte or osmotic agent), stabilizers and/or solubilizers.

The pharmaceutical composition according to the invention is a stable composition.

A pharmaceutical composition is "stable" if the active agent retains physical stability and/or chemical stability and/or biological activity thereof during the specified shelf life at storage temperature, for example, of 2-8 °C. Preferably, the active agent retains both physical and chemical stability, as well as biological activity. Storage period is adjusted based on the results of stability test in accelerated or natural aging conditions.

The pharmaceutical composition according to the present invention is suitable for parenteral administration as sterile formulations intended for administration in a subject body through the breach in skin or mucosal barriers, bypassing the gastrointestinal tract by virtue of injection, infusion and implantation. In particular, it is contemplated that parenteral administration includes, inter alia, subcutaneous, intraperitoneal, intramuscular, intravenous, intraarterial, intrathecal, intraventricular, intraurethral, intracranial, intrasynovial, transdermal injection or infusion; and kidney dialytic infusion techniques. Intra-tumor delivery, for example, intra-tumor injection, may also be employed. Regional perfusion is also contemplated.

In some embodiments, the pharmaceutical composition is administered intravenously.

In some embodiments, intravenous administration is carried out by using infusion, prolonged infusion, or long-lasting continuous infusion.

In some embodiments, the pharmaceutical composition is administered subcutaneously.

In some embodiments, subcutaneous administration is carried out by using subcutaneous injection.

In some embodiments, the pharmaceutical composition is an injectable dosage form.

In some embodiments, the injectable dosage form is an infusion solution.

In some embodiments, the injectable dosage form is a solution for subcutaneous administration.

Injectable formulations may be manufactured without limitation, in unit dosage form, such as in ampoules, vials, plastic containers, pre-filled syringes, autoinjection devices.

In some embodiments, the pharmaceutical composition is a pharmaceutical composition provided in dry, i.e. powder or granular, form for reconstitution with a suitable solvent (e.g., sterile pyrogen-free water) prior to administration. Such medicinal formulation may be prepared by, for example, lyophilization, i.e. a process, which is known in the art as freeze drying, and which involves freezing a product followed by removal of solvent from frozen material.

In some embodiments, the pharmaceutical composition is a lyophilizate for preparing a solution for infusion.

In some embodiments, the pharmaceutical composition is a lyophilizate for preparing a solution for subcutaneous administration.

In some embodiments, the pharmaceutical composition is a concentrate for preparing a solution for infusion.

In some embodiments, the pharmaceutical composition is a concentrate for preparing a solution for subcutaneous administration.

In one aspect, the present invention relates to a pharmaceutical composition that comprises a bispecific antibody according to the present invention, that specifically binds to CD3 and a tumor antigen, and at least one other therapeutically active compound.

In one aspect, the present invention relates to a pharmaceutical composition that comprises a bispecific antibody according to the present invention, that specifically binds to CD3 and the tumor antigen BCMA, and at least one other therapeutically active compound.

In one aspect, the present invention relates to a pharmaceutical composition that comprises a bispecific antibody according to the present invention, that specifically binds to CD3 and a tumor antigen, in a therapeutically effective amount and at least one other therapeutically active compound.

In one aspect, the present invention relates to a pharmaceutical composition that comprises a bispecific antibody according to the present invention, that specifically binds to CD3 and the tumor antigen in a therapeutically effective amount, in a therapeutically effective amount and at least one other therapeutically active compound.

In one aspect, the present invention relates to a pharmaceutical composition for treating a disease or disorder mediated by a tumor antigen, which comprises any above bispecific antibody and at least one other therapeutically active compound.

In one aspect, the present invention relates to a pharmaceutical composition comprising any above bispecific antibody and further at least one other therapeutically active compound.

In one aspect, the present invention relates to a pharmaceutical composition for treating a disease or disorder mediated by a tumor antigen, which comprises any above bispecific antibody and further at least one other therapeutically active compound.

In one aspect, the present invention relates to a pharmaceutical composition for treating a disease or disorder mediated by a tumor antigen, which comprises any above bispecific antibody and at least one other therapeutically active compound, which is an antibody, a small molecule, a hormone therapy agent or a combination thereof.

In one aspect, the present invention relates to a pharmaceutical composition for treating a disease or disorder mediated by a tumor antigen, which comprises any above bispecific antibody in a therapeutically effective amount and at least one other therapeutically active compound.

In one aspect, the present invention relates to a pharmaceutical composition comprising any above bispecific antibody in a therapeutically effective amount and further at least one other therapeutically active compound.

In one aspect, the present invention relates to a pharmaceutical composition for treating a disease or disorder mediated by a tumor antigen, which comprises any above bispecific antibody in a therapeutically effective amount and further at least one other therapeutically active compound.

In one aspect, the present invention relates to a pharmaceutical composition for treating a disease or disorder mediated by a tumor antigen, which comprises any above bispecific antibody in a therapeutically effective amount and at least one other therapeutically active compound, which is an antibody, a small molecule, a hormone therapy agent or a combination thereof.

In some embodiments of the pharmaceutical composition, the disease or disorder mediated by a tumor antigen is selected from the group: multiple myeloma, chronic lymphocytic leukemia, acute B-lymphoblastic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma.

*Therapeutic use of bispecific antibody according to the present invention*

In one aspect, the bispecific antibody of the present invention that specifically binds to CD3 and a tumor antigen is used in the treatment of disorders mediated by the activity of a tumor antigen.

In one aspect, the bispecific antibody of the present invention that specifically binds to CD3 and the tumor antigen BCMA is used in the treatment of disorders mediated by the activity of the tumor antigen BCMA.

In some embodiments, the subject of treatment, or patient, is a mammal, preferably a human subject. Said subject may be either male or female, of any age.

In one aspect, the present invention relates to a method for treating a disease or disorder mediated by a tumor antigen, which comprises administering to a subject in need of such treatment any above bispecific antibody or above pharmaceutical composition in a therapeutically effective amount.

In one aspect, the present invention relates to a method for treating a disease or disorder mediated by a tumor antigen, comprising administering in a subject in need of such treatment any above bispecific antibody and at least one other therapeutically active compound in a therapeutically effective amount.

In some embodiments of the method for treating, the disease or disorder mediated by a tumor antigen is selected from the group: multiple myeloma, chronic lymphocytic leukemia, acute B-lymphoblastic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma.

In some embodiments of the method for treatment, the other therapeutically active compound is an antibody, small molecule, hormone therapy agent, or a combination thereof.

In one aspect, the present invention relates to the use of the above bispecific antibody or above pharmaceutical composition for the treatment, in a subject in need of such treatment, of a disease or disorder mediated by a tumor antigen.

In one aspect, the present invention relates to the use of the above bispecific antibody and at least one other therapeutically active compound for treating a disease or disorder mediated by a tumor antigen.

In some embodiments of the use, the disease or disorder mediated by a tumor antigen is selected from the group: multiple myeloma, chronic lymphocytic leukemia, acute B-lymphoblastic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma.

In some embodiments of the use, the other therapeutically active compound is an antibody, small molecule, hormone therapy agent, or a combination thereof.

The uses or methods used herein relating to the bispecific antibody according to the present invention with one or more other therapeutic agents are contemplated to mean, refer to and include the following:

1) simultaneous administration of such combination of the bispecific antibody of the present invention and a therapeutic agent to a patient in need of treatment, when such components are formulated together into a single dosage form which releases said components at substantially the same time to said patient,

2) simultaneous administration of such combination of the bispecific antibody of the present invention and a therapeutic agent to a patient in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at substantially the same time by said patient, whereupon said components are released at substantially the same time to said patient,

3) sequential administration of such combination of the bispecific antibody of the present invention and a therapeutic agent to a patient in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at consecutive times by said patient with a significant time interval between each administration, whereupon said components are released at substantially different times to said patient; and

4) sequential administration of such combination of the bispecific antibody of the present invention and a therapeutic agent to a patient in need of treatment, when such components are formulated together into a single dosage form which releases said components in a controlled manner, whereupon they are concurrently, consecutively, and/or overlappingly released at the same and/or different times to said patient, where each part may be administered by either the same or a different route.

The bispecific antibody of the present invention may be administered without further therapeutic treatment, i.e. as an independent therapy.

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in combination with proteasome inhibitors.

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in combination with antitumor immunomodulators (for example, lenalidomide, pomalidomide).

In some embodiments of the method for treating or the use (for example, lenalidomide, pomalidomide).

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in combination with cytostatic chemotherapy (cyclophosphamide, etoposide, and others).

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in combination with tyrosine kinase inhibitors.

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in combination with BCL2 inhibitor drugs.

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in combination with exportin 1 antagonist drugs.

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in combination with glucocorticosteroids.

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in combination with antitumor monoclonal antibodies (for example, daratumumab).

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in combination with targeted therapy.

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in combination with cytokine agonist monoclonal antibodies (for example, IL15SA, IL2).

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in combination with cytokine antagonist monoclonal antibodies (for example, anti-IL6R, anti-TNF).

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in combination with G-CSF (granulocyte-colony stimulating factor) drugs.

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in a complex with hematopoietic stem cell transplantation.

In some embodiments of the method for treating or the use, the bispecific antibody of the present invention may be administered in a complex with radiation therapy.

In some embodiments of the method for treating or the use, the suitable dose of the bispecific antibody according to the present invention will be in the range of 0.1 to 200 mg/kg.

**Brief description of drawings**

Figure 1 is an electrophoregram of bispecific antibodies that specifically bind to CD3 and the tumor antigen CD20 by 7.5% SDS-PAGE under non-reducing conditions.

1. Protein molecular weight marker;
2. 001;
3. 002;
4. 003;
5. 004;
6. 005;

7. 006;
8. 007;
9. 008;
10. 009;
11. 010;
12. 011;
13. 012;
14. 013;
15. 014.

Figure 2 is an electrophoregram of bispecific antibodies that specifically bind to CD3 and the tumor antigen CD20 by 12.5% SDS-PAGE under reducing conditions.

1. Protein molecular weight marker;
2. 001;
3. 002;
4. 003;
5. 004;
6. 005;
7. 006;
8. 007;
9. 008;
10. 010.

Figure 3 is an electrophoregram of bispecific antibodies that specifically bind to CD3 and the tumor antigen CD20 by 12.5% SDS-PAGE under reducing conditions.

1. Protein molecular weight marker;
2. 011;
3. 012;
4. 013;
5. 014.

Figure 4 is an electrophoregram of a bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 by 7.5% SDS-PAGE under non-reducing conditions.

1. Protein molecular weight marker;
2. bispecific antibody 019 (10 μg);
3. bispecific antibody 019 (40 μg).

Figure 5 is an electrophoregram of a bispecific antibody that specifically binds to CD3 and the tumor antigen CD20 by 12.5% SDS-PAGE under reducing conditions.

1. Protein molecular weight marker;

2. bispecific antibody 019.

Figure 6 is an electrophoregram of purified products of bispecific anti-CD3/anti-AXL antibodies. Electrophoresis was performed in 12.5% PAAG under non-denaturing conditions in the presence of SDS, gels were stained with Coomassie G-250.

M – molecular weight markers (kDa);

1 – first antibody to CD3/AXL;

2 – second antibody to CD3/AXL.

Figure 7 is an electrophoregram of purified products of bispecific anti-CD3/anti-AXL antibodies. Electrophoresis was performed in 7.5% PAAG under reducing conditions in the presence of SDS, gels were stained with Coomassie G-250.

M – molecular weight markers (kDa);

1 – first antibody to CD3/AXL;

2 – second antibody to CD3/AXL.

Figure 8 is a graph showing the results of checking for a specific activity of the first and second bispecific antibodies to CD3/AXL on a reporter cell line, wherein:

● first bispecific antibody to CD3/AXL+ MDA-MB-231, EC50=138 ng/ml;

■ second bispecific antibody to CD3/AXL+ MDA-MB-231, EC50=81 ng/ml;

▲ first bispecific antibody to CD3/AXL;

♦ second bispecific antibody to CD3/AXL.

Figure 9 is an electrophoregram of antibodies 01, 06, 016, 021 and 022 in 7.5 % polyacrylamide gel under denaturing non-reducing conditions following the first stage of purification on Protein A sorbent.

M – molecular weight marker;

1- 01-001;

2- 01-006;

3- 01-016;

4- 01-021;

5- 01-022.

Figure 10 is an electrophoregram of antibodies 024, 025, 026, 027, 030, 033 and 037 in 7.5 % polyacrylamide gel under denaturing non-reducing conditions following the first stage of purification on Protein A sorbent.

M – molecular weight marker;

1- 01-024;

2- 01-025;

3- 01-026;

4- 01-027;

5- 01-030;

6- 01-033;

7- 01-037.

Figure 11 is an electrophoregram of bispecific antibodies, that specifically bind to CD3 and the tumor antigen BCMA, 02-001, 02-002, 02-003, 02-004, 02-005, 02-006, 02-007, 02-008, 02-009 and 02-010 in 7.5% polyacrylamide gel under denaturing non-reducing conditions following the first stage of purification on Protein A sorbent.

M – molecular weight marker:

1- 02-001;

2- 02-002;

3- 02-003;

4- 02-004;

5- 02-005;

6 - 02-006;

7- 02-007;

8- 02-008;

9- 02-009;

10- 02-010.

Figure 12 is an electrophoregram of bispecific antibody 02-007 that specifically binds to CD3 and the tumor antigen BCMA in a 7.5% polyacrylamide gel under denaturing non-reducing conditions following the first stage of purification on Protein A sorbent.

M – molecular weight marker;

1- 02-007.

Figure 13 is an electrophoregram of bispecific antibody 02-011 that specifically binds to CD3 and the tumor antigen BCMA in a 7.5% polyacrylamide gel under denaturing non-reducing conditions following the first stage of purification on Protein A sorbent.

M – molecular weight marker;

1- 02-011.

Figure 14 is an electrophoregram of bispecific antibody 02-012 that specifically binds to CD3 and the tumor antigen BCMA in a 7.5% polyacrylamide gel under denaturing non-reducing conditions following the first stage of purification on Protein A sorbent.

M – molecular weight marker;

1- 02-012.

**Examples**

The following examples are provided for better understanding of the invention. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended embodiments.

**Materials and general methods**

General information regarding the nucleotide sequences of human immunoglobulin light and heavy chains is given in: Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991). Amino acids of antibody chains are numbered according to the EU numbering scheme (Edelman, G.M., et al., Proc. Natl. Acad. Sci. USA 63 (1969) 78-85; Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD, (1991).

*Recombinant DNA techniques*

Standard methods were used to manipulate DNA as described in Sambrook, J. et al, Molecular cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. The molecular biological reagents were used according to the manufacturer protocols.

*Gene synthesis*

Desired gene segments were prepared from oligonucleotides made by chemical synthesis. The gene segments of 300-1400 bp long, which were flanked by singular restriction sites, were assembled by annealing and ligation of oligonucleotides including PCR amplification and subsequently cloned via the restriction sites. The DNA sequences of the subcloned gene fragments were confirmed by DNA sequencing.

*DNA sequence determination*

DNA sequences were determined by Sanger sequencing.

*DNA and protein sequence analysis and sequence data management*

The Unipro's UGENE suite version 1.29 and SnapGene Viewer were used for sequence creation, mapping, analysis, annotation and illustration.

*Expression vectors*

For the expression of the antibodies described in the application materials, variants of expression plasmids intended for expression of antibodies in prokaryotic cells *(E.coli)*, transient expression in eukaryotic cells (e.g., in CHO cells) were applied. Beside the antibody expression cassette the vectors contained: an origin of replication which allows replication of said plasmid in *E. coli*, genes which confer resistance in *E. coli* to various antibiotics (e.g. to ampicillin, kanamycin).

The fusion genes comprising the described antibody chains as described below were generated by PCR and/or gene synthesis and assembled with known recombinant methods and techniques by connection of the according nucleic acid segments, e.g. using unique restriction sites in the corresponding vectors. The subcloned nucleic acid sequences were verified by DNA sequencing. For transient transfections, larger quantities of the plasmids were prepared by plasmid preparation from transformed *E. coli* cultures.

**Example 1. Design and preparation of genetic constructs of bispecific antibodies that specifically bind to CD3 and tumor antigen CD20**

The first chain of the first antigen-binding fragment, which specifically binds to CD3, of any bispecific antibody selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014 or 019 comprises a heavy chain variable domain with a b2M protein sequence and a IgG1 crystallizable fragment with the substitutions L240A, L241A to reduce antibody effector properties; M258Y, S260T, T262E to increase antibody elimination time; S360C, T372W to allow for proper heterodimerization of the heavy chains of the bispecific antibody. The second chain of the first antigen-binding fragment, which specifically binds to CD3, of any bispecific antibody selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014 or 019 comprises a light chain variable domain with a CD1b protein sequence.

The chains of the second antigen-binding fragment, which specifically binds to CD20, of any bispecific antibody selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014 or 019 comprise the variable domains of a heavy and light chain with the constant domains of the heavy and light chains, respectively. The heavy chain constant domains comprise the substitutions L238A, L239A to reduce antibody effector properties; M256Y, S258T, T260E to increase antibody elimination time; Y353C, T358S, L372A, Y411V to allow for proper heterodimerization of the heavy chains of the bispecific antibody.

Cloning was performed by the standard technique. For the chains of the first antigen-binding fragment, which specifically binds to CD3, of any bispecific antibody selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014 or 019, generated were PCR products of variable domains, of b2M and CD1b dimerizing domains and of constant domains with overlaps, whereupon the PCR products of the domains were combined in PCR using primers

comprising restriction sites. Full-length sequences of the chains of the first antigen-binding fragment that specifically binds to CD3 were cloned into the vector pEE-HCLALA_IgG1 using SalI/BamHI restriction sites. For the chains of the second antigen-binding fragment, which specifically binds to CD20, of any bispecific antibody selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014 or 019, generated were PCR products comprising genes of the variable domains of antibody heavy and light chains with primers comprising restriction sites. The heavy chain variable domain was cloned into the vector pEE-HCholeLALAYTE_IgG1 using SalI/NheI restriction sites. The light chain variable domain was cloned into the vector pEE-CK using SalI/BsiWI restriction sites. The resulting gene constructs were transferred for transient generation of proteins in the CHO-T cell line.

**Example 2. Production of recombinant bispecific antibodies that specifically bind to CD3 and tumor antigen CD20 in suspension culture of mammalian cells**

Full-size bispecific antibodies selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014 or 019 were produced in established cell line obtained from Chinese hamster ovary cells (CHO line). Cells were cultured in a serum-free medium in baffled flasks on orbital incubator shakers at a temperature of +37C, humidity of 70% and in the presence of 5% $CO_2$ at 150 revolutions per minute. For transient expression, cells at a concentration of 2-2.2×$10^6$ cells/ml were transfected using linear polyethyleneimine. DNA/PEI ratio was 1:3/1:10. 9 days after transfection, culture liquid was separated from cells by filtration through a 0.5/0.22 μm deep-bed filter.

Antibodies were purified by affinity chromatography. Cleared culture liquid was passed through a column at a load of 10-20 mg of protein per 1 ml of sorbent, which was pre-equilibrated with phosphate buffered saline (PBS, pH 7.4). The column was then washed with PBS to remove non-specifically binding components. Bound antibodies were eluted using 0.1 M glycine buffer (pH 3.5). Viral inactivation was then performed. The protein was then transferred to 20 mM acetate buffer (pH 5.0) using dialysis cassettes to conduct additional purification on a cation exchange sorbent.

Purity of bispecific antibodies selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014 or 019 was controlled using polyacrylamide gel electrophoresis and size-exclusion HPLC. Electrophoresis was performed in 7.5% polyacrylamide gel under denaturing non-reducing conditions, and also in 12.5% polyacrylamide gel under denaturing reducing conditions. Protein purity was determined by the intensity of band staining at a protein load of 10 μg per lane. The electrophoregram is shown in Figures 1–5.

**Example 3. Affinity of interaction between bispecific antibodies selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013 or 014 and human CD3 antigen (epsilon and delta subunits)**

Interaction between bispecific antibodies selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013 or 014 and human CD3 antigen (epsilon and delta subunits) was tested on the ForteBio Octet RED384 instrument using AR2G biosensors (ForteBio). The test used recombinant human CD3 antigen (epsilon and delta subunits). The experiment consisted of the following steps: activating sensors, loading protein onto sensors, quenching unreacted activated groups, recording baselines, recording analyte association, recording dissociation. Measurements were carried out at 30 °C. The sensors were activated in an aqueous solution comprising 20 mM EDC and 10 mM sNHS for 300 s. The human CD3 antigen (epsilon and delta subunits) was loaded onto the biosensor surface in 10 mM sodium acetate buffer solution with pH 5.0 for 900 s. The concentration of loading protein was 10 μg/ml. Unreacted active centers on the sensor surface were quenched in 1M aqueous solution of ethanolamine with pH 8.5 for 300 s (pH value was adjusted by adding hydrochloric acid). The baseline and all subsequent steps of the experiment were carried out in a kinetic buffer solution. At the association step (step duration was 300 s), the antigen-loaded sensors were dipped into wells with the solution of analyte (test antibody) prepared in a kinetic buffer solution. The analyte concentration was 10 μg/ml. At the dissociation step (600 s), the sensors were dipped into wells with a kinetic buffer, where the baseline was recorded.

Binding curves were processed using the Octet Data Analysis (Version 9.0) software using the 1:1 interaction model. Table 1 shows processing results.

Table 1 – Kinetic constants for interaction between bispecific antibodies selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013 or 014 and human CD3 antigen

| Bispecific antibody | Analyte concentration, nM | Response*, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|---|
| 001 | 68.2 | 0.3714 | 1.42E-09 | 1.11E+05 | 1.58E-04 | 0.9948 |
| 002 | 68.2 | 0.3574 | 1.29E-09 | 1.12E+05 | 1.45E-04 | 0.9959 |
| 003 | 68.2 | 0.3822 | 3.12E-09 | 1.08E+05 | 3.37E-04 | 0.9936 |
| 004 | 68.2 | 0.4146 | 2.99E-09 | 1.22E+05 | 3.65E-04 | 0.9961 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 005 | 68.2 | 0.2716 | 9.30E-09 | 6.51E+04 | 6.05E-04 | 0.9929 |
| 006 | 68.2 | 0.2749 | 7.57E-09 | 7.44E+04 | 5.63E-04 | 0.991 |
| 007 | 68.2 | 0.4294 | 2.55E-09 | 1.15E+05 | 2.92E-04 | 0.9971 |
| 008 | 68.2 | 0.3578 | 3.24E-09 | 1.15E+05 | 3.71E-04 | 0.9944 |
| 009 | 68.2 | 0.2791 | 3.19E-09 | 1.19E+05 | 3.78E-04 | 0.9852 |
| 010 | 68.2 | 0.2969 | 3.16E-09 | 1.15E+05 | 3.62E-04 | 0.9926 |
| 011 | 68.2 | 0.3169 | 3.77E-09 | 1.39E+05 | 5.25E-04 | 0.9917 |
| 012 | 68.2 | 0.2501 | 1.13E-08 | 1.01E+05 | 1.15E-03 | 0.9879 |
| 013 | 68.2 | 0.2187 | 1.30E-08 | 9.68E+04 | 1.26E-03 | 0.987 |
| 014 | 68.3 | 0.3245 | 3.25E-09 | 1.32E+05 | 4.31E-04 | 0.9917 |
| Control antibody Regn1979 | 68.7 | 0.1632 | 3.46E-09 | 7.02E+04 | 2.43E-04 | 0.9862 |
| *Response – signal level achieved at the end of association step; | | | | | | |

Bispecific antibodies interact with human CD3 with nanomolar affinity.

**Example 4. Affinity of interaction of bispecific antibodies selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013 or 014 to cynomolgus CD3 antigen (epsilon and delta subunits)**

The interaction of bispecific antibodies selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013 or 014 with cynomolgus CD3 antigen (epsilon and delta subunits) was tested on the ForteBio Octet RED384 instrument using AR2G biosensors (ForteBio). The aim of the experiment was to obtain estimated values of antigen-antibody interaction constants (equilibrium dissociation constant KD, association rate constant kon, dissociation rate constant kdis). The test used the recombinant cynomolgus CD3 antigen (epsilon and delta subunits). The

experiment consisted of the following steps: activating sensors, loading protein onto sensors, quenching unreacted activated groups, recording baselines, recording analyte association, recording dissociation. Measurements were carried out at 30 °C. The sensors were activated in an aqueous solution comprising 20 mM EDC (*N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride*) and 10 mM sNHS (*N-hydroxysulfosuccinimide*) for 300 s. The cynomolgus CD3 antigen (epsilon and delta subunits) was loaded onto the biosensor surface in 10 mM sodium acetate buffer solution with pH 5.0 for 900 s. The concentration of loading protein was 10 μg/ml. Unreacted active centers on the sensor surface were quenched in 1M aqueous solution of ethanolamine with pH 8.5 for 300 s (pH value was adjusted by adding hydrochloric acid). The baseline and all subsequent steps of the experiment were carried out in a kinetic buffer solution. At the association step (step duration was 300 s), the antigen-loaded sensors were dipped into wells with the solution of analyte (test antibody) prepared in a kinetic buffer solution. At the dissociation step (600 s), the sensors were dipped into wells with a kinetic buffer, where the baseline was recorded.

Binding curves were processed using the Octet Data Analysis (Version 9.0) software using the 1:1 interaction model. The sensorgrams showed a rapid drop in response, and therefore, the dissociation phase was processed based on drop in response lasting 30 seconds. In view of significant deviation of the observed sensorgrams from the model curves (parameter R^2 < 0.95), the resulting values of the kinetic constants are applicable only as estimated values. Table 2 shows processing results.

Table 2 – Kinetic constants for interaction between bispecific antibodies selected from 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013 or 014 and cynomolgus CD3 antigen

| Bispecific antibody | Analyte concentration, nM | Response*, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|---|
| 001 | 2046 | 0.3512 | 5.44E-07 | 6.74E+04 | 3.67E-02 | 0.8023 |
| 002 | 2046 | 0.3328 | 5.52E-07 | 8.54E+04 | 4.72E-02 | 0.8586 |
| 003 | 2046 | 0.3535 | 5.23E-07 | 8.47E+04 | 4.43E-02 | 0.8427 |
| 004 | 2046 | 0.3476 | 5.02E-07 | 8.27E+04 | 4.15E-02 | 0.8083 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 005 | 2046 | 0.2062 | 6.18E-07 | 9.68E+04 | 5.98E-02 | 0.7999 |
| 006 | 2046 | 0.2021 | 8.26E-07 | 7.00E+04 | 5.78E-02 | 0.8312 |
| 007 | 2046 | 0.3533 | 5.14E-07 | 7.81E+04 | 4.01E-02 | 0.812 |
| 008 | 2046 | 0.2351 | 1.02E-06 | 3.16E+04 | 3.23E-02 | 0.726 |
| 009 | 2046 | 0.2056 | 4.92E-07 | 1.45E+05 | 7.11E-02 | 0.8414 |
| 010 | 2046 | 0.407 | 1.08E-06 | 3.32E+04 | 3.58E-02 | 0.7264 |
| 011 | 2046 | 0.2765 | 8.37E-07 | 5.94E+04 | 4.97E-02 | 0.8078 |
| 012 | 2046 | 0.2836 | 4.64E-06 | 6.03E+03 | 2.80E-02 | 0.6466 |
| 013 | 2046 | 0.1189 | 4.30E-06 | 8.31E+03 | 3.57E-02 | 0.6959 |
| 014 | 2048 | 0.319 | 8.40E-07 | 4.75E+04 | 3.99E-02 | 0.7721 |
| Control antibody Regn1979 | 2060 | 0.1579 | 3.00E-06 | 7.74E+03 | 2.32E-02 | 0.6734 |
| *Response – signal level achieved at the end of association step; | | | | | | |

Bispecific antibodies interact with human CD3 with micromolar or submicromolar affinity.

**Example 5. Determination of affinity of bispecific antibody 019, that specifically binds to CD3 and tumor antigen CD20, to human CD3E/D antigen and cynomolgus CD3D & CD3E antigen on Forte Bio Octert RED 384**

Affinity of interaction between bispecific antibody 019 and the human and cynomolgus CD3E/D antigen was tested by biolayer interferometry on the Octet Red384 instrument (ForteBio). The test used the human CD3E/D antigen and cynomolgus CD3D&CD3E antigen.

The test was conducted using covalent immobilization of protein (antigens) on AR2G biosensors as shown in the example above, except that for the analysis of binding to the human CD3E/D antigen there were selected 4 analyte concentrations as follows: 136.7 nM, 68.4 nM, 34.2

nM, 17.1 nM, and for the analysis of binding to the cynomolgus CD3D&CD3E antigen there were selected 5 analyte concentrations as follows: 3076 nM, 2051 nM, 1367 nM, 911.1 nM, 607.6 nM.

Following measurement, the sensors were regenerated in 10 mM glycine solution with HCl (pH 1.8) to remove bound antibodies (3 regeneration cycles - 5 s regeneration, followed by 5 s neutralization in kinetic buffer). The baseline, association, dissociation and regeneration phases were then repeated. A total of 4 sets of sensorgrams (4 replicates) were produced as follows: 1 set before regeneration and 3 sets following regeneration. To obtain numerical values of kinetic constants (kon - the on/association rate constant, kdis - the dissociation rate constant, KD - the equilibrium dissociation constant), the resulting sensorgrams were processed according to the 1:1 (one-to-one) interaction model using the Global Fit method (selection of one set of kon, kdis, KD constants to analyze multiple sensorgrams of distinct concentrations) of the ForteBio Octet Data Analysis 9.0 software. The final kon, kdis, KD values were obtained by averaging the corresponding values for three replicates.

Table 3 – Kinetic constants for interaction between bispecific antibody 019 and human CD3 antigen

| Bispecific antibody | Antigen | KD, M | RSD (KD), % | kon, 1/Ms | RSD (kon), % | kdis, 1/s | RSD (kdis), % |
|---|---|---|---|---|---|---|---|
| 019 | Human CD3E/D | 1.14E-08 | 2.65 | 4.44E+04 | 6.29 | 5.07E-04 | 5.27 |

Bispecific antibody 019 interacts with the human CD3E/D antigen, the KD value is 11.4 nM (Table 3).

Table 4 shows results of checking for a nonspecific interaction between the analyte and antigen-free sensors.

Table 4

| Sensors | Analyte | Concentration, nM | Response, nm | Notes |
|---|---|---|---|---|
| Antigen-free AR2G | bispecific antibody 019 | 136.7 | -0.004 | No interaction between test antibody and antigen-free sensors (negative control) was detected. |

Table 5 – Kinetic constants for interaction between bispecific antibody 019 and cynomolgus CD3 antigen

| Bispecific antibody | Antigen | KD, M | RSD (KD), % | kon, 1/Ms | RSD (kon), % | kdis, 1/s | RSD (kdis), % |
|---|---|---|---|---|---|---|---|
| 019 | cyno CD3E&CD3D/ Fc | 1.80E -06 | 35.14 | 1.13E +04 | 40.63 | 1.85E -02 | 5.5 |

The bispecific antibody 019 shows a low signal level in specific interaction with the cynomolgus CD3D & CD3E antigen, the KD value is 1800 nM. Based on the resulting sensorgrams, one may conclude that there is interaction between bispecific antibody 019 and the recombinant cynomolgus CD3D & CD3E protein, whereas no high-affinity binding was detected (Table 5).

Table 6 shows the results of checking for a nonspecific interaction between the analyte and sensors.

Table 6

| Sensors | Analyte | Concentration, nM | Response, nm | Notes |
|---|---|---|---|---|
| AR2G loaded with non-specific IL4R-His protein | bispecific antibody 019 | 3076 | 0.0204 | No interaction between test antibody and sensor loaded with nonspecific IL4R-His protein (negative control) was detected. |

**Example 6. Determination of affinity of bispecific antibody 019 on Forte Bio Octet RED 384 to human CD20**

Affinity of bispecific antibody 019 to the CD20 antigen was tested by biolayer interferometry on the Octet Red384 instrument (ForteBio). The biotinylated peptide [NH2]CEPANPSEKNSPSTQYCYSIQS[CH2CH2]biotin comprising a CD20 sequence fragment in the amino acid sequence (hereinafter referred to as CD20 peptide) was used as an antigen.

The test was conducted using non-covalent attachment of biotinylated peptide (antigen) on streptavidin biosensors. The experiment included the following main steps: antigen loading onto sensors, baseline recording, analyte association recording, dissociation recording. The antigen was loaded onto the biosensor surface in a kinetic buffer for 300 s. The concentration of loading antigen (CD20 peptide) was 10 μg/ml. To check for a nonspecific interaction between the analyte (bispecific antibody 019) and the sensors, we used a CD20 peptide-free sensor (at the loading step, the sensor was dipped in a kinetic buffer; all other steps were analogous to the antigen-loaded sensor). The baseline and all subsequent steps of the experiment were carried out in a kinetic buffer. At the association step (step duration was 300 s), sensors loaded with CD20 peptide were dipped into wells containing a solution of analyte (bispecific antibody 019) prepared in a kinetic

buffer. 4 analyte concentrations were selected for analysis as follows: 2051 nM, 1367 nM, 911.1 nM, 607.6 nM. At the dissociation step (step duration was 600 s), the sensors were immersed into wells with a kinetic buffer, in which the baseline was recorded. The measurements were carried out at a temperature of 30 °C; tangential stirring at 1000 revolutions per minute was employed in all steps. Reference sensors went through all the steps analogously to the sensors used to record analyte sensorgrams, with the exception of the association step - at the association step, the sensors were dipped into a kinetic buffer without analyte (the signals of reference sensors was measured in parallel with the recording of the main sensorgrams). The reference signal was subtracted from the signal received on sensors interacting with the analyte during the processing of sensorgrams. Following measurement, the sensors were regenerated in 10 mM glycine solution with HCl (pH 1.8) to remove bound antibodies (3 regeneration cycles - 5 s regeneration, followed by 5 s neutralization in kinetic buffer). The baseline, association, dissociation and regeneration phases were then repeated. A total of 4 sets of sensorgrams were produced as follows: 1 set before regeneration and 3 sets following regeneration. No significant effect of regeneration on the values of constants was revealed, therefore, average values for three replicates were obtained following regeneration. Studied association time – 300 s, dissociation time – 20 s (association and dissociation times used for model processing of sensorgrams). To obtain numerical values of kinetic constants (kon - the on/association rate constant, kdis - the dissociation rate constant, KD - the equilibrium dissociation constant), the resulting sensorgrams were processed according to the 1:1 (one-to-one) interaction model using the Global Fit method (selection of one set of kon, kdis, KD constants to analyze multiple sensorgrams of distinct concentrations) of the ForteBio Octet Data Analysis 9.0 software. The final kon, kdis, KD values were obtained by averaging the corresponding values for three replicates.

Table 7 – Kinetic constants for interaction between bispecific antibody 019 and the CD20 peptide

| Bispecific antibody | Antigen | KD, M | RSD (KD), % | kon, 1/Ms | RSD (kon), % | kdis, 1/s | RSD (kdis), % |
|---|---|---|---|---|---|---|---|
| 019 | CD20_peptide | 7.69E-07 | 12.41 | 1.93E+05 | 8.2 | 1.48E-01 | 12.9 |

Bispecific antibody 019 interacts with the CD20 peptide antigen, the KD value is 769 nM. Based on the resulting data, one may conclude that there is interaction between bispecific antibody 019 and the CD20 peptide. (see Table 7).

**Example 7. Analysis of T cell activation by bispecific antibodies that specifically bind to CD3 and tumor antigen CD20**

T cell activation was determined by the ability of bispecific anti-CD3/CD20 antibodies to stimulate NFAT-dependent luciferase production in the model CD3-positive cell line *Jurkat-NFAT Luc cl.1* carrying a luciferase gene under the control of *NFAT* elements, in the presence of CD20-positive *Raji* cells. Luciferase activity was detected by adding a mixture of a luciferase substrate and a lysis buffer. The level of activation of NFAT signaling pathway. was determined based on the luminescence level.

*Jurkat-NFAT Luc cl.1* cells were cultured in suspension in a complete growth medium, 2 mM L-glutamine, 10% HI FBS in culture vials supplemented with selective antibiotic Hygromycin B up to 0.25 mg/ml. *Raji* cells were cultured in suspension in a complete growth medium in culture vials.

On the day of analysis, the antibodies were diluted to a concentration of 0.1 mg/ml in a medium for quantification. Antibody solutions were then titrated in U-bottom plates in increments of 5, 9 points; point 10 – without adding antibodies (background). Titrated samples were transferred to the inner wells of white culture plates at 25 µl each in two replicates. In an assay medium, prepared was a solution of aCD28 antibody, which is involved in the activation of *Jurkat-NFAT Luc cl.1*, at a concentration of 600 ng/ml. Prepared was a *Raji* suspension in a solution of aCD28 at a concentration of $8\times10^5$ cells/ml, and the suspension was added to the inner wells of the plates at 25 µl/well. Prepared was *Jurkat NFAT-Luc cl.1* suspension in an assay medium at a concentration of $8\times10^5$ cells/ml, the suspension was added to the inner wells at 25 µl/well.

The plates were stirred and incubated at 37 °C in a $CO_2$ incubator for 18-24 hours.

At the end of incubation, Bio-Glo substrate was added to analyzed wells of the plate at 75 µl/well. The luminescence level was measured in relative luminescence units at the integration time of 100 ms. Next, the results were statistically processed.

Table 8 shows analysis results.

Table 8 - EC50 values for bispecific antibodies that specifically bind to CD3 and the tumor antigen CD20 upon activation of Jurkat NFAT-luc Cl.1 in the presence of CD20-positive Raji cells

| Bispecific antibody | EC50, µg/ml | Standard error of mean |
|---|---|---|
| 001 | 33.9 | 4.1 |
| 002 | 38.8 | 4.4 |
| 003 | 35.0 | 4.0 |
| 004 | 29.9 | 3.0 |
| 005 | 57.8 | 5.7 |
| 006 | 64.8 | 6.8 |
| 007 | 31.4 | 3.7 |

| | | |
|---|---|---|
| 008 | 29.4 | 3.6 |
| 010 | 26.7 | 3.1 |
| 011 | 28.5 | 3.3 |
| 012 | 52.2 | 6.2 |
| 013 | 54.3 | 6.5 |
| 014 | 20.1 | 2.6 |
| 019 | 86.4 | 6.9 |

According to the resulting data, all test bispecific antibodies that specifically bind to CD3 and the tumor antigen CD20 are capable of activating the NFAT signaling pathway in the *Jurkat-NFAT Luc cl.* cell line, and therefore may stimulate T cell-dependent cellular cytotoxicity with respect to CD20-positive cells.

**Example 8. T-cell dependent cellular cytotoxicity (TDCC) analysis for anti-CD3/CD20 bispecific antibody 019**

TDCC was measured by cytofluorimetric determination of the level of depletion of labeled target cells following incubation with effector cells in the presence of test antibody.

PBMCs were isolated from whole blood from a healthy donor by Ficoll density gradient centrifugation; T cells were then isolated by magnetic separation. *Raji* Burkitt lymphoma cells highly expressing CD20 were selected as target cells. To label the target cells, selected was a green fluorescent dye which has long aliphatic tails that incorporate into the membrane. Staining was carried out using a stock dye solution at a concentration of 7 μM.

Test antibody 019 was diluted to a concentration of 200 μg/ml, the antibody was then titrated by sequential dilutions: 9 points in increments of 5. Diluted antibody was added to a 96-well flat-bottom plate at 25 μl/well. To the wells with titrated antibody added were stained *Raji* at a concentration of 1 x 10^6 cells/ml at 25 μl/well and T cells at a concentration of 1.5x10^6 cells/ml at 50 μl/well. then stirred for 40 seconds at 600 rpm. The plate was incubated at 37 °C, 5% CO2 for 22 hours. Immediately before loading the flow cytometer, to the samples were added 0.2 μl of viability stain at a concentration of 25 mg/ml and 35 μl of particles for absolute cell counting. TDCC level was measured based on reduction of absolute number of living *Raji* cells in the wells containing test antibody.

Next, the results were statistically processed.

Table 9 shows analysis results.

Table 9 - EC50 for bispecific antibodies that specifically bind to CD3 and tumor antigen CD20 upon depletion CD20-positive Raji cells involving human PBMCs

| Bispecific antibody | EC50, ng/ml | Standard error of mean |
|---|---|---|

| | | |
|---|---|---|
| 019 | 32.3 | 6.5 |

The results of the analysis show a significant 3.7X fold depleting activity of anti-CD3/CD20 bispecific antibody 019 against *Raji*.

**Example 9. Production of sequences of bispecific antibodies that specifically bind to CD3 and tumor antigen AXL**

The first bispecific antibody that specifically binds to CD3 and the tumor antigen AXL includes a first antigen-binding fragment that specifically binds to CD3 that consists of a light chain with the amino acid sequence of SEQ ID NO: 25 and a heavy chain with the amino acid sequence of SEQ ID NO: 38 (hereinafter the first antibody to CD3/AXL).

The second bispecific antibody that specifically binds to CD3 and the tumor antigen AXL includes a first antigen-binding fragment that specifically binds to CD3 that consists of a light chain with the amino acid sequence of SEQ ID NO: 158 and a heavy chain with the amino acid sequence of SEQ ID NO: 159 (hereinafter the second antibody to CD3/AXL).

Heavy and light chain variable domain genes of antibodies to AXL and CD3 were synthesized *de novo*. To this end, synthesized were oligonucleotides of 55-60 bp each forming a completely overlapping gene sequence. Each gene was assembled by two-round PCR. The gene of the heavy chain variable domain and the Fc fragment of human IgG1, the light chain variable domain and CK were fused using PCR and/or gene synthesis and assembly using known restriction and ligation methods.

The genes of the heavy and light chains of the antibody were cloned into pEE plasmids to generate protein in the IgG1 format in mammalian cells. Heterodimerization of the heavy chains of the bispecific anti-AXL/anti-CD3 is provided by introduction of knob-into-hole substitutions in the Fc region.

To produce antibodies with improved properties, the Fc heavy chain constant domain was modified by introducing the point mutations M252Y, S254T, T256 (YTE) and L234A L235A (AA). The set of YTE mutations makes it possible to achieve prolonged pharmacokinetics, whereas the introduction of the AA mutation reduces the complement-dependent cytotoxicity of resulting antibodies. Cloned nucleic acid sequences were confirmed by DNA sequencing. The resulting plasmids were produced in desired quantities in *E.coli* cells and purified using a plasmid DNA isolation kit.

The resulting gene constructs were transferred for transient production of proteins in CHO cell line.

**Example 10. Production of full-length bispecific antibodies that specifically bind to CD3 and tumor antigen AXL**

The aforementioned full-length first and second antibodies to CD3/AXL were produced in established cell line obtained from Chinese hamster ovary cells (CHO-T line) using transient transfection in two replicates. Suspension culturing was conducted in orbital shake bioreactors using serum-free media. For transient expression, cells at a concentration of 2-2.2×$10^6$ cells/ml were transfected using linear polyethyleneimine. DNA/PEI ratio was 1:7. On day 10 of culturing, the cell suspension was centrifuged under 2000 g for 15 min and filtered through 0.22 μm filter.

Antibodies were purified by affinity chromatography. Protein was then eluted. The resulting samples were neutralized with 25 μl of 1M phosphate buffer (pH 8).

Polyacrylamide gel electrophoresis and size-exclusion HPLC were used to control the purity of the antibodies to CD3/AXL. Electrophoresis was carried out in 7.5% polyacrylamide gel under reducing conditions and in 12.5% polyacrylamide gel under non-reducing conditions. Purity of the antibodies to CD3/AXL was determined by the intensity of band staining at a protein load of 10 μg per lane. Electropherograms are shown in Figures 6-7. Size-exclusion HPLC was performed on a column in a mobile phase of 0.05M NaH2PO4, 0.3 M NaCl pH=7.0.

**Example 11. Cellular *in vitro* assay to determine anti-CD3 specific activity of bispecific antibodies that specifically bind to CD3 and tumor antigen AXL**

The assay used Jurkat-NFAT-Luc cell line created on the basis of the Jurkat cell line comprising a gene encoding firefly luciferase under the control of the NFAT promoter, and also MDA-MB-231 cell line, a natural expressor of the AXL receptor.

The test was performed in a 96-well culture plate designed for luminescence assays. The suspension in each well contained 20 000 Jurkat-NFAT-Luc cells, 20000 MDA-MB-231 cells and the test bispecific antibody to CD3/AXL at a concentration indicated in the graph. The final volume of cell suspension in a well was 75 μl, all suspension components were prepared in RPMI-1640 medium comprising 10% fetal bovine serum. After adding all the components, the plate was incubated for 16 hours at 37°C, 5%$CO_2$; then, using a luminescence assay kit, the luminescence intensity in the wells was measured.

The results are shown in Figure 8 and indicate the presence of anti-CD3 specific activity of the first and second bispecific antibodies to CD3/AXL in the presence of target cells, the AXL receptor expressors. EC50 for both bispecific antibodies are similar, equaling to 138 ng/ml for the first bispecific antibody to CD3/AXL and 81 ng/ml for the second bispecific antibody to CD3/AXL.

**Example 12. Production of recombinant antigens in suspension culture of mammalian cells**

Sequences of extracellular domains of the human BCMA receptor (SEQ https://www.uniprot.org/uniprot/Q02223 (1-54 aa)) were cloned into pEE plasmids for protein

generation in mammalian cells with TEV-Fc, Avi-His, Avi-His-TEV-HSA tags via SalI/NotI restriction sites. The plasmids were cultured in required quantities in *E.Coli* cells and purified using the Qiagen kit.

Antigens were produced in established cell line obtained from Chinese hamster ovary (CHO-T) cells. Suspension culturing was carried out in flasks on an orbital incubator shaker. To express the target antigens, the cells were transfected using linear polyethylenimine. 9 days after transfection, culture liquid was separated from cells by filtration through a 0.5/0.22 μm deep-bed filter.

The antigen BCMA-TEV-Fc was isolated from the culture fluid and purified using a column with Protein A affinity chromatography sorbent. The cleared culture liquid was passed through a column equilibrated with phosphate buffered saline (PBS, pH 7.4). Bound antigen was eluted using 0.1 M glycine buffer (pH 2.5.). The resulting target protein was then dialyzed into PBS (pH 7.4), DTT was added, the mixture was filtered through 0.22 μm, transferred into tubes and stored at -70°C. BCMA-Avi-His, BCMA-Avi-His-TEV-HSA antigens were isolated from the culture fluid and purified using a column with a Ni-NTA affinity chromatography sorbent. The cleared culture liquid was passed through a column equilibrated with phosphate buffered saline (PBS, pH 7.4). Bound antigen was eluted with PBS supplemented with 500 mM imidazole (pH 7.4). The protein was then dialyzed into PBS. Thereafter, the protein was applied onto a gel filtration column. The resulting protein was filtered through a 0.22 μm filter, transferred into tubes and stored at -70 °C.

**Example 13. Cloning of variable domain genes of antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 into expression plasmid**

Variable domain genes of antibodies were cloned into expression plasmids pLL and pET22 according to a standard protocol using the restriction ligation method.

Subsequently, expression vectors comprising antibody fragments were transformed into *E. coli* BL21-DE3 and BL21-Gold strains for comparative analysis of affinity of variable antibody fragments from display libraries to antigen by ELISA using an automated platform.

**Example 14. Generation and primary analysis of Fabs and scFvs of antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 specifically binding human BCMA**

Fabs and scFvs were generated according to the standard technique: *E. coli* BL21-DE3 and BL21-Gold bacterial cells were transformed with expression vectors containing scFv and Fab genes, respectively, and the subsequent addition of an inducer triggered transcription of lac operon, thus, while culturing the resulting transformants, causing expression of scFvs and Fabs which were

exported into periplasmic space. ELISA was then performed to verify scFv and Fab binding to substrate-immobilized antigen hBCMA-Avi-His at a concentration of 0.2 μg/ml in 0.1 M $NaHCO_3$ (pH 9.0) (the antigen was immobilized overnight at 4 °C). All subsequent steps were carried out at room temperature according to the standard ELISA protocol using a high-throughput automated platform based on robotic systems. Washing was performed after each step, with 300 μl/well 1x PBST in three replicates. Non-specific binding sites on the plate were blocked with 1% fat-free milk in 1x PBST; analyte (*E. coli* supernatants) was added following washing at 60 μl/well. Immune complexes were detected using peroxidase-conjugated goat anti-Myc-tag antibodies (1:20000). Substrate-chromogenic mixture was stained by adding 50 μl of TMB substrate solution for 15 minutes. 25 μl of 1% $H_2SO_4$ was used to stop the reaction. The color signal was measured at a wavelength of 450 nm. Antibody binding level was proportional to the signal produced. Clones in which a colour signal exceeded the signal from control antibody were tested in ELISA against non-specific binding.

Thus, all test Fabs and scFvs of antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 exhibit specific binding to hBCMA-Avi-His.

**Example 15. Analysis of nonspecific binding of Fabs and scFvs of antibodies 01–001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 to other antigens**

ELISA was also employed to analyze non-specific binding of scFvs and Fabs in question to different antigens. The test was performed as described above, except that as immobilization antigens used were hCD16-Avi-His-FLAG, cynoIL4R-Fc in 0.1 M NaHCO3 (pH 9.0) (antigens were immobilized overnight at 4 °C). hBCMA-Avi-His, rhesusBCMA were used as controls for specific binding (antigens were immobilized overnight at 4°C). All subsequent steps were carried out according to the standard ELISA protocol using a high-throughput automated platform.

All tested Fabs and scFvs of antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 exhibit specific binding to hBCMA-Avi-His, rhesusBCMA and do not exhibit non-specific interaction with hCD16-Avi-His-FLAG and cynoIL4R-Fc.

**Example 16. Analysis of Fabs and scFvs of antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 for dissociation rates on ForteBio**

Fabs were measured on the ForteBio Octet RED 384 system using FAB2G biosensors (ForteBio). Fab samples were loaded onto sensors from supernatants for 16-18 hours at temperature conditions of (5±3) °C. Following loading, the sensors were transferred to a kinetic

buffer solution with 500 mM NaCl; this buffer solution was used to prepare all subsequent steps of experiments with Fab samples. The measurements were carried out at 30 °C. The sensors were equilibrated in a kinetic buffer solution for at least 10 minutes, then the baseline was recorded (60 s). Following the baseline recording, the sensors with immobilized Fab were dipped into wells containing the solution of analyte (human recombinant antigen BCMA-Avi-His-TEV-HSA), where the antigen-Fab complex was associated for 300 seconds. The human BCMA concentration was 391.2 nM (30 μg/ml). The complex dissociation in a kinetic buffer solution was then detected for 600 seconds. To check for a nonspecific interaction between the analyte and the sensors (negative control), used were sensors free of antibody Fabs. At the loading step, the negative control sensors were dipped into Fab-free supernatant, all other steps were analogous to sensors loaded with Fab samples).

scFvs were measured on the Forte Bio Octert RED 384 system using SAX biosensors (ForteBio). For measurements, the human BCMA was biotinylated in a molar antigen/biotin ratio of 1:1.5. The interaction kinetics analysis was carried out at 30 °C. The analysis was carried out using a kinetic buffer solution. The biotinylated human BCMA was loaded onto sensors for 600 seconds, the concentration of the antigen solution in a kinetic buffer solution was 20 μg/ml. Following loading, the baseline was recorded for 120 seconds. Following the baseline recording, the sensors with immobilized antigen were dipped into wells containing the solution of analyte (antibody scFvs), where the antigen-scFv complex was associated for 150 seconds (antibody scFvs for the association step were diluted with a kinetic buffer solution to a concentration of 1200 nM). The antigen-scFv complex dissociation in a kinetic buffer solution was then detected for 600 seconds. The reference signal was recorded simultaneously with recording of antibody sensorgrams and was subtracted while processing the sensorgrams. The reference signal was the signal of a sensor dipped into an analyte-free kinetic buffer solution at the association step, all other steps were analogous to sensors recording the test signal. Antigen-free sensors were used to check for a nonspecific interaction between the analyte (antibody scFvs) and sensors. At the loading step, the negative control sensors were dipped into an antigen-free sodium kinetic buffer solution, all other steps were analogous to antigen-loaded sensors).

Binding curves were analyzed using the Octet Data Analysis (Version 9.0) software using the 1:1 interaction model. Analysis result for Fabs and scFvs of antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 is shown in Table 10. These Fabs and scFvs were further used to create full-length antibodies.

Table 10 – Results of analysis of interaction kinetics between Fabs and scFvs of antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 and the human BCMA on ForteBio Octet RED384.

| Antibody | *Response, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|
| 01-001 (Fab) | 0.8314 | 1,04E-08 | 3,54E+04 | 3,67E-04 | 0,9919 |
| 01-006 (Fab) | 0,2955 | 5,04E-09 | 4,45E+04 | 2,24E-04 | 0,9718 |
| 01-016 (Fab) | 0,5322 | 8,46E-09 | 4.31E+04 | 3.65E-04 | 0,9876 |
| 01-021 (Fab) | 0,641 | 3,93E-09 | 6.78E+04 | 2,66E-04 | 0,9765 |
| 01-022 (scFv) | 0,1622 | 1,44E-08 | 7,11E+04 | 1,03E-03 | 0,9403 |
| 01-024 (scFv) | 0,1739 | 1,50E-09 | 3,80E+05 | 5,70E-04 | 0,8803 |
| 01-025 (scFv) | 0,1833 | 1,55E-08 | 8.26E+04 | 1.28E-03 | 0,9721 |
| 01-026 (scFv) | 0,1148 | 5,96E-08 | 4,17E+04 | 2,48E-03 | 0,9843 |
| 01-027 (scFv) | 0,1712 | 3,38E-09 | 2,84E+05 | 9,60E-04 | 0,8347 |
| 01-030 (scFv) | 0,1721 | 8,42E-09 | 1.12E+05 | 9,39E-04 | 0,9351 |
| 01-033 (scFv) | 0,1502 | 6,67E-09 | 1.12E+05 | 7,46E-04 | 0,9493 |
| 01-037 (scFv) | 0,1931 | 1,34E-08 | 7,07E+04 | 9,49E-04 | 0.9486 |
| **Negative Control for Fab | 0.0368 | | | | |
| ***Negative control for scFv | $\leq$ 0.0272 | | | | |

*Response – signal level achieved at the end of association phase;

**negative control for Fabs – signal of non-loaded FAB2G sensors while interacting with human BCMA;

***negative control for scFvs – signal of non-loaded SAX sensors with scFv sample solutions; measured for all samples in the scFv format, the highest value observed was 0.0272 nm.

All test Fabs and scFvs of antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 exhibit specific binding to the human antigen BCMA-Avi-His-TEV-HSA.

**Example 17. Production of genetic constructs to synthesize full-length antibodies specifically binding BCMA**

Cloning was performed by the standard technique. Generated were PCR products comprising heavy and light chain variable domain genes of antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 with primers comprising restriction sites. The heavy chain variable domain was cloned into the vector pEE-HCLALA IgG1 using Sal1/Nhe1 restriction sites. The light chain variable domain was cloned into the vector pEE-

CK using Sal1/BsiW1 restriction sites. The resulting gene constructs were transferred for transient generation of proteins in the CHO-T cell line.

**Example 18. Production of full-length antibodies specifically binding BCMA**

Full-length antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 were produced in established cell line obtained from Chinese hamster ovary cells (CHO-T) using transient transfection in two replicates. Suspension culturing was conducted in orbital shake bioreactors using serum-free media. For transient expression, cells at a concentration of 2-2.2×$10^6$ cells/ml were transfected using linear polyethyleneimine. DNA/PEI ratio was 1:7. On day 10 of culturing, the cell suspension was centrifuged under 2000 g for 15 min and filtered through 0.22 μm filter.

Antibodies were purified by affinity chromatography columns using a robotic station. The column was equilibrated with a buffer containing 50 mM NaPB (sodium phosphate buffer), 150 mM NaCl (pH 7.5), the filtered culture liquid with antibodies was applied, the column was then washed with 8 volumes of a buffer containing 50 mM NaPB, 150 mM NaCl and 4 volumes of 50 mM NaPB (pH 7.5). Protein was eluted with 6 column volumes of a solution of 50 mM NaPB, 100 mM NaCl (pH 3). The resulting samples were neutralized with 25 μl of 1M phosphate buffer (pH 8).

Polyacrylamide gel electrophoresis and size-exclusion HPLC were used to control the purity of the antibodies. Electrophoresis was performed in 7.5% polyacrylamide gel under denaturing non-reducing conditions. Protein purity was determined by the intensity of band staining at a protein load of 10 μg per lane. Electrophoregrams are shown in Fig. 9 and Fig. 10. Size-exclusion HPLC was performed on a column in a mobile phase of 0.05M NaH2PO4, 0.3 M NaCl pH=7.0.

**Example 19. Determination of affinity of full-length antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 to human BCMA on Forte Bio**

Experiments to test affinity of antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 to human BCMA were conducted on the ForteBio Octet RED 384 system. Antibodies at a concentration of 20 μg/ml were immobilized onto the surface of Protein A biosensors (ForteBio). The analysis was performed at 30 °C using a kinetic buffer solution. After setting the baseline in a kinetic buffer solution, the sensors with immobilized antibodies were dipped into wells containing the solution of analyte (BCMA), where the association of the complex took place for 300 seconds. For each test antibody, a sensorgram for the solution of human BCMA at a concentration of 10 μg/ml (130.4 nM) and a reference signal (reference sensorgram) of a BCMA-free kinetic buffer solution were recorded. The complex

dissociation in a buffer solution was then detected for 600 seconds. To check for a nonspecific interaction between the analyte and the sensors (negative control), antibody-free sensors were used. At the loading step, the negative control sensors were dipped into antibody-free sodium acetate buffer, all other steps were analogous to antibody-loaded sensors.

Binding curves, after subtracting a reference signal, were analyzed using the Octet Data Analysis (Version 9.0) software using 1:1 interaction model (Table 11).

Table 11 – Kinetic constants for antibodies/human BCMA interaction.

| Antibody | *Response, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|
| 01-001 | 1,0255 | 1,89E-09 | 8,37E+04 | 1.58E-04 | 0,9954 |
| 01-006 | 0,4332 | 5,16E-09 | 9,83E+04 | 5,08E-04 | 0,9851 |
| 01-016 | 1,0352 | 3,44E-09 | 8,05E+04 | 2,77E-04 | 0.9951 |
| 01-021 | 1,3325 | 2,15E-09 | 1,02E+05 | 2,18E-04 | 0,9924 |
| 01-022 | 0,6207 | 3,08E-09 | 1,05E+05 | 3,25E-04 | 0,9877 |
| 01-024 | 0,6046 | 5,34E-10 | 1,83E+05 | 9,77E-05 | 0,9973 |
| 01-025 | 0,5664 | 3,97E-09 | 8,15E+04 | 3,24E-04 | 0.9936 |
| 01-026 | 0,4853 | 6,20E-09 | 6,92E+04 | 4,29E-04 | 0.9936 |
| 01-027 | 0,6213 | 5,92E-10 | 1,80E+05 | 1,07E-04 | 0,9974 |
| 01-030 | 1,1529 | 1,82E-09 | 9,54E+04 | 1,73E-04 | 0,9946 |
| 01-033 | 1,0012 | 1,09E-09 | 9,95E+04 | 1,09E-04 | 0.9944 |
| 01-037 | 0,4618 | 2,91E-09 | 9,29E+04 | 2,70E-04 | 0.9842 |
| **Negative control | -0.0142 | | | | |

*Response – signal level achieved at the end of association step,

**negative control – verification of nonspecific interaction between the analyte (human BCMA) and sensors not loaded with antibodies.

Thus, all test anti-BCMA antibodies specifically bind to human BCMA (Table 11).

**Example 20. Verification of interactions between full-length antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 and rhesus BCMA on Forte Bio instrument.**

This experiment aimed to confirm interaction between antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 and the rhesus BCMA. Interaction between antibodies and the rhesus BCMA was verified on the ForteBio Octet RED384 system using AR2G biosensors (ForteBio). The experiment consisted of the following steps: activating sensors, loading protein onto sensors, quenching unreacted activated groups, recording

baselines, recording analyte association, recording dissociation. Measurements were carried out at 30 °C. The sensors were activated in an aqueous solution comprising 20 mM EDC and 10 mM sNHS for 300 s. The rhesus BCMA antigen was loaded onto the biosensor surface in 10 mM sodium acetate buffer solution with pH 5.0 for 300 s. The concentration of loading protein was 10 μg/ml. Unreacted active centers on the sensor surface were quenched in 1M aqueous solution of ethanolamine with pH 8.5 for 300 s (pH value was adjusted by adding hydrochloric acid). To check for a nonspecific interaction between the analyte and the sensors (negative control), used was an antigen-free sensor (at the loading step, the sensor was dipped into 10 mM sodium acetate buffer solution with pH 5.0; all other steps were analogous to antigen-loaded sensors). The baseline and all subsequent steps of the experiment were carried out in a kinetic buffer solution. At the association step (step duration was 300 s), the rhesus BCMA-loaded sensors were immersed into wells with the solution of analyte (test antibody) prepared in a kinetic buffer solution. The analyte concentration was 2.5 μg/ml. At the dissociation step, the sensors were immersed into wells with a kinetic buffer, where the baseline was recorded.

Binding curves were processed using the Octet Data Analysis (Version 9.0) software using the 1:1 interaction model. All anti-BCMA antibodies exhibit a signal of binding to the rhesus BCMA at the association step and a slow signal decay at the dissociation step, thus confirming high avidity while interaction with this antigen.

Table 12 – Results of processing of sensorgrams for antibody-rhesus BCMA binding

| Antibody | *Response, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|
| 01-001 | 1,7867 | <1.0E-12 | 3,06E+05 | <1.0E-07 | 0,9595 |
| 01-006 | 1,4718 | 2,83E-11 | 4,85E+05 | 1,37E-05 | 0.9998 |
| 01-016 | 1,8205 | <1.0E-12 | 3,23E+05 | <1.0E-07 | 0,9611 |
| 01-021 | 1,8126 | <1.0E-12 | 3,54E+05 | <1.0E-07 | 0,9489 |
| 01-022 | 1,8809 | <1.0E-12 | 3,31E+05 | <1.0E-07 | 0,9534 |
| 01-024 | 1,8102 | 7,32E-12 | 4,96E+05 | 3,63E-06 | 0.9997 |
| 01-025 | 1,2699 | 4,02E-12 | 5,92E+05 | 2,38E-06 | 0,9992 |
| 01-026 | 1,4956 | <1.0E-12 | 4,91E+05 | <1.0E-07 | 0.9994 |
| 01-027 | 0,6259 | <1.0E-12 | 1.10E+06 | <1.0E-07 | 0,9897 |
| 01-030 | 1,1718 | <1.0E-12 | 8,22E+05 | <1.0E-07 | 0.9928 |
| 01-033 | 1,3062 | <1.0E-12 | 5,55E+05 | <1.0E-07 | 0,9993 |
| 01-037 | 1,5779 | <1.0E-12 | 5,27E+05 | <1.0E-07 | 0.9997 |
| **Negative control | ≤ 0.0150 | | | | |

The given values of KD, Kon and Kdis constants can only be used to compare antibodies in view of the use of a bivalent analyte (antibody); the value of Kdis constant <1.0E-07 (1/s) and the corresponding thereto value of KD <1.0E-12 (M) means that, within the framework of the applied measurement and processing technique, the given sample showed exceeded limit of sensitivity of the dissociation rate constant for the model curve describing the sensorgram;
*Response – signal level achieved at the end of association phase;
**negative control – checking for a nonspecific interaction between the analyte (antibody) and antigen-free sensors; measured for all antibody samples; the highest value observed was 0.0150 nm.

Thus, all test anti-BCMA antibodies specifically bind to the rhesus BCMA antigen (Table 12).

**Example 21. Checking for inhibition of interaction between APRIL and human BCMA by antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 on Forte Bio.**

The test was carried out on the ForteBio Octet RED 384 instrument using AR2G biosensors (ForteBio). The experiment included the following main steps: loading the human APRIL/TNFSF13 antigen onto sensors, checking for binding of the APRIL/TNFSF13 antigen to a mixture of human BCMA and test antibody (premix). A human BCMA solution free of antibodies served as a positive control. Blocking of interaction was determined by comparing the premix signal (response) and positive control signal.

The sensors were activated in an aqueous solution comprising 20 mM EDC and 10 mM sNHS for 300 s. The APRIL/TNFSF13 antigen was loaded onto the biosensor surface in 10 mM sodium acetate buffer with pH 5.0 for 900 s. The concentration of loading protein APRIL/TNFSF13 was 10 μg/ml. To check for a nonspecific interaction between the analyte and the sensors, used was an antigen-free sensor (at the loading step, the sensor was dipped into sodium acetate buffer with pH 5.0; all other steps were analogous to the antigen-loaded sensor). Unreacted active centers on the sensor surface were quenched in 1M aqueous solution of ethanolamine with pH 8.5 (pH was adjusted by adding hydrochloric acid) for 300 s. All steps of the experiment following the quenching step were carried out in a kinetic buffer. At the association step (step duration was 300 s), protein-loaded sensors were immersed into wells with the solution of analyte. A solution comprising 710 nM test antibody and 71 nM human BCMA in a kinetic buffer solution was used as an analyte at the association step. A solution (analyte) with human BCMA at a concentration of 71 nM served as a positive control. Measurements were carried out at 30 °C. Reference sensors went through all the steps analogously to the sensors used to record analyte sensorgrams, with the exception of the association step - at the association step, the sensors were

dipped into a kinetic buffer without analyte (the signals of reference sensors was measured in parallel with the recording of the main sensorgrams). The reference signal was subtracted from the analyzed signal during processing of the sensorgrams.

The sensorgrams were processed using ForteBio Octet Data Analysis 9.0 software. Table 13 shows experiment results.

Table 13 – Results of checking for inhibition of interaction between APRIL/TNFSF13 and human BCMA by antibodies

| Antibodies | Protein loaded onto sensors | Analyte (antibody + human BCMA premix) | *Response, nm | Notes |
|---|---|---|---|---|
| 01-001 | APRIL/TNFSF 13 | 710 nM 01-001 and 71 nM BCMA | -0.0081 | 01-001 inhibits binding of BCMA to APRIL/TNFSF13 |
| 01-006 | APRIL/TNFSF 13 | 710 nM 01-006 and 71 nM BCMA | 0.0464 | 01-006 inhibits binding of BCMA to APRIL/TNFSF13 |
| 01-016 | APRIL/TNFSF 13 | 710 nM 01-016 and 71 nM BCMA | -0.0062 | 01-016 inhibits binding of BCMA to APRIL/TNFSF13 |
| 01-021 | APRIL/TNFSF 13 | 710 nM 01-021 and 71 nM BCMA | -0.0015 | 01-021 inhibits binding of BCMA to APRIL/TNFSF13 |
| 01-022 | APRIL/TNFSF 13 | 710 nM 01-022 and 71 nM BCMA | 0.013 | 01-022 inhibits binding of BCMA to APRIL/TNFSF13 |
| 01-024 | APRIL/TNFSF 13 | 710 nM 01-024 and 71 nM BCMA | 0.0151 | 01-024 inhibits binding of BCMA to APRIL/TNFSF13 |
| 01-025 | APRIL/TNFSF 13 | 710 nM 01-025 and 71 nM BCMA | 0.0259 | 01-025 inhibits binding of BCMA to APRIL/TNFSF13 |
| 01-026 | APRIL/TNFSF 13 | 710 nM 01-026 and 71 nM BCMA | 0.0111 | 01-026 inhibits binding of BCMA to APRIL/TNFSF13 |

| | | | | |
|---|---|---|---|---|
| 01-027 | APRIL/TNFSF 13 | 710 nM 01-027 and 71 nM BCMA | 0.017 | 01-027 inhibits binding of BCMA to APRIL/TNFSF13 |
| 01-030 | APRIL/TNFSF 13 | 710 nM 01-030 and 71 nM BCMA | -0.0066 | 01-030 inhibits binding of BCMA to APRIL/TNFSF13 |
| 01-033 | APRIL/TNFSF 13 | 710 nM 01-033 and 71 nM BCMA | -0.0078 | 01-033 inhibits binding of BCMA to APRIL/TNFSF13 |
| 01-037 | APRIL/TNFSF 13 | 710 nM 01-037 and 71 nM BCMA | 0.0304 | 01-037 inhibits binding of BCMA to APRIL/TNFSF13 |
| Positive control | APRIL/TNFSF 13 | 71 nM BCMA | 0.6588 | Positive control, demonstrates binding of BCMA to APRIL/TNFSF13 without inhibiting antibody |
| **Negative control | Sensor was not loaded | 71 nM BCMA | -0.0461 | Demonstrates no binding of analyte (71 nM BCMA) to sensors free of APRIL/TNFSF13 antigen |
| *Response – signal level achieved at the end of association phase; **negative control – checking for a nonspecific interaction between BCMA (antibodies) and antigen-free sensors; positive control – signal of binding (Response) of human BCMA to APRIL/TNFSF13 without inhibiting antibody. | | | | |

Thus, all test anti-BCMA antibodies inhibit binding of APRIL/TNFSF13 to the human BCMA antigen (Table 13).

**Example 22. Analysis of binding of anti-BCMA antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 to human BCMA on RPMI8226 cell surface by flow cytometry.**

Ability of anti-BCMA antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 to bind to the human BCMA antigen on cell-surface

was measured by flow cytometry on the RPMI8226 cell line (multiple myeloma) stably expressing the BCMA receptor on the surface thereof.

25 000 cells in a well were incubated in a staining buffer for 30 minutes at +4°C with serial dilutions of test antibodies. After the specified period of time, the cells were washed 2 times with a cold (+4°C) staining buffer; bound antibodies were detected by staining with anti-human Fc secondary antibodies labelled with Phycoerythrin for 30 minutes at +4°C. Wells without introduced antibodies and only with secondary antibodies were used as a control. After that period of time, the cells were washed 2 times with a cold (+4°C) staining buffer and resuspended in 150 µl of a cold (+4°C) staining buffer. The samples were then analyzed on a flow cytometer. Forward and side scatter (FSC and SSC) gating was performed to distinguish the target cell population; thereafter, forward scatter height and forward scatter area (FSC-H and FSC-A) gating was performed to identify single (singlet) cells in the population.

Half-effective concentration $EC_{50}$ was calculated by plotting a four-parameter logistic regression model, the data is shown in Table 14.

Table 14 – $ED_{50}$ of anti-BCMA antibodies while binding to RPMI8226 cell surface.

| Antibody | $EC_{50}$ (µg/ml) |
|---|---|
| 01-001 | 0.59 |
| 01-006 | 0.45 |
| 01-016 | 1.05 |
| 01-021 | 0.49 |
| 01-022 | 0.76 |
| 01-024 | 0.22 |
| 01-025 | 0.70 |
| 01-026 | 1.75 |
| 01-027 | 0.25 |
| 01-030 | 0.33 |
| 01-033 | 0.39 |
| 01-037 | 0.79 |

Conclusion: the resulting data show that all test antibodies bind to the BCMA target on multiple myeloma cell surface.

**Example 23. Analysis of nonspecific binding of anti-BCMA antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 on BCMA-negative K562 cell surface by flow cytometry.**

The presence of nonspecific binding of anti-BCMA antibodies 01-001, 01-006, 01-016, 01-021, 01-022, 01-024, 01-025, 01-026, 01-027, 01-030, 01-033, 01-037 was determined by flow cytometry on the K562 cell line (chronic myelogenous leukemia) which is characterized by no BCMA expression on the surface thereof.

25,000 K562 cells in a well were incubated in a staining buffer (PBS+$NaN_3$+5% BSA) for 30 minutes at +4°C with diluted test antibodies. After the specified period of time, the cells were washed 2 times with a cold (+4°C) staining buffer; bound antibodies were detected by staining with anti-human Fc secondary antibodies labelled with Phycoerythrin for 30 minutes at +4°C. Wells without introduced antibodies and only with secondary antibodies were used as a control. After that period of time, the cells were washed 2 times with a cold (+4°C) staining buffer and resuspended in 150 µl of a cold (+4°C) staining buffer. The samples were then analyzed on a flow cytometer. Forward and side scatter (FSC and SSC) gating was performed to distinguish the target cell population; thereafter, forward scatter height and forward scatter area (FSC-H and FSC-A) gating was performed to identify single (singlet) cells in the population.

RPMI8226 was used as a positive control for cell-surface binding of test antibodies to BCMA. Sample preparation was carried out analagously to that described above for K562.

Nonspecific binding of antibodies was evaluated by comparing fluorescent signal levels from antibodies at the same concentration in the variants for K562 and RPMI8226 cell lines, the data is shown in Table 15.

Table 15 – Data on cell-surface binding of anti-BCMA antibodies to K562 (BCMA-negative line) and RPMI8226 (BCMA-positive line).

| Antibody | Fluorescent signal level (sample/background) | |
|---|---|---|
| | RPMI8226 ($BCMA^+$) | K562 ($BCMA^-$) |
| 01-001 | 10.66 | 0.97 |
| 01-006 | 9.61 | 1.04 |
| 01-016 | 7.87 | 0.85 |
| 01-021 | 9.99 | 1.00 |
| 01-022 | 9.57 | 1.05 |
| 01-024 | 19.91 | 1.53 |
| 01-025 | 15.02 | 1.23 |
| 01-026 | 12.10 | 1.34 |
| 01-027 | 19.11 | 1.53 |
| 01-030 | 16.46 | 1.13 |

| | | |
|---|---|---|
| 01-033 | 14.66 | 1.13 |
| 01-037 | 11.68 | 1.21 |

Conclusion: the results show that all test antibodies do not exhibit nonspecific binding to the BCMA-negative cell line.

**Example 24. Design of humanized bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA**

Antibody 01-016 was selected solely for illustrative purposes. This antibody 01-016 has been optimized to increase the humanization degree.

In total, 10 humanized variants of the antibody VH fragment were developed, each including 4 point substitutions. Table 16 shows the list of substitutions.

Table 16. List of mutations used for humanization. Substitution position numbering is ordinal

| **VH of second antigen-binding fragment, which specifically binds to BCMA, of bispecific antibody** | **Substitutions** |
|---|---|
| 02-001 | F45L, F60Y, A75S, K87R |
| 02-002 | F45L, D56T, F60Y, A75S |
| 02-003 | F45L, F60Y, A75S, P88A |
| 02-004 | F45L, D56T, F60Y, K87R |
| 02-005 | V24A, F45L, A75S, K87R |
| 02-006 | F45L, F60Y, K87R, P88A |
| 02-007 | V24A, F60Y, A75S, K87R |
| 02-008 | F45L, D56T, F60Y, P88A |
| 02-009 | F45L, D56T, A75S, K87R |
| 02-010 | V24A, D56T, F60Y, A75S |

The results of annotation of humanized heavy chains show that the target values have been achieved: v-gene score of each chain is at least 0.85, and also the most similar germline sequence relates to *Homo sapiens* (Table 17).

Table 17. Annotation of humanized chains

| **VH of second antigen-binding fragment, which specifically binds to BCMA, of bispecific antibody** | **Closest reference** | **V-gene score** |
|---|---|---|
| 02-001 | *H. sapiens* | 85.9 |
| 02-002 | *H. sapiens* | 85.9 |

| | | |
|---|---|---|
| 02-003 | *H. sapiens* | 85.9 |
| 02-004 | *H. sapiens* | 85.9 |
| 02-005 | *H. sapiens* | 85.9 |
| 02-006 | *H. sapiens* | 85.9 |
| 02-007 | *H. sapiens* | 85.9 |
| 02-008 | *H. sapiens* | 85.9 |
| 02-009 | *H. sapiens* | 85.9 |
| 02-010 | *H. sapiens* | 85.9 |

The resulting heavy chain variable fragments of the second antigen-binding fragment that specifically binds to BCMA were used to generate bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA.

**Example 25. Preparation of genetic constructs for synthesis of full-length bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA**

In order to produce the genetic constructs of the heavy chains of anti-BCMA, sequences of humanized variable domains were assembled *de novo* and cloned into the pEE_VH vector.

In order to produce the genetic construct of the light chain of anti-BCMA, a sequence of the light chain variable domain was amplified and cloned into the pEE_VL vector.

In order to generate full-length bispecific antibodies, the aforementioned constructs were used together with constructs carrying sequences of heavy and light chains of the first antigen-binding fragment, which specifically binds to CD3, which are shown in Example 1.

**Example 26. Production of full-length bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA.**

Full-length antibodies 02-001, 02-002, 02-003, 02-004, 02-005, 02-006, 02-007, 02-008, 02-009, 02-010 were produced in the cells of established cell line obtained from Chinese hamster ovary cells (CHO-T line) using transient transfection in two replicates. Suspension culturing was conducted in orbital shake bioreactors using serum-free media. For transient expression, cells at a concentration of 2-2.2×$10^6$ cells/ml were transfected using linear polyethyleneimine. DNA/PEI ratio was 1:7. On day 10 of culturing, the cell suspension was centrifuged under 2000 g for 15 min and filtered through 0.22 μm filter.

Antibodies were purified by affinity chromatography columns using a robotic station. The column was equilibrated with a buffer containing 50 mM NaPB (sodium phosphate buffer), 150 mM NaCl (pH 7.5), the filtered culture liquid with antibodies was applied, the column was then washed with 8 volumes of a buffer containing 50 mM NaPB, 150 mM NaCl and 4 volumes of 50 mM NaPB (pH 7.5). Protein was eluted with 6 column volumes of a solution of 50 mM NaPB,

100 mM NaCl (pH 3). The resulting samples were neutralized with 25 µl of 1M phosphate buffer (pH 8).

Polyacrylamide gel electrophoresis and size-exclusion HPLC were used to control the purity of the bispecific antibodies. Electrophoresis was performed in 7.5% polyacrylamide gel under denaturing non-reducing conditions. Protein purity was determined by the intensity of band staining at a protein load of 10 µg per lane. Electropherograms are shown in Fig. 11. Size-exclusion HPLC was performed on a column in a mobile phase of 0.05M NaH2PO4, 0.3 M NaCl pH=7.0.

**Example 27. Determination of affinity of bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA to human BCMA on Forte Bio**

Experiments to test binding affinity of antibodies to BCMA-Avi-his-TEV-HSA antigen. Antibodies at a concentration of 10 µg/ml were immobilized on the surface of Protein A biosensors (ForteBio) for 300 s. The analysis was performed at 30 °C using a kinetic buffer solution. After setting the baseline in a kinetic buffer solution (120 s), the sensors with immobilized antibodies were dipped into wells containing the solution of analyte (BCMA), where the association of the complex took place for 300 seconds. For each test antibody, a sensorgram for the solution of antigen (BCMA-Avi-his-TEV-HSA) at a concentration of 5 µg/ml (65.2 nM) or 10 µg/ml (130.4 nM) (Table 18) and a reference signal (reference sensorgram) of an antigen-free kinetic buffer solution were recorded. The complex dissociation in a buffer solution was then detected for 600 seconds. To check for a nonspecific interaction between the analyte and the sensors (negative control), antibody-free sensors were used. At the loading step, the negative control sensors were dipped into antibody-free sodium acetate buffer, all other steps were analogous to antibody-loaded sensors.

Table 18 – Kinetic constants for interaction between antibodies and the human BCMA antigen (BCMA-Avi-his-TEV-HSA)

| Bispecific antibody | Analyte concentration, nM | *Response, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|---|
| 02-001 | 130.4 | 0.3528 | 6.79E-09 | 6.12E+04 | 4.16E-04 | 0.9958 |
| 02-002 | 130.4 | 0.1302 | 8,64E-09 | 3.32E+04 | 2.87E-04 | 0.9922 |
| 02-003 | 130.4 | 0.3617 | 6.36E-09 | 6.24E+04 | 3.97E-04 | 0.9951 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 02-004 | 130.4 | 0.1321 | 5.79E-09 | 3.24E+04 | 1.88E-04 | 0.9941 |
| 02-005 | 130.4 | 0.8029 | 2.73E-09 | 1.07E+05 | 2.92E-04 | 0.9928 |
| 02-006 | 130.4 | 0.3613 | 6.54E-09 | 6.59E+04 | 4.31E-04 | 0.9928 |
| 02-007 | 130.4 | 0.4077 | 4.49E-09 | 7.53E+04 | 3.38E-04 | 0.9953 |
| 02-008 | 130.4 | 0.143 | 5.20E-09 | 3.71E+04 | 1.93E-04 | 0.9947 |
| 02-009 | 130.4 | 0.4675 | 6.35E-09 | 5.77E+04 | 3.66E-04 | 0.9966 |
| 02-010 | 130.4 | 0.152 | 6.82E-09 | 2.75E+04 | 1.88E-04 | 0.9977 |
| *Response – signal level achieved at the end of association step, negative control – verification of nonspecific interaction between the analyte (antigen) and antibody-free sensors. | | | | | | |

Thus, all test antibodies specifically bind to human BCMA (Table 18).

**Example 28. Determination of affinity of bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA to human CD3 antigen (epsilon and delta subunits) on Forte Bio**

The test employed a recombinant human CD3 antigen, CD3-Fc. The test was carried out on the ForteBio Octet RED384 instrument using AR2G biosensors (ForteBio). The experiment consisted of the following steps: activating sensors, loading protein onto sensors, quenching unreacted activated groups, recording baselines, recording analyte association, recording dissociation. Measurements were carried out at 30 °C. The sensors were activated in an aqueous solution comprising 20 mM EDC and 10 mM sNHS for 300 s. The CD3-Fc antigen was loaded onto the biosensor surface in 10 mM sodium acetate buffer solution with pH 5.0 for 300 s. The concentration of loading protein was 10 μg/ml. Unreacted active centers on the sensor surface were quenched in 1M aqueous solution of ethanolamine with pH 8.5 for 300 s (pH value was adjusted by adding hydrochloric acid). The baseline and all subsequent steps of the experiment were carried out in a kinetic buffer solution. At the association step (step duration was 300 s), the CD3-Fc antigen-loaded sensors were dipped into wells with the solution of analyte (test antibody) prepared in a kinetic buffer solution. The concentration of the analyte (test antibody) was 10 μg/ml. At the dissociation step, the sensors were dipped into wells with a kinetic buffer (900 s), where the

baseline was recorded. To check for a nonspecific interaction between the analyte and the sensors (negative control), used was an antigen-free sensor (at the loading step, the sensor was dipped into 10 mM sodium acetate buffer solution with pH 5.0; all other steps were analogous to antigen-loaded sensors). The signal of non-loaded AR2G sensors (negative control) was measured for all antibody samples, the highest value observed was 0.0079 nm.

Binding curves were processed using the Octet Data Analysis (Version 9.0) software using the 1:1 interaction model. Table 19 shows processing results.

Table 19 - Kinetic constants for interaction between antibodies and CD3-Fc

| Bispecific antibody | Analyte concentration, nM | *Response, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|---|
| 02-001 | 68.4 | 0.3291 | 6.64E-09 | 6.06E+04 | 4.02E-04 | 0.9995 |
| 02-002 | 68.4 | 0.3313 | 6.50E-09 | 6.26E+04 | 4.07E-04 | 0.9994 |
| 02-003 | 68.4 | 0.3445 | 6.04E-09 | 6.49E+04 | 3.92E-04 | 0.9995 |
| 02-004 | 68.4 | 0.3493 | 5.55E-09 | 6.83E+04 | 3.79E-04 | 0.9994 |
| 02-005 | 68.4 | 0.3367 | 6.43E-09 | 6.52E+04 | 4.19E-04 | 0.9996 |
| 02-006 | 68.4 | 0.3339 | 5.39E-09 | 6.78E+04 | 3.65E-04 | 0.9996 |
| 02-007 | 68.4 | 0.3096 | 6.19E-09 | 6.00E+04 | 3.71E-04 | 0.9995 |
| 02-008 | 68.4 | 0.3514 | 6.11E-09 | 6.58E+04 | 4.02E-04 | 0.9997 |
| 02-009 | 68.4 | 0.3386 | 5.06E-09 | 6.70E+04 | 3.39E-04 | 0.9996 |
| 02-010 | 68.4 | 0.3213 | 5.46E-09 | 6.60E+04 | 3.60E-04 | 0.9995 |
| *Response – signal level achieved at the end of association phase; Negative control – signal of non-loaded AR2G sensors was measured for all antibody samples, the highest value observed was 0.0079 nm. | | | | | | |

Thus, all test antibodies specifically bind to human CD3ED-Fc (Table 19).

**Example 29. Determination of ability of bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA to activate reporter T lymphocytic Jurkat NFAT-luc Cl.1 line in the presence of RPMI8226 multiple myeloma cells**

Ability of anti-BCMA/anti-CD3 antibodies to activate T cell signaling by means of binding to the CD3 subunit of the T cell receptor (TCR) in the presence of a target multiple myeloma cell line RPMI8226 was measured by way of detecting a luminescent signal following incubating the antibodies with reporter T lymphocytic human T cells, Jurkat NFAT-luc Cl.1, in the presence of RPMI8226 multiple myeloma cells.

20 000 RPMI8226 cells and 20 000 Jurkat NFAT-luc Cl.1 cells were incubated in a growth medium with serial dilutions of test antibodies for 24 hours. After the specified period of time, to the cells was added a luciferase detection reagent, incubation was carried out for 8 minutes in the dark, followed by recording of the luminescent signal using a plate reader.

Half-effective concentration $EC_{50}$ was calculated using a four-parameter logistic regression model, the data is shown in Table 20.

Table 20 – $EC_{50}$ value for bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA while activation of Jurkat NFAT-luc Cl.1 in the presence of RPMI8226 multiple myeloma cells

| Bispecific antibody | $EC_{50}$ (ng/ml) |
|---|---|
| 02-001 | 17.626 |
| 02-002 | 125.74 |
| 02-003 | 26.317 |
| 02-004 | 136.05 |
| 02-005 | 8.2472 |
| 02-006 | 22.217 |
| 02-007 | 9.9637 |
| 02-008 | 101.47 |
| 02-009 | 60.208 |
| 02-010 | 79.606 |

Thus, all tested bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA are capable of activating the Jurkat NFAT-luc Cl.1 reporter cell line in the presence of multiple myeloma.

**Example 30. Determination of affinity of bispecific antibodies, which specifically**

**bind to CD3 and tumor antigen BCMA, to rhesus BCMA antigen on Forte Bio**

The test employed a recombinant rhesus BCMA antigen. The test was carried out on the ForteBio Octet RED384 instrument using AR2G biosensors (ForteBio). The experiment consisted of the following steps: activating sensors, loading protein onto sensors, quenching unreacted activated groups, recording baselines, recording analyte association, recording dissociation. Measurements were carried out at 30 °C. The sensors were activated in an aqueous solution comprising 20 mM EDC and 10 mM sNHS for 300 s. The Rhesus BCMA antigen was loaded onto the biosensor surface in 10 mM sodium acetate buffer solution with pH 5.0 for 300 s. The concentration of loading protein was 10 μg/ml. Unreacted active centers on the sensor surface were quenched in 1M aqueous solution of ethanolamine with pH 8.5 for 300 s (pH value was adjusted by adding hydrochloric acid). The baseline and all subsequent steps of the experiment were carried out in a kinetic buffer solution. At the association step (step duration was 300 s), the Rhesus BCMA antigen-loaded sensors were dipped into wells with the solution of analyte (test antibody) prepared in a kinetic buffer solution. The concentration of the analyte (test antibody) was 10 μg/ml. At the dissociation step (900 s), the sensors were dipped into wells with a kinetic buffer, where the baseline was recorded. To check for a nonspecific interaction between the analyte and the sensors, used were antigen-free sensors (at the loading step, the sensor was dipped into 10 mM sodium acetate buffer solution with pH 5.0; all other steps were analogous to antigen-loaded sensors). The signal of non-loaded AR2G sensors (negative control) was measured for all antibody samples, the highest value observed was 0.0044 nm.

Binding curves were processed using the Octet Data Analysis (Version 9.0) software using the 1:1 interaction model. Table 21 shows processing results.

Table 21 – Kinetic constants for antibodies/Rhesus BCMA interaction

| Bispecific antibody | Analyte concentration, nM | *Response, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|---|
| 02-005 | 68.4 | 1.2517 | 1.87E-09 | 2.28E+05 | 4.25E-04 | 0.9887 |
| 02-007 | 68.4 | 1.1943 | 2.28E-09 | 2.12E+05 | 4.84E-04 | 0.9907 |
| *Response – signal level achieved at the end of association phase;<br>Negative control – signal of non-loaded AR2G sensors was measured for all antibody samples, the highest value observed was 0.0044 nm. | | | | | | |

Thus, all test antibodies specifically bind to the rhesus BCMA antigen (Table 21).

**Example 31. Determination of affinity of bispecific antibodies, which specifically bind**

**to CD3 and tumor antigen BCMA, to cynomolgus CD3 antigen (epsilon and delta subunits) on Forte Bio.**

The test employed a recombinant cynomolgus CD3D&CD3E antigen. The test was carried out on the ForteBio Octet RED384 instrument using AR2G biosensors (ForteBio). The experiment consisted of the following steps: activating sensors, loading protein onto sensors, quenching unreacted activated groups, recording baselines, recording analyte association, recording dissociation. Measurements were carried out at 30 °C. The sensors were activated in an aqueous solution comprising 20 mM EDC and 10 mM sNHS for 300 s. The cynomolgus CD3D&CD3E antigen was loaded onto the biosensor surface in 10 mM sodium acetate buffer solution with pH 5.0 for 300 s. The concentration of loading protein was 10 μg/ml. Unreacted active centers on the sensor surface were quenched in 1M aqueous solution of ethanolamine with pH 8.5 for 300 s (pH value was adjusted by adding hydrochloric acid). The baseline and all subsequent steps of the experiment were carried out in a kinetic buffer solution. At the association step (step duration was 15 s), the cynomolgus CD3D&CD3E antigen-loaded sensors were dipped into wells with the solution of analyte (test antibody) prepared in a kinetic buffer solution. The concentration of the analyte (test antibody) was 440 μg/ml, 370 μg/ml (for the control antibody). At the dissociation step (300 s), the sensors were dipped into wells with a kinetic buffer, where the baseline was recorded.

To check for a nonspecific interaction between the analyte and the sensors, used were antigen-free sensors (at the loading step, the sensor was dipped into 10 mM sodium acetate buffer solution with pH 5.0; all other steps were analogous to antigen-loaded sensors). The signal of non-loaded AR2G sensors (negative control) was measured for all antibody samples. In view of the high concentration of analyte (antibodies) and the relatively low dilution levels of samples in a kinetic buffer, performed was an additional negative control involving checking of the response of the excipients from the antibody storage buffer to sensors loaded with test antigen (placebo control). Placebo samples for each test antibody were prepared by diluting the sample storage solution (without antibodies) in a kinetic buffer solution to a dilution level corresponding to the sample in question.

Binding curves were processed using Octet Data Analysis (version 9.0) using 1:1 interaction model; the dissociation phase was processed using 15 s out of 300 s of dissociation time because of a rapid drop in the response. The results of processing are shown in Table 22.

Table 22 - Kinetic constants for interaction between antibodies and the cynomolgus CD3D&CD3E antigen

| Bispecific antibody | Analyte concentration, nM | *Response, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|---|
| 02-005 | 3008 | 0.1621 | 3.85E-07 | 3.71E+05 | 1.43E-01 | 0.9093 |
| 02-007 | 3008 | 0.1251 | 4.15E-07 | 2.33E+05 | 9.67E-02 | 0.925 |
| *Response – signal level achieved at the end of association phase; Negative control – signal of non-loaded AR2G sensors was measured for all antibody samples; placebo control – response of excipients from the antibody storage solution in a kinetic buffer while interacting with antigen-loaded sensors (dilution level corresponds to the dilution level of test samples). | | | | | | |

Thus, all test antibodies specifically bind to the cynomolgus CD3D&CD3E antigen (Table 22).

**Example 32. Aggregation stability of bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA.**

The test consisted of the following steps: determination of aggregation temperature (DLS), subjecting of samples to thermal stress at 40 °C for 144 hours, analysis of stressed samples and of control intact samples by (SE) HPLC, particle size analysis of stressed samples and control samples by DLS.

Table 23 shows the results of determination of mean particle size, of hydrodynamic radius of the target fraction, of polydispersity of the target fraction and of scattering intensity of the target fraction by DLS for the intact sample (not subjected to stress) and a stressed sample. Table 24 shows the results of SE HPLC measurement on an intact (non-stressed) sample and a stressed sample.

Table 23 - Results of testing bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA by DLS

| | Intact sample | | | | Sample following thermal stress at 40°C 144 h | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Bispecific antibody | Radius of target fraction, $R_h$, nm | PD of target fraction, % | Intensity fraction of target fraction I, % | Mean radius of particles, nm | Radius of target fraction, $R_h$, nm | PD of target fraction, % | Intensity fraction of target fraction I, % | Mean radius of particles, nm | Aggregation temperature, °C |
| | ≤ 7 nm | ≤ 30 % | ≥ 70 % | ≤ 7 nm | ≤ 7 nm | ≤ 30 % | ≥ 70 % | ≤ 10 nm | ≥ 60°C |
| 02-005 | 5.5 | 11.9 | 95.3 | 5.8 | 5.3 | 10.7 | 90.1 | 6 | 56.03 |
| 02-007 | 5.5 | 11.9 | 96.4 | 5.7 | 5.2 | 10 | 95.4 | 5.8 | 57.21 |

Table 24 - SE HPLC data, peak areas per fragment, target fraction (monomer) and aggregates

| Bispecific antibody | Type of fraction | Total area of fraction peaks (Intact sample), % | Total area of fraction peaks (Sample following thermal stress at 40 °C, 144 h), % | Change in fraction content following stress |
|---|---|---|---|---|
| Norms | For monomer | ≥ 75 % | Not determined | -5% to 1% |
| 02-005 | Aggregates | 6.154 | 6.492 | |
| | Monomer | 89.728 | 87.694 | -2.034 |
| | Fragments | 4.117 | 5.815 | |
| 02-007 | Aggregates | 6.258 | 6.009 | |
| | Monomer | 89.537 | 87.823 | -1.714 |
| | Fragments | 4.204 | 5.271 | |

Conclusion: the test bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA have sufficient aggregation stability.

**Example 33. Determination of ability of bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA to non-specifically activate reporter T lymphocytic Jurkat NFAT-luc Cl.1 line without RPMI8226 multiple myeloma cells.**

Ability of bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA to activate T cell signaling by means of binding to the CD3 subunit of the T cell receptor (TCR) in the absence of the target RPMI8226 multiple myeloma cell line was measured by way of

detecting a luminescent signal following incubation of the antibodies with reporter T-lymphocytic human T cells, Jurkat NFAT-luc Cl.1.

20 000 Jurkat NFAT-luc Cl.1 were incubated in a culture medium for 24 hours with either test antibodies or Hit3a, an anti-CD3 antibody, which was used as a positive control in the test. After the specified period of time, to the cells was added a luciferase detection reagent, incubation was carried out for 8 minutes in the dark, followed by recording of the luminescent signal using a plate reader.

Nonspecific activation of antibodies was measured by comparing the levels of the luminescent signal from antibodies and Hit3a at the same concentration; the data is shown in Table 25.

Table 25 – Data on activation of Jurkat NFAT-luc Cl.1 cell line following incubation with test humanized anti-BCMA/anti-CD3 antibodies and with anti-CD3 antibody Hit3a. These antibodies were used at the same concentration

| Bispecific antibody | Luminescent signal intensity |
|---|---|
| 02-005 | 5301.5 |
| 02-007 | 5657 |
| Hit3a | 10824 |

Thus, test bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA do not activate Jurkat NFAT-luc Cl.1 reporter cells in the absence of multiple myeloma cells.

**Example 34. Determination of ability of bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA to specifically induce depletion of NCI-H929 multiple myeloma cells.**

Ability of bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA to induce depletion of NCI-H929 multiple myeloma cells with involvement of effector cells within human peripheral blood mononuclear cells (hereinafter PBMCs) was measured colorimetrically by way of detecting changes in optical density following incubation of these antibodies with human PBMCs in the presence of NCI-H929 multiple myeloma cells.

30 000 NCI-H929 cells and 150 000 PBMCs were incubated in a growth serum-free medium with serial dilutions of test antibodies for 24 hours. After the specified time, to the cells was added a lactate dehydrogenase detection reagent, incubation was carried out for 10 minutes in the dark, followed by recording of optical density at a wavelength of 490 nm using a plate reader.

Half-effective concentration $EC_{50}$ was calculated by plotting a four-parameter logistic regression model, the data is shown in Table 26.

Table 26 - $EC_{50}$ of bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA while depletion of NCI-H929 multiple myeloma cells involving human PBMCs

| Bispecific antibody | $EC_{50}$ (ng/ml) |
|---|---|
| 02-005 | 5.72 |
| 02-007 | 4.25 |

Thus, test bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA induce depletion of NCI-H929 multiple myeloma cells.

**Example 35. Production of genetic constructs for synthesis of full-length bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA**

Further, for illustrative purposes only, analysed are bispecific antibody 02-007 that specifically binds to CD3 and the tumor antigen BCMA, and bispecific antibodies 02-011 and 02-012 that specifically bind to CD3 and the tumor antigen BCMA. Bispecific antibodies 02-011 and 02-012 were produced based on antibody 01-016 as well, wherein 02-011 has a Crossmab format and 02-012 has a scFv-Fab format.

The method for producing full-length bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA has been described above in Example 25.

**Example 36. Production of full-length bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA**

Full-length antibodies 02-007, 02-011, 02-012 were produced in established cell line obtained from Chinese hamster ovary cells (CHO-T line) by transient transfection in two replicates. Suspension culturing was conducted in orbital shake bioreactors using serum-free media. For transient expression, cells at a concentration of 1.9-2.2×106 cells/ml were transfected using linear polyethyleneimine. DNA/PEI ratio was 1:6-1:7. On day 10 of culturing, the cell suspension was filtered through a 0.22 μm filter.

Antibodies were purified by affinity chromatography using Protein A ligand. The column with sorbent was equilibrated with 20 mM Na Citrate buffer (pH 7.5), filtered culture liquid with antibodies was applied, the column was then washed with 20 mM Na Citrate (pH 7.5) until achieving a minimum absorption at a wavelength of 280nm. Elution was carried out with a linear gradient of 20 mM Na Citrate (pH 3.5). Resulting samples were titrated with 4 N HCl until pH 3.5 was achieved and incubated for 30 minutes, then neutralized with 1M Tris-OH pH 11, until pH 7.5 was achieved. Next, the samples were dialyzed against 20 mM sodium phosphate buffer pH 6.7 for additional purification. For the next stage of purification, proteins obtained following affinity chromatography were applied onto a column with a multimodal sorbent pre-equilibrated with 20 mM sodium phosphate pH 6.7 buffer. In order to remove non-specific impurities following application of the sample, the column was washed with an equilibration buffer until achieving a minimum absorption at a wavelength of 280 nm. Elution was carried out with a linear gradient of

sodium phosphate with increase in a molarity thereof to a final concentration of 0.3 M. Fractions comprising the target antibody were combined and dialyzed against a buffer with 20 mM sodium phosphate pH 6.7 buffer. To the resulting protein products was added trehalose to a concentration of 100 mg/ml, whereupon they were filtered. For long-term storage, the products were aliquoted and stored at -80 °C.

Purity of resulting antibodies was determined by vertical protein electrophoresis in the presence of sodium dodecyl sulfate under reducing and non-reducing conditions in 12.5% and 7.5% polyacrylamide gel (Fig. 12, Fig. 13, Fig. 14). Electrophoresis under non-reducing conditions used 2 loads as follows: 10 μg per lane to determine the relative molecular weight of a molecule; 40 μg per lane to determine the purity of a product. Further, in order to evaluate product purity, size-exclusion HPLC was performed on a column using a mobile phase of 0.1M Na2HPO4, 0.2 M NaCl pH = 6.9 with elution time of 25 min and the detector's operating wavelength of 280 nm.

**Example 37. Determination of affinity of bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA**

Experiments to test binding affinity of antibodies to Avi-His-hBCMA-HSA antigen. Antibodies at a concentration of 10 μg/ml were immobilized on the surface of Protein A biosensors (ForteBio) for 300 s. The analysis was performed at 30 °C using a kinetic buffer solution. After setting the baseline in a kinetic buffer solution (120 s), the sensors with immobilized antibodies were dipped into wells containing the solution of analyte (BCMA), where the association of the complex took place for 300 seconds. For each test antibody, a sensorgram for the solution of antigen (Avi-His-hBCMA-HSA) at concentrations of 5 μg/ml (65.2 nM), 2.5 μg/ml (32.6 nM), 1.25 μg/ml (16.3 nM) and a reference signal (reference sensorgram) of an antigen-free kinetic buffer solution were recorded. The complex dissociation in a buffer solution was then detected for 600 seconds. To check for a nonspecific interaction between the analyte and the sensors (negative control), antibody-free sensors were used. At the loading step, the negative control sensors were dipped into antibody-free sodium acetate buffer, all other steps were analogous to antibody-loaded sensors.

Binding curves, after subtracting a reference signal, were analyzed using the Octet Data Analysis (Version 9.0) software using 1:1 interaction model based on 3 sensorgrams (Table 130), sensorgrams are shown in Table 27.

Table 27 – Kinetic constants for antibodies/human BCMA interaction

| Bispecific antibody | Analyte concentration, nM | *Response, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| 02-007 | 65.2 | 0.2684 | 3.53E-09 | 1.08E+05 | 3.79E-04 | 0.9918 |
| | 32.6 | 0.1661 | | | | |
| | 16.3 | 0.1169 | | | | |
| 02-11 | 65.2 | 0.5802 | 1.54E-09 | 9.86E+04 | 1.52E-04 | 0.9989 |
| | 32.6 | 0.4316 | | | | |
| | 16.3 | 0.2863 | | | | |
| 02-12 | 65.2 | 0.5471 | 2.31E-09 | 9.87E+04 | 2.28E-04 | 0.9989 |
| | 32.6 | 0.3724 | | | | |
| | 16.3 | 0.2373 | | | | |
| *Response – signal level achieved at the end of association step, negative control – verification of nonspecific interaction between the analyte (antigen) and antibody-free sensors. | | | | | | |

Thus, all test antibodies specifically bind to the human BCMA antigen (Table 27).

**Example 38. Determination of affinity of bispecific antibodies, which specifically bind to CD3 and tumor antigen BCMA, to human CD3 antigen (epsilon and delta subunits)**

The test employed a recombinant human CD3 antigen, CD3ED-Fc. The test was carried out on the ForteBio Octet RED384 instrument using AR2G biosensors (ForteBio). The experiment consisted of the following steps: activating sensors, loading protein onto sensors, quenching unreacted activated groups, recording baselines, recording analyte association, recording dissociation. Measurements were carried out at 30 °C. The sensors were activated in an aqueous solution comprising 20 mM EDC and 10 mM sNHS for 300 s. The CD3ED-Fc antigen was loaded onto the biosensor surface in 10 mM sodium acetate buffer solution with pH 5.0 for 300 s. The concentration of loading protein was 10 μg/ml. Unreacted active centers on the sensor surface were quenched in 1M aqueous solution of ethanolamine with pH 8.5 for 300 s (pH value was adjusted by adding hydrochloric acid). The baseline and all subsequent steps of the experiment were carried out in a kinetic buffer solution. At the association step (step duration was 300 s), the CD3ED-Fc antigen-loaded sensors were dipped into wells with the solution of analyte (test antibody) prepared in a kinetic buffer solution. The concentration of the analyte (test antibody) was 10 μg/ml, 5 μg/ml, 2.5 μg/ml. At the dissociation step, the sensors were dipped into wells with a kinetic buffer (600 s), where the baseline was recorded. To check for a nonspecific interaction between the analyte and the sensors (negative control), used were antigen-free sensors (at the loading step, the sensor was dipped into 10 mM sodium acetate buffer solution with pH 5.0; all other steps were analogous to antigen-loaded sensors).

Binding curves were processed using the Octet Data Analysis (Version 9.0) software using

the 1:1 interaction model based on 3 sensorgrams. The results of processing are shown in Table 28.

Table 28 – Kinetic constants for antibodies/CD3ED-FC antigen interaction

| Bispecific antibody | Analyte concentration, nM | Response, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|---|
| 02-007 | 68.4 | 0.2863 | 1.14E-08 | 4.14E+04 | 4.73E-04 | 0.9985 |
| | 34.2 | 0.1758 | | | | |
| | 17.1 | 0.1032 | | | | |
| 02-011 | 68.8 | 0.3044 | 9.59E-09 | 4.58E+04 | 4.39E-04 | 0.9979 |
| | 34.4 | 0.1897 | | | | |
| | 17.2 | 0.0994 | | | | |
| | 80 | 0.3200 | 9.23E-09 | 3.87E+04 | 3.57E-04 | 0.9982 |
| 02-012 | 80 | 0.2762 | 1.31E-08 | 3.38E+04 | 4.42E-04 | 0.9982 |
| | 40 | 0.1877 | | | | |
| | 20 | 0.0969 | | | | |
| *Response – signal level achieved at the end of association phase; Negative control – signal from non-loaded AR2G sensors. | | | | | | |

Thus, all test antibodies specifically bind to human CD3ED-Fc (Table 28).

**Example 39. Determination of affinity of bispecific antibodies, which specifically bind to CD3 and tumor antigen BCMA, to rhesus BCMA antigen**

The test employed a recombinant rhesus BCMA antigen. The test was carried out on the ForteBio Octet RED384 instrument using AR2G biosensors (ForteBio). The experiment consisted of the following steps: activating sensors, loading protein onto sensors, quenching unreacted activated groups, recording baselines, recording analyte association, recording dissociation. Measurements were carried out at 30 °C. The sensors were activated in an aqueous solution comprising 20 mM EDC and 10 mM sNHS for 300 s. The Rhesus BCMA antigen was loaded onto the biosensor surface in 10 mM sodium acetate buffer solution with pH 5.0 for 300 s. The concentration of loading protein was 10 μg/ml. Unreacted active centers on the sensor surface were quenched in 1M aqueous solution of ethanolamine with pH 8.5 for 300 s (pH value was adjusted by adding hydrochloric acid). The baseline and all subsequent steps of the experiment were carried out in a kinetic buffer solution. At the association step (step duration was 300 s), the Rhesus BCMA antigen-loaded sensors were dipped into wells with the solution of analyte (test antibody) prepared in a kinetic buffer solution. The concentration of the analyte (test antibody) was 10 μg/ml, 5 μg/ml,

2.5 μg/ml. At the dissociation step (600 s), the sensors were dipped into wells with a kinetic buffer, where the baseline was recorded. To check for a nonspecific interaction between the analyte and the sensors, used were antigen-free sensors (at the loading step, the sensor was dipped into 10 mM sodium acetate buffer solution with pH 5.0; all other steps were analogous to antigen-loaded sensors).

Binding curves were processed using the Octet Data Analysis (Version 9.0) software using the 1:1 interaction model (based on 3 sensorgrams). Table 29 shows processing results.

Table 29 – Kinetic constants for antibodies/Rhesus BCMA interaction

| Bispecific antibody | Analyte concentration, nM | *Response, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|---|
| 02-007 | 34.2 | 1.6793 | 1.17E-09 | 2.29E+05 | 2.69E-04 | 0.9994 |
| | 17.1 | 1.2401 | | | | |
| | 8.54 | 0.7791 | | | | |
| 02-011 | 34.4 | 1.7919 | 6.31E-10 | 2.07E+05 | 1.30E-04 | 0.9995 |
| | 17.2 | 1.3611 | | | | |
| | 8.6 | 0.7604 | | | | |
| 02-012 | 40 | 1.3476 | 6.25E-10 | 1.85E+05 | 1.16E-04 | 0.9998 |
| *Response – signal level achieved at the end of association phase; Negative control – signal from non-loaded AR2G sensors. | | | | | | |

Thus, all test antibodies specifically bind to the rhesus BCMA antigen (Table 29).

**Example 40. Determination of affinity of bispecific antibodies, which specifically bind to CD3 and tumor antigen BCMA, to cynomolgus CD3 antigen (epsilon and delta subunits)**

The test employed a recombinant cynomolgus CD3D&CD3E antigen. The test was carried out on the ForteBio Octet RED384 instrument using AR2G biosensors (ForteBio). The experiment consisted of the following steps: activating sensors, loading protein onto sensors, quenching unreacted activated groups, recording baselines, recording analyte association, recording dissociation. Measurements were carried out at 30 °C. The sensors were activated in an aqueous solution comprising 20 mM EDC and 10 mM sNHS for 300 s. The cynomolgus CD3D&CD3E antigen was loaded onto the biosensor surface in 10 mM sodium acetate buffer solution with pH 5.0 for 300 s. The concentration of loading protein was 10 μg/ml. Unreacted active centers on the sensor surface were quenched in 1M aqueous solution of ethanolamine with pH 8.5 for 300 s (pH value was adjusted by adding hydrochloric acid). The baseline and all subsequent steps of the

experiment were carried out in a kinetic buffer solution. At the association step (step duration was 15 s), the cynomolgus CD3D&CD3E antigen-loaded sensors were dipped into wells with the solution of analyte (test antibody) prepared in a kinetic buffer solution. The concentration of the analyte (test antibody) was 1000 μg/ml, 500 μg/ml, 250 μg/ml; for the control antibody: 500 μg/ml, 250 μg/ml, 125 μg/ml. At the dissociation step (300 s), the sensors were dipped into wells with a kinetic buffer, where the baseline was recorded.

To check for a nonspecific interaction between the analyte and the sensors, used were antigen-free sensors (at the loading step, the sensor was dipped into 10 mM sodium acetate buffer solution with pH 5.0; all other steps were analogous to antigen-loaded sensors). In view of the high concentration of analyte (antibodies) and the relatively low dilution levels of samples in a kinetic buffer, performed was an additional negative control involving checking of the response of the excipients from the antibody storage buffer to sensors loaded with test antigen (placebo control). Placebo samples for each test antibody were prepared by diluting the sample storage solution (without antibodies) in a kinetic buffer solution to a dilution level corresponding to the sample in question.

Binding curves were processed using Octet Data Analysis (version 9.0) using 1:1 interaction model; the dissociation phase was processed using 45 s out of 300 s of dissociation time because of a rapid drop in the response. Table 30 shows processing results.

Table 30 - Kinetic constants for interaction between antibodies and the cynomolgus CD3D&CD3E antigen

| Bispecific antibody | Analyte concentration, nM | *Response, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|---|
| 02-007 | 6835 | 0.1394 | 4.30E-06 | 8.49E+04 | 3.65E-01 | 0.9781 |
| | 3418 | 0.0900 | | | | |
| | 1709 | 0.0487 | | | | |
| 02-011 | 6882 | 0.1424 | 2.50E-06 | 1.09E+05 | 2.73E-01 | 0.9736 |
| | 3441 | 0.0951 | | | | |
| | 1721 | 0.0531 | | | | |
| 02-012 | 8013 | 0.1351 | 8.17E-07 | 1.39E+05 | 1.14E-01 | 0.9092 |
| | 4006 | 0.0904 | | | | |

*Response – signal level achieved at the end of association phase;
Negative control – signal of non-loaded AR2G sensors was measured for all antibody samples;
placebo control – response of excipients from the antibody storage solution in a kinetic buffer while

| |
|---|
| interacting with antigen-loaded sensors (dilution level corresponds to the dilution level of test samples). |

Thus, all test antibodies specifically bind to the cynomolgus CD3D&CD3E antigen (Table 30).

**Example 41. Checking for simultaneous interaction between two distinct antigens (BCMA and CD3) and bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA**

Simultaneous interaction between two distinct human antigens (BCMA and CD3 epsilon and delta subunits) and bispecific antibodies was tested by biolayer interferometry on the Octet Red384 instrument (ForteBio). The test used Avi-His-hBCMA and CD3ED-Fc antigens.

The test was conducted using covalent immobilization of protein (antigens) on AR2G biosensors (Amine Reactive Second-Generation (AR2G) Biosensors, ForteBio). The experiment included the following main steps: loading BCMA (Avi-His-hBCMA) onto sensors, loading test antibody onto BCMA-loaded sensors, interaction with analyte – CD3 (CD3ED-Fc). Thus, the interaction signal at the last step of the experiment is detected in the presence of a bispecific antibody capable of simultaneously binding to BCMA present on sensors and CD3 present in solution.

Sensors were activated in an aqueous solution comprising 20 mM EDC and 10 mM sNHS for 300 s. BCMA antigen (Avi-His-hBCMA) was loaded onto the biosensor surface in a sodium acetate buffer with pH 5.0 for 300 s. The concentration of loading BCMA protein (Avi-His-hBCMA) was 10 μg/ml. Unreacted active centers on the sensor surface were quenched in 1M aqueous solution of ethanolamine with pH 8.5 for 300 s. All steps of the experiment following the quenching step were carried out in a kinetic buffer. Test antibody was loaded onto sensors with immobilized BCMA (Avi-His-hBCMA) for 300 s, the antibody concentration was 10 μg/ml. At the association phase (interaction with CD3), the sensors were dipped into a solution with a concentration of CD3 (CD3ED-Fs) of 10 μg/ml (137.1 nM) and into a kinetic buffer solution (reference signal, subtracted during processing). To test nonspecific interaction of CD3 analyte (negative control), used was an antibody-free sensor with immobilized BCMA (at the antibody loading step, the sensor was dipped into a kinetic buffer, all other steps were analogous to the sensor in question). Sensorgrams were processed using ForteBio Octet Data Analysisn 9.0 software. Simultaneous interaction with two different antigens was determined by analyzing the response at the end of the phase of interaction with CD3 (CD3ED-Fc). Table 31 shows the results.

Table 31 – Results of experiment to check for simultaneous interaction between bispecific antibodies and two different antigens (BCMA and CD3)

| Protein loaded onto sensors | Bispecific antibody | Analyte | Response, nm | Notes |
|---|---|---|---|---|
| BCMA (Avi-His-hBCMA) | 02-007 | CD3 (CD3ED-Fc) | 0.1168 | There is interaction between CD3ED-Fc antigen and antibody 02-007 bound to sensors loaded with Avi-His-hBCMA antigen |
| BCMA (Avi-His-hBCMA) | 02-011 | CD3 (CD3ED-Fc) | 0.1339 | There is interaction between CD3ED-Fc antigen and antibody 02-011 bound to sensors loaded with Avi-His-hBCMA antigen |
| BCMA (Avi-His-hBCMA) | 02-012 | CD3 (CD3ED-Fc) | 0.08 | There is interaction between CD3ED-Fc antigen and antibody 02-012 bound to sensors loaded with Avi-His-hBCMA antigen |

Thus, test antibodies 02-007, 02-011, 02-012 demonstrate simultaneous binding to two different antigens: BCMA (Avi-His-hBCMA) and CD3 (CD3ED-Fc) (Table 31).

**Example 42. Checking of inhibition of interaction between BCMA and APRIL by bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA on Forte Bio Octert RED 384**

Inhibition of interaction between BCMA and APRIL by bispecific antibodies was tested by biolayer interferometry on the Octet Red384 instrument (ForteBio). The test used human antigens Avi-His-hBCMA-HSA and APRIL.

The test was conducted using covalent immobilization of protein (antigens) on AR2G biosensors. The experiment included the following main steps: loading the APRIL antigen onto sensors, checking for binding of the APRIL antigen to a mixture of BCMA and test antibody (premix). BCMA solution free of antibodies served as a positive control. Blocking of interaction was determined by comparing the premix signal (response) and positive control signal.

Sensors were activated in an aqueous solution comprising 20 mM EDC and 10 mM sNHS for 300 s. APRIL antigen was loaded onto the biosensor surface in a sodium acetate buffer with pH 5.0 for 300 s. The concentration of loading SIRPa protein was 20 μg/ml. To check the nonspecific interaction between the analyte and the sensors, used was an antigen-free sensor (at the loading step, the sensor was dipped into sodium acetate buffer with pH 5.0; all other steps are analogous to antigen-loaded sensor). Unreacted active centers on the sensor surface were quenched in 1M aqueous solution of ethanolamine with pH 8.5 for 300 s. All steps of the experiment following the quenching step were carried out in a kinetic buffer. At the association phase (phase

duration was 300 s), protein-loaded sensors were dipped into wells with a solution of analyte. A solution comprising 70 nM test antibody and 28 nM human BCMA in a kinetic buffer solution was used as an analyte at the association phase. A solution (analyte) with BCMA at a concentration of 28 nM served as a positive control. Measurements were carried out at 30 °C. Reference sensors went through all the steps analogously to the sensors used to record analyte sensorgrams, with the exception of the association step - at the association step, the sensors were dipped into a kinetic buffer without analyte (the signals of reference sensors was measured in parallel with the recording of the main sensorgrams). The reference signal was subtracted from the signal received on sensors interacting with the analyte during the processing of sensorgrams. The results of the experiment are shown in Table 32.

At the association phase, the positive control exhibited a binding signal for BCMA of 0.297 nm, whereas the analyte comprising BCMA and test antibody (for all test antibodies) showed no binding signal for APRIL.

Table 32 - Results of experiment to check for inhibition of interaction between BCMA and APRIL by bispecific antibodies

| Protein loaded onto sensors | Analyte | Response, nm | Notes |
|---|---|---|---|
| human APRIL/TNFSF13 | 70 nM 02-007// 28 nM Avi-His-hBCMA-HSA | 0.0136 | No binding signal, antibody 02-007 inhibits interaction between BCMA and APRIL |
| human APRIL/TNFSF13 | 70 nM 02-011 // 28 nM Avi-His-hBCMA-HSA | 0.0131 | No binding signal, antibody 02-011 inhibits interaction between BCMA and APRIL |
| human APRIL/TNFSF13 | 70 nM 02-012)// 28 nM Avi-His-hBCMA-HSA | 0.0098 | No binding signal, antibody 02-012 inhibits interaction between BCMA and APRIL |

Thus, test antibodies 02-007, 02-011, 02-012 inhibit the interaction between BCMA with APRIL.

**Example 43. Comparative analysis of affinity of bispecific antibodies, which specifically bind to CD3 and tumor antigen BCMA, to FcRn**

Affinity to the FcRn receptor was compared using ForteBio Octet RED 384. Biotinylated

FcRn at a concentration of 5 μg/ml was immobilized on the surface of streptavidin SA biosensors (ForteBio) for 14.4 seconds (until the loading signal level of 0.4 – 0.5 nm). The analysis was performed at 30 °C using a kinetic buffer solution pH 6.0. After setting the baseline in the kinetic buffer solution pH 6.0 (60 s), the sensors with immobilized antibodies were dipped into wells containing a solution of analyte (test antibody), where the association of the complex took place for 30 seconds. For each test antibody, recorded were a sensorgram for the solution of analyte (antibody) at concentrations of 10 μg/ml, 5 μg/ml, 2.5 μg/ml and a reference signal (reference sensorgram) of an antibody-free kinetic buffer solution pH 6.0. The complex dissociation in a buffer solution pH 6.0 was then detected for 90 seconds. To check for a nonspecific interaction between the analyte and the sensors (negative control), used were FcRn-free sensors. At the loading step, the negative control sensors were dipped into antibody-free sodium acetate buffer, all other steps were analogous to antibody-loaded sensors.

Binding curves after subtracting the reference signal were analyzed using three sensorgrams (Table 33). In view of the presence of bivalent interaction contribution, numerical values of kinetic constants may depend on FcRn loading density and analyte concentration.

Table 33 - Kinetic constants for interaction between antibodies BCD248 and FcRn

| Bispecific antibody | Analyte concentration, nM | *Response, nm | KD, M | Kon, 1/Ms | Kdis, 1/s | $R^2$ |
|---|---|---|---|---|---|---|
| 02-007 | 68.4 | 0.5882 | 1.62E-09 | 9.61E+05 | 1.56E-03 | 0.9967 |
| | 34.2 | 0.4085 | | | | |
| | 17.1 | 0.2346 | | | | |
| 02-011 | 68.8 | 0.5101 | 1.88E-09 | 1.10E+06 | 2.06E-03 | 0.9959 |
| | 34.4 | 0.3702 | | | | |
| | 17.2 | 0.2312 | | | | |
| 02-012 | 80 | 0.446 | 2.13E-09 | 9.45E+05 | 2.02E-03 | 0.9966 |
| | 40 | 0.3235 | | | | |
| | 20 | 0.1976 | | | | |
| *Response – signal level achieved at the end of association step, negative control – checking for nonspecific interaction between the analyte (antibodies) and FcRn-free sensors. | | | | | | |

Thus, all test antibodies interact with FcRn receptors.

**Example 44. Aggregation stability of bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA**

Determination of aggregation stability of monoclonal bispecific antibodies by size-exclusion (SE) HPLC and dynamic light scattering (DLS).

The test consisted of the following steps: determination of aggregation temperature (DLS), subjecting of samples to thermal stress at 40 °C for 144 hours, analysis of stressed samples and of control intact samples by (SE) HPLC, particle size analysis of stressed samples and control samples by DLS.

Table 34 shows the results of determination of mean particle size, of hydrodynamic radius of the target fraction, of polydispersity of the target fraction and of scattering intensity of the target fraction by DLS for the intact sample (not subjected to stress) and a stressed sample. Table 35 shows the results of SE HPLC determination on an intact (non-stressed) sample and a stressed sample.

Table 34 – Results of testing antibodies by DLS

| | Intact sample | | | | Sample following thermal stress at 40°C 144 h | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Bispecific antibody | Radius of target fraction, $R_h$, nm | PD of target fraction, % | Intensity fraction of target fraction I, % | Mean radius of particles, nm | Radius of target fraction, $R_h$, nm | PD of target fraction, % | Intensity fraction of target fraction I, % | Mean radius of particles, nm | Aggregation temperature, °C |
| | ≤ 7 nm | ≤ 30 % | ≥ 70 % | ≤ 7 nm | ≤ 7 nm | ≤ 30 % | ≥ 70 % | ≤ 10 nm | ≥ 60°C |
| 02-007 | 6.8 | 11.6 | 95.9 | 5.5 | 5.8 | 11.3 | 96.9 | 5.6 | 58.23 |
| 02-011 | 5.3 | 11.2 | 100 | 5.2 | 4.2 | 11.2 | 82.9 | 5.8 | 57.74 |
| 02-012 | 5.1 | 6.9 | 100 | 5.1 | 5.3 | 11.3 | 100 | 5.4 | n/a |

Table 35 – SE HPLC data, peak areas per fragment, target fraction (monomer) and aggregates

| Bispecific antibody | Type of fraction | Total area of fraction peaks (Intact sample), % | Total area of fraction peaks (Sample following thermal stress at 40 °C, 144 h), % | Change in fraction content following stress |
|---|---|---|---|---|
| Norms | For monomer | ≥ 75 % | Not determined | -5% to 1% |
| 02-007 | Aggregates | 1.373 | 1.704 | |
| | Monomer | 96.377 | 96.001 | -0.376 |
| | Fragments | 2.252 | 2.295 | |
| 02-011 | Aggregates | 0.241 | 2.006 | |
| | Monomer | 98.17 | 94.382 | -3.788 |

| | | | | |
|---|---|---|---|---|
| | Fragments | 1.589 | 3.611 | |
| 02-012 | Aggregates | 11.595 | 14.943 | |
| | Monomer | 87.988 | 83.417 | -4.571 |
| | Fragments | 0.417 | 1.64 | |

General conclusion: test bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA have sufficient aggregation stability.

**Example 45. Analysis of ability of bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA to activate reporter T lymphocytic Jurkat NFAT-luc Cl.1 line in the presence of RPMI8226 multiple myeloma cells.**

Ability of anti-BCMA/anti-CD3 antibodies to activate T cell signaling by means of binding to the CD3 subunit of the T cell receptor (TCR) in the presence of a target multiple myeloma cell line RPMI8226 was measured by way of detecting a luminescent signal following incubating the antibodies with reporter T lymphocytic human T cells, Jurkat NFAT-luc Cl.1, in the presence of RPMI8226 multiple myeloma cells.

20 000 RPMI8226 cells and 20 000 Jurkat NFAT-luc Cl.1 cells were incubated in a growth medium with serial dilutions of test antibodies for 24 hours. After the specified period of time, to the cells was added a luciferase detection reagent, incubation was carried out for 8 minutes in the dark, followed by recording of the luminescent signal using a plate reader.

Half-effective concentration $EC_{50}$ was calculated using a four-parameter logistic regression model, the data is shown in Table 36.

Table 36 – $EC_{50}$ of anti-BCMA/anti-CD3 antibodies upon activation of Jurkat NFAT-luc Cl.1 in the presence of multiple myeloma cells RPMI8226

| Bispecific antibody | $EC_{50}$ (ng/ml) |
|---|---|
| 02-007 | 5.85 |
| 02-011 | 13.25 |
| 02-012 | 17.11 |

Thus, all test bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA are capable of activating the Jurkat NFAT-luc Cl.1 reporter cell line in the presence of multiple myeloma.

**Example 46. Analysis of ability of bispecific antibodies that specifically bind to CD3 and tumor antigen BCMA to non-specifically activate reporter T lymphocytic Jurkat NFAT-luc Cl.1 line without RPMI8226 multiple myeloma cells**

Ability of anti-BCMA/anti-CD3 antibodies to activate T cell signaling by means of binding to the CD3 subunit of the T cell receptor (TCR) in the absence of a target multiple

myeloma cell line RPMI8226 was measured by way of detecting a luminescent signal following incubating the antibodies with reporter T lymphocytic human T cells, Jurkat NFAT-luc Cl.1.

20 000 Jurkat NFAT-luc Cl.1 were incubated in a culture medium for 24 hours with either test antibodies or Hit3a, an anti-CD3 antibody, which was used as a positive control in the test. After the specified period of time, to the cells was added a luciferase detection reagent, incubation was carried out for 8 minutes in the dark, followed by recording of the luminescent signal using a plate reader.

Nonspecific activation of antibodies was measured by comparing the levels of the luminescent signal from antibody and Hit3a at the same concentration; the data is shown in Table 37.

Table 37 – Data on activation of Jurkat NFAT-luc Cl.1 cell line following incubation with test anti-BCMA/anti-CD3 antibodies and with anti-CD3 antibody Hit3a. These antibodies were used at the same concentration

| Bispecific antibody | Luminescent signal intensity |
|---|---|
| 02-007 | 2955 |
| 02-011 | 3287 |
| 02-012 | 2832 |
| Hit3a | 11942 |

Thus, test bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA do not activate Jurkat NFAT-luc Cl.1 reporter cells in the absence of multiple myeloma cells.

**Example 47. Analysis of ability of bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA to specifically induce depletion of NCI-H929 multiple myeloma cells**

Ability of anti-BCMA/anti-CD3 antibodies to induce depletion of NCI-H929 multiple myeloma cells involving effector cells within human peripheral blood mononuclear cells (PBMCs) was measured colorimetrically by way of detecting changes in optical density following incubation of the antibodies with human PBMCs in the presence of NCI-H929 multiple myeloma cells.

30 000 NCI-H929 cells and 150 000 PBMCs were incubated in a growth serum-free medium with serial dilutions of test antibodies for 24 hours. After the specified time, to the cells was added a lactate dehydrogenase detection reagent, incubation was carried out for 10 minutes in the dark, followed by recording of optical density at a wavelength of 490 nm using a plate reader.

Half-effective concentration $EC_{50}$ was calculated by plotting a four-parameter logistic regression model, the data is shown in Table 38.

Table 38 - $EC_{50}$ of anti-BCMA/anti-CD3 antibodies while depletion of NCI-H929 multiple myeloma cells involving human PBMCs. Shown is data from 6 donors

| Bispecific antibody | $EC_{50}$ (ng/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Donor 1 | Donor 2 | Donor 3 | Donor 4 | Donor 5 | Donor 6 |
| 02-007 | 10.22 | 2.33 | 2.88 | 7.68 | 1.48 | 3.34 |
| 02-011 | 3.94 | 4.84 | 2.00 | 6.52 | 1.33 | 2.56 |
| 02-012 | 3.23 | 3.63 | 1.61 | 7.31 | 0.58 | 1.70 |

To measure the nonspecific activity of the antibody, depletion of BCMA-negative K562 line cells was analyzed. Sample preparation and data collection were carried out according to the procedure described for the NCI-H929 line. To analyze the ability of antibodies to non-specifically induce depletion of K562 cells, determined was the presence or absence of dose-dependent effect (i.e., dependence of increase in LDH in the medium on increasing concentrations of test antibodies). The results are shown in Table 39.

Table 39 – No dose-dependent effect of anti-BCMA/anti-CD3 antibodies on depletion of BCMA-negative K562 cells involving human PBMCs. For comparison, shown is data on the presence of a dose-dependent effect of anti-BCMA/anti-CD3 antibodies against BCMA-positive NCI-H929 cells

| | Presence of dose-dependent effect | |
|---|---|---|
| Bispecific antibody | H929 | K562 |
| 02-007 | + | - |
| 02-011 | + | - |
| 02-012 | + | - |

Thus, all test bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA are capable of inducing depletion BCMA-positive NCI-H929 multiple myeloma cells and do not exhibit activity against BCMA-negative K562 cells.

**Example 48. Analysis of ability of bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA to induce depletion of NCI-H929 multiple myeloma cells in the presence of April and BAFF ligands**

Ability of anti-BCMA/anti-CD3 antibody to induce depletion of NCI-H929 multiple myeloma cells involving effector cells within human peripheral blood mononuclear cells (hereinafter PBMCs) in the presence of April and BAFF ligands was measured colorimetrically by way of detecting changes in optical density following incubation of the antibody with human PBMCs in the presence of NCI-H929 multiple myeloma cells.

30 000 NCI-H929 cells and 150 000 PBMCs were incubated in a serum-free culture medium for 24 hours with serial dilutions of test antibody supplemented with BAFF at a

concentration of 1 ng/ml or supplemented with April at concentrations from 12.5 to 100 ng/ml. After the specified time, to the cells was added a lactate dehydrogenase detection reagent, incubation was carried out for 10 minutes in the dark, followed by recording of optical density at a wavelength of 490 nm using a plate reader.

Half-maximal effective concentration $EC_{50}$ was calculated using a four-parameter logistic regression model. Effect of BAFF and various concentrations of April on antibody characteristics was evaluated by way of comparing antibody $EC_{50}$ under different conditions and calculating the relative activity.

The data is shown in Tables 40 and 41.

Table 40 – Relative activity of anti-BCMA/anti-CD3 antibodies while depletion of NCI-H929 multiple myeloma cells involving human PBMCs in the presence of BAFF ligand

| | Relative activity |
|---|---|
| Bispecific antibody | + 1 ng/ml BAFF |
| 02-007 | 0.72 |
| 02-011 | 0.86 |
| 02-012 | 1.08 |
| EC50 under standard conditions (i.e. in the absence of BAFF) were used as a reference | |

Table 41 – Relative activity of anti-BCMA/anti-CD3 antibodies while depletion of NCI-H929 multiple myeloma cells involving human PBMCs in the presence of April ligand

| | Relative activity | | | |
|---|---|---|---|---|
| Bispecific antibody | + 12.5 ng/ml April | + 25 ng/ml April | + 50 ng/ml April | + 100 ng/ml April |
| 02-007 | 0.95 | 0.75 | 0.77 | 0.35 |
| 02-011 | 1.18 | 0.89 | 0.87 | 0.33 |
| 02-012 | 1.04 | 0.56 | 0.49 | 0.38 |
| $EC_{50}$ under standard conditions (i.e. in the absence of April) were used as a reference | | | | |

Thus, the BAFF ligand does not significantly affect the efficiency of test antibodies against the depletion of NCI-H929 multiple myeloma cells; of note, increase in April concentrations leads to decreased efficiency of test antibodies pertaining to depletion of NCI-H929 multiple myeloma cells.

**Example 49. Analysis of ability of bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA to induce proliferation of NCI-H929 multiple myeloma cells**

Ability of anti-BCMA/anti-CD3 antibodies to induce proliferation of multiple myeloma cells was evaluated by direct count of NCI-H929 cells following incubation with test antibodies.

10 000 NCI-H929 cells were incubated in a serum-free culture medium for 24 hours with analyzed antibodies at a concentration of 250 nM or with 250 nM April ligand which was used as a positive control. After the specified time, to the cells were added specialized cell counting microspheres, and analysis on a flow cytometer was conducted. Table 42 shows cell concentrations.

Table 42 – Concentration of NCI-H929 multiple myeloma cells following incubation with anti-BCMA/anti-CD3 antibodies or April ligand at a concentration of 250 nM

| Bispecific antibody | Concentration, cells/µl |
|---|---|
| 02-007 | 2.4 |
| 02-011 | 2.2 |
| Positive control (+ 250 nM April) | 2.9 |
| Negative control (cells without adding April and test antibodies) | 2.2 |

Thus, test bispecific antibodies that specifically bind to CD3 and the tumor antigen BCMA do not induce proliferation of multiple myeloma cells.

**Claims**

1. An isolated bispecific antibody that specifically binds to CD3 and a tumor antigen, and comprises:

1) a first antigen-binding fragment that specifically binds to CD3 and comprises:

(a) a light chain variable domain comprising:

(i) CDR1 with the amino acid sequence RASQSX$_1$SSNLA,

X$_1$=I or V;

(ii) CDR2 with the amino acid sequence of SEQ ID NO: 3, and

(iii) CDR3 with the amino acid sequence QX$_2$YX$_3$NWPLT, where

X$_2$=H or Q;

X$_3$=N or I; and

(b) a heavy chain variable domain comprising:

(i) CDR1 with the amino acid sequence of SEQ ID NO: 6,

(ii) CDR2 with the amino acid sequence of SEQ ID NO: 7, and

(iii) CDR3 with the amino acid sequence DNSGYGHYYYGX$_4$DI, wherein

X$_4$=M or L; and

2) a second antigen-binding fragment that specifically binds to a tumor antigen.

2. The isolated bispecific antibody according to claim 1, wherein the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 1 or SEQ ID NO: 2.

3. The isolated bispecific antibody according to claim 1, wherein the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 4 or SEQ ID NO: 5.

4. The isolated bispecific antibody according to claim 1, wherein the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 8 or SEQ ID NO: 9.

5. The isolated bispecific antibody according to claim 1, comprising the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 1 or SEQ ID NO: 2;

CDR2 with the amino acid sequence of SEQ ID NO: 3; and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 4 or SEQ ID NO: 5.

6. The isolated bispecific antibody according to claim 1, comprising the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6;

CDR2 with the amino acid sequence of SEQ ID NO: 7; and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 8 or SEQ ID NO: 9.

7. The isolated bispecific antibody according to claim 1, comprising

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 1 or SEQ ID NO: 2;

CDR2 with the amino acid sequence of SEQ ID NO: 3; and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 4 or SEQ ID NO: 5; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6;

CDR2 with the amino acid sequence of SEQ ID NO: 7; and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 8 or SEQ ID NO: 9.

8. The isolated bispecific antibody according to claim 7, comprising

(i) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8; or

(ii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 1,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8; or

(iii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 5; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 9; or

(iv) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 1,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 5; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 9.

9. The isolated bispecific antibody according to claim 1, wherein the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 or SEQ ID NO: 156.

10. The isolated bispecific antibody according to claim 1, wherein the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 or SEQ ID NO: 157.

11. The isolated bispecific antibody according to claim 1, wherein:

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 or SEQ ID NO: 156; and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 or SEQ ID NO: 157.

12. The isolated bispecific antibody according to any one of claims 1-11, wherein the first antigen-binding fragment that specifically binds to CD3 is designed in scFv, Fab, scFab or CrossMab format.

13. The isolated bispecific antibody according to any one of claims 1-11, wherein the second antigen-binding fragment that specifically binds to a tumor antigen is designed in scFv, Fab, scFab or CrossMab format.

14. The isolated bispecific antibody according to claim 11, wherein:

(i) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 10 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 16; or

(ii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 16; or

(iii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 10 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 17; or

(iv) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 10 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 18; or

(v) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 10 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19; or

(vi) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19; or

(vii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 12 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 18; or

(viii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 13 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 20; or

(ix) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 14 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 20; or

(x) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 13 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 21; or

(xi) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 13 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 22; or

(xii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 13 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 23; or

(xiii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 14 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 23; or

(xiv) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 15 and

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 22; or

(xv) (a) the light chain variable domain of the first antigen-binding fragment, designed in CrossMab format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 156 and

(b) the heavy chain variable domain of the first antigen-binding fragment, designed in CrossMab format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 157.

15. The isolated bispecific antibody according to any one of claims 1-12 and 14, wherein the antibody that specifically binds to CD3 and a tumor antigen is a full-length IgG antibody.

16. The isolated bispecific antibody according to claim 15, wherein the full-length IgG antibody is of human IgG1, IgG2, IgG3 or IgG4 isotype.

17. The isolated bispecific antibody according to claim 16, wherein the full-length IgG antibody is of human IgG1 isotype.

18. The isolated bispecific antibody according to claim 17, wherein the antibody comprises mutations L234A and L235A according to the EU numbering scheme for amino acids of antibodies in the CH2 region.

19. The isolated bispecific antibody according to claim 17, wherein the antibody comprises mutations M252Y, S254T, T256E according to the EU numbering scheme for amino acids of antibodies in the CH2 region.

20. The isolated bispecific antibody according to claim 17, wherein the antibody comprises mutations S354C and T366W according to the EU numbering scheme for amino acids of antibodies in the CH3 region.

21. The isolated bispecific antibody according to claim 17, wherein the antibody comprises mutations Y349C, T366S, L368A and Y407V according to the EU numbering scheme for amino acids of antibodies in the CH3 region.

22. The isolated bispecific antibody according to claim 1, wherein the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 158, SEQ ID NO: 160 or SEQ ID NO: 162.

23. The isolated bispecific antibody according to claim 1, wherein the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 159, SEQ ID NO: 161 or SEQ ID NO: 163.

24. The isolated bispecific antibody according to claim 1, wherein

a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 158, SEQ ID NO: 160 or SEQ ID NO: 162; and

b) wherein the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises an amino acid sequence that is selected from the group: SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 159, SEQ ID NO: 161 or SEQ ID NO: 163.

25. The isolated bispecific antibody according to claim 24, wherein

(i) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 24 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 30; or

(ii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 25 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 30; or

(iii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 24 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3, of the first antigen-binding fragment that specifically binds to CD3, comprises the amino acid sequence of SEQ ID NO: 31; or

(iv) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 24 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 32; or

(v) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 24 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 33; or

(vi) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 25 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 33; or

(vii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 26 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 32; or

(viii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 27 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 34; or

(ix) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 28 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 34; or

(x) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 27 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 35; or

(xi) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 27 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 36; or

(xii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 27 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 37; or

(xiii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 28 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 37; or

(xiv) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 29 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 36; or

(xv) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 25 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 38; or

(xvi) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159; or

(xvii) (a) the light chain of the first antigen-binding fragment, designed in CrossMab format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 160 and

(b) the heavy chain of the first antigen-binding fragment, designed in CrossMab format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 161; or

(xviii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 162 and

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 163.

26. The isolated bispecific antibody according to claim 1, wherein the second antigen-binding fragment that specifically binds to a tumor antigen is selected from the group: GUCY2C, AXL, GD2, CD19, EpCAM, EGFR, CD20, CEA, HER2/neu, PSMA, BCMA, MUC17, CD38, FLT3, ENPP3, TPBG, FAP, CD70, Cadherin-19, Mesothelin, CD33, ROR1, IL-3Rα, Cadherin-17, P-cadherin, MUC1, B7-H6, DLL3, B7-H3, CD28, CD155, IRTA2, GPRC5D, RNF43, IL-1RAP, Globo H, Glypican 3, CLDN18, TPBG, LYPD1, PD-L1, GPNMB, NY-ESO-1, MAGE-A4, TACSTD-2, DR5, CD8, TMEFF2, PRLR, CD22, HLA-G, Robo1, EphA2, Prominin-1, HMW-MAA, A33, TAG-72, HER3, IL-23R, CD2, MIF, PD-1, Epha10, GCC, ALP, LFL2, MAGE-A4, STEAP2, CA125, TYRP1, 4-1BB, FRA, CD44v6, Robo4, TIM-3, CLEC12A, B7-H4, FCRL5, SSTR2, c-Kit, MOSPD2, PSA or CD26.

27. The isolated bispecific antibody according to claim 1, wherein the tumor antigen is BCMA, and wherein:

a second antigen-binding fragment that specifically binds to the tumor antigen BCMA and comprises:

(a) a light chain variable domain comprising:

(i) CDR1 with the amino acid sequence $X_7GX_8X_9SNIGX_{10}X_{11}X_{12}X_{13}VX_{14}$, wherein

$X_7$=S or T;

$X_8$=S, G or T;

$X_9$=S, T, I or R;

$X_{10}$=0 or A;

$X_{11}$=S, H, N, G or T;

$X_{12}$=N, R or Y;

$X_{13}$=T, A, I or D;

$X_{14}$=N or H;

(ii) CDR2 with the amino acid sequence $X_{15}X_{16}X_{17}X_{18}$RPS, wherein

$X_{15}$=N, R, K, S or G;

$X_{16}$=D, N, G, T or H;

$X_{17}$=S, N or T;

$X_{18}$=Q or N; and

(iii) CDR3 with the amino acid sequence $X_{19}X_{20}$WD$X_{21}X_{22}X_{23}X_{24}X_{25}$W$X_{26}$, wherein

$X_{19}$=A or S;

$X_{20}$=A, S, T or V;

$X_{21}$=G, D, H or S;

$X_{22}$=S, D or R;

$X_{23}$=L or V;

$X_{24}$=N, T, R or S;

$X_{25}$=V, A, G or N;

$X_{26}$=M, V or L; and

(b) a heavy chain variable domain comprising:

(i) CDR1 with the amino acid sequence S$X_1X_2$MS, wherein

$X_1$=S or G;

$X_2$=A, L or V;

(ii) CDR2 with the amino acid sequence $X_3$YNGGS$X_0$RAG$X_4X_5$DSV$X_6$G, wherein

$X_0$=D or T;

$X_3$=G or C;

$X_4$=F or Y;

$X_5$=A or T;

$X_6$=E or K; and

(iii) CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 83 or SEQ ID NO: 130.

28. The isolated bispecific antibody according to claim 27, wherein the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56 or SEQ ID NO: 122.

29. The isolated bispecific antibody according to claim 27, wherein the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises CDR2 with an amino acid sequence that is selected from the group: SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 123.

30. The isolated bispecific antibody according to claim 27, wherein the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO:73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76 or SEQ ID NO: 124.

31. The isolated bispecific antibody according to claim 27, wherein the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79 or SEQ ID NO: 125.

32. The isolated bispecific antibody according to claim 27, wherein the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises CDR2 with an amino acid sequence that is selected from the group: SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128 or SEQ ID NO: 129.

33. The isolated bispecific antibody according to claim 27, comprising the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56 or SEQ ID NO: 122;

CDR2 with an amino acid sequence that is selected from the group: SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 123; and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO:73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76 or SEQ ID NO: 124.

34. The isolated bispecific antibody according to claim 27, comprising the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79 or SEQ ID NO: 125;

CDR2 with an amino acid sequence that is selected from the group: SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128 or SEQ ID NO: 129; and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 83 or SEQ ID NO: 130.

35. The isolated bispecific antibody according to claim 27, comprising

(a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56 or SEQ ID NO: 122;

CDR2 with an amino acid sequence that is selected from the group: SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 123; and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO:73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76 or SEQ ID NO: 124; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with an amino acid sequence that is selected from the group: SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79 or SEQ ID NO: 125;

CDR2 with an amino acid sequence that is selected from the group: SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128 or SEQ ID NO: 129; and

CDR3 with an amino acid sequence that is selected from the group: SEQ ID NO: 83 or SEQ ID NO: 130.

36. The isolated bispecific antibody according to claim 35, comprising:

(i) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 49,

CDR2 with the amino acid sequence of SEQ ID NO: 57 and

CDR3 with the amino acid sequence of SEQ ID NO: 66; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 77,

CDR2 with the amino acid sequence of SEQ ID NO: 80 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(ii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 50,

CDR2 with the amino acid sequence of SEQ ID NO: 58 and

CDR3 with the amino acid sequence of SEQ ID NO: 67; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 81 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(iii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 51,

CDR2 with the amino acid sequence of SEQ ID NO: 59 and

CDR3 with the amino acid sequence of SEQ ID NO: 68; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 79,

CDR2 with the amino acid sequence of SEQ ID NO: 80 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(iv) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 52,

CDR2 with the amino acid sequence of SEQ ID NO: 60 and

CDR3 with the amino acid sequence of SEQ ID NO: 69; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 80 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(v) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 53,

CDR2 with the amino acid sequence of SEQ ID NO: 61 and

CDR3 with the amino acid sequence of SEQ ID NO: 70; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 81 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(vi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 53,

CDR2 with the amino acid sequence of SEQ ID NO: 61 and

CDR3 with the amino acid sequence of SEQ ID NO: 71; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 82 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(vii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 54,

CDR2 with the amino acid sequence of SEQ ID NO: 63 and

CDR3 with the amino acid sequence of SEQ ID NO: 73; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 81 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(viii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 53,

CDR2 with the amino acid sequence of SEQ ID NO: 61 and

CDR3 with the amino acid sequence of SEQ ID NO: 71; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 81 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(ix) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 55,

CDR2 with the amino acid sequence of SEQ ID NO: 64 and

CDR3 with the amino acid sequence of SEQ ID NO: 74; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 80 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(x) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 49,

CDR2 with the amino acid sequence of SEQ ID NO: 65 and

CDR3 with the amino acid sequence of SEQ ID NO: 75; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 80 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(xi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 56,

CDR2 with the amino acid sequence of SEQ ID NO: 58 and

CDR3 with the amino acid sequence of SEQ ID NO: 76; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 81 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(xii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 51,

CDR2 with the amino acid sequence of SEQ ID NO: 62 and

CDR3 with the amino acid sequence of SEQ ID NO: 72; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 78,

CDR2 with the amino acid sequence of SEQ ID NO: 81 and

CDR3 with the amino acid sequence of SEQ ID NO: 83; or

(xiii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 126 and

CDR3 with the amino acid sequence of SEQ ID NO: 130; or

(xiv) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 127 and

CDR3 with the amino acid sequence of SEQ ID NO: 130; or

(xv) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 128 and

CDR3 with the amino acid sequence of SEQ ID NO: 130; or

(xvi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 129 and

CDR3 with the amino acid sequence of SEQ ID NO: 130.

37. The isolated bispecific antibody according to claim 27, wherein the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises an amino acid sequence that is selected from the group: SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94 or SEQ ID NO: 131.

38. The isolated bispecific antibody according to claim 27, wherein the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises an amino acid sequence that is selected from the group: SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141 or SEQ ID NO: 142.

39. The isolated bispecific antibody according to claim 27, wherein

(a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises an amino acid sequence that is selected from the group: SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94 or SEQ ID NO: 131; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises an amino acid sequence that is selected from the group:

SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141 or SEQ ID NO: 142.

40. The isolated bispecific antibody according to claim 39, wherein

(i) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 84; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 95; or

(ii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 85; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 96; or

(iii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 86; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 97; or

(iv) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 87; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 98; or

(v) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 88;

and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 99; or

(vi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 89; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 100; or

(vii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 91; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 99; or

(viii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 89; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 99; or

(ix) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 92; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 101; or

(x) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 93; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 102; or

(xi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 94; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 99; or

(xii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 90; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 99; or

(xiii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 132; or

(xiv) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 133; or

(xv) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 134; or

(xvi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 135; or

(xvii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO:136; or

(xviii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO:137; or

(xix) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 138; or

(xx) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 139; or

(xxi) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 140; or

(xxii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 141; or

(xxiii) (a) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(b) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 142.

41. The isolated bispecific antibody according to claim 27, wherein the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises an amino acid sequence that is selected from the group: SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 143 or SEQ ID NO: 144.

42. The isolated bispecific antibody according to claim 27, wherein the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises an amino acid sequence that is selected from the group: SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154 or SEQ ID NO: 155.

43. The isolated bispecific antibody according to claim 27, wherein

(a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises an amino acid sequence that is selected from the group: SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 143 or SEQ ID NO: 144; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises an amino acid sequence that is selected from the group: SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154 or SEQ ID NO: 155.

44. The isolated bispecific antibody according to claim 43, wherein

(i) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 103; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 114; or

(ii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 104; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 115; or

(iii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 105; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 116; or

(iv) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 106; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 117; or

(v) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 107; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 118; or

(vi) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 108; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 119; or

(vii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 110; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 118; or

(viii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 108; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 118; or

(ix) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 111; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 120; or

(x) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 112; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 121; or

(xi) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 113; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 118; or

(xii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 109; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 118; or

(xiii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 145; or

(xiv) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 146; or

(xv) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 147; or

(xvi) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 148; or

(xvii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 149; or

(xviii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 150; or

(xix) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 151; or

(xx) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 152; or

(xxi) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 153; or

(xxii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 154; or

(xxiii) (a) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 144; and

(b) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 155.

45. The isolated bispecific antibody according to claim 27, comprising:

(i) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 126 and

CDR3 with the amino acid sequence of SEQ ID NO: 130; or

(ii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 127 and

CDR3 with the amino acid sequence of SEQ ID NO: 130; or

(iii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 128 and

CDR3 with the amino acid sequence of SEQ ID NO: 130; or

(iv) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 129 and

CDR3 with the amino acid sequence of SEQ ID NO: 130; or

(v) (a) the light chain variable domain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 6,

CDR2 with the amino acid sequence of SEQ ID NO: 7 and

CDR3 with the amino acid sequence of SEQ ID NO: 8;

(b) the heavy chain variable domain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 2,

CDR2 with the amino acid sequence of SEQ ID NO: 3 and

CDR3 with the amino acid sequence of SEQ ID NO: 4;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 122,

CDR2 with the amino acid sequence of SEQ ID NO: 123 and

CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA, comprising:

CDR1 with the amino acid sequence of SEQ ID NO: 125,

CDR2 with the amino acid sequence of SEQ ID NO: 126 and

CDR3 with the amino acid sequence of SEQ ID NO: 130.

46. The isolated bispecific antibody according to claim 27, comprising:

(i) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 132; or

(ii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 133; or

(iii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 134; or

(iv) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 135; or

(v) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO:136; or

(vi) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO:137; or

(vii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 138; or

(viii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 139; or

(ix) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 140; or

(x) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 141; or

(xi) (a) the light chain variable domain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 156;

(b) the heavy chain variable domain of the first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 157;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 142; or

(xii) (a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 11;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 19;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 142.

47. The isolated bispecific antibody according to claim 27, comprising:

(i) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 145; or

(ii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 146; or

(iii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 147; or

(iv) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 148; or

(v) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 149; or

(vi) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 150; or

(vii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 151; or

(viii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 152; or

(ix) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 153; or

(x) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 158;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 159;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 143; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 154; or

(xi) (a) the light chain of the first antigen-binding fragment, designed in CrossMab format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 160;

(b) the heavy chain of the first antigen-binding fragment, designed in CrossMab format, that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 161;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 144; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 155; or

(xii) (a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 162;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 163;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 144; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 155.

48. An isolated bispecific antibody that specifically binds to CD3 and the tumor antigen BCMA, and comprises:

1) a first antigen-binding fragment, designed in CrossMAb format, that specifically binds to CD3, and comprises:

(a) a light chain variable domain comprising:

(i) CDR1 with the amino acid sequence of SEQ ID NO: 6;

(ii) CDR2 with the amino acid sequence of SEQ ID NO: 7, and

(iii) CDR3 with the amino acid sequence of SEQ ID NO: 8; and

(b) a heavy chain variable domain comprising:

(i) CDR1 with the amino acid sequence of SEQ ID NO: 2,

(ii) CDR2 with the amino acid sequence of SEQ ID NO: 3, and

(iii) CDR3 with the amino acid sequence of SEQ ID NO: 4; and

2) a second antigen-binding fragment that specifically binds to the tumor antigen BCMA and comprises:

(a) a light chain variable domain comprising:

(i) CDR1 with the amino acid sequence of SEQ ID NO: 122;

(ii) CDR2 with the amino acid sequence of SEQ ID NO: 123; and

(iii) CDR3 with the amino acid sequence of SEQ ID NO: 124; and

(b) a heavy chain variable domain comprising:

(i) CDR1 with the amino acid sequence of SEQ ID NO: 125;

i) CDR1 with the amino acid sequence of SEQ ID NO: 126; and

(iii) CDR3 with the amino acid sequence of SEQ ID NO: 130.

49. The isolated bispecific antibody according to claim 48, wherein

(a) the light chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 156;

(b) the heavy chain variable domain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 157;

(c) the light chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 131; and

(d) the heavy chain variable domain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 142.

50. The isolated bispecific antibody according to any one of claims 48-49, wherein the antibody that specifically binds to CD3 and the tumor antigen BCMA is a full-length IgG antibody.

51. The isolated bispecific antibody according to claim 50, wherein the full-length IgG antibody is of human IgG1, IgG2, IgG3 or IgG4 isotype.

52. The isolated bispecific antibody according to claim 51, wherein the full-length IgG antibody is of human IgG1 isotype.

53. The isolated bispecific antibody according to claim 52, wherein the antibody comprises mutations L234A and L235A according to the EU numbering scheme for amino acids of antibodies in the CH2 region.

54. The isolated bispecific antibody according to claim 52, wherein the antibody comprises mutations M252Y, S254T, T256E according to the EU numbering scheme for amino acids of antibodies in the CH2 region.

55. The isolated bispecific antibody according to claim 52, wherein the antibody comprises mutations S354C and T366W according to the EU numbering scheme for amino acids of antibodies in the CH3 region.

56. The isolated bispecific antibody according to claim 52, wherein the antibody comprises mutations Y349C, T366S, L368A and Y407V according to the EU numbering scheme for amino acids of antibodies in the CH3 region.

57. The isolated bispecific antibody according to claim 48, wherein

(a) the light chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 160;

(b) the heavy chain of the first antigen-binding fragment that specifically binds to CD3 comprises the amino acid sequence of SEQ ID NO: 161;

(c) the light chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 144; and

(d) the heavy chain of the second antigen-binding fragment that specifically binds to BCMA comprises the amino acid sequence of SEQ ID NO: 155.

58. An isolated nucleic acid that encodes the bispecific antibody according to any one of claims 1-57.

59. The isolated nucleic acid according to claim 58, wherein the nucleic acid is DNA.

60. An expression vector comprising the nucleic acid according to any one of claims 58-59.

61. A method for producing a host cell to produce the bispecific antibody according to any one of claims 1-57, comprising transformation of the cell by the vector according to claim 60.

62. A host cell for producing the antibody or antigen-binding fragment thereof according to any one of claims 1-57, comprising the nucleic acid according to any one of claims 58-59.

63. A method for producing the bispecific antibody according to any one of claims 1-57, comprising culturing the host cell according to claim 62 in a culture medium under conditions sufficient to produce said bispecific antibody, followed by isolating and purifying the resulting antibody.

64. A pharmaceutical composition for treating a disease or disorder mediated by a tumor antigen comprising the bispecific antibody according to any one of claims 1-57 in a therapeutically effective amount in combination with one or more pharmaceutically acceptable excipients.

65. The pharmaceutical composition according to claim 64, wherein the disease or disorder mediated by a tumor antigen is selected from the group: multiple myeloma, chronic lymphocytic leukemia, acute B-lymphoblastic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma.

66. A pharmaceutical composition for treating a disease or disorder mediated by a tumor antigen, comprising the bispecific antibody according to any one of claims 1-57 and at least one other therapeutically active compound.

67. The pharmaceutical composition according to claim 66, wherein the disease or disorder mediated by a tumor antigen is selected from the group: multiple myeloma, chronic lymphocytic leukemia, acute B-lymphoblastic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma.

68. The pharmaceutical composition according to any one of claims 66-67, wherein the other therapeutically active compound is an antibody, a small molecule, a hormone therapy agent or combination thereof.

69. A method for treating a disease or disorder mediated by a tumor antigen, comprising administering to a subject in need of such treatment the bispecific antibody according to any one of claims 1-57 or the pharmaceutical composition according to any one of claims 64-68 in a therapeutically effective amount.

70. The method for treating a disease or disorder according to claim 69, wherein the disease or disorder mediated by a tumor antigen is selected from the group: multiple myeloma, chronic lymphocytic leukemia, acute B-lymphoblastic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma.

71. A method for treating a disease or disorder mediated by a tumor antigen, comprising administering to a subject in need of such treatment the bispecific antibody according to any one of claims 1-57 and at least one other therapeutically active compound in a therapeutically effective amount.

72. The method for treating a disease or disorder according to claim 71, wherein the disease or disorder mediated by a tumor antigen is selected from the group: multiple myeloma, chronic lymphocytic leukemia, acute B-lymphoblastic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma.

73. The method for treating a disease or disorder according to any one of claims 71-72, wherein the other therapeutically active compound is an antibody, a small molecule, a hormone therapy agent or combination thereof.

74. Use of the bispecific antibody according to any one of claims 1-57 or the pharmaceutical composition according to any one of claims 64-68 for treating in a subject in need of such treatment a disease or disorder mediated by a tumor antigen.

75. The use according to claim 74, wherein the disease or disorder mediated by a tumor antigen is selected from the group: multiple myeloma, chronic lymphocytic leukemia, acute B-lymphoblastic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma.

76. Use of the bispecific antibody according to any one of claims 1-57 and at least one other therapeutically active compound for treating a disease or disorder mediated by a tumor antigen.

77. The use according to claim 76, wherein the disease or disorder mediated by a tumor antigen is selected from the group: multiple myeloma, chronic lymphocytic leukemia, acute B-lymphoblastic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma.

78. The use according to claim 76, wherein the other therapeutically active compound is an antibody, small molecule, hormone therapy agent, or any combination thereof.