

Description

ANTI-CLEAVED MUTANT CALR-CD3 BISPECIFIC ANTIBODY AND
PHARMACEUTICAL COMPOSITION

Technical Field

[0001]

The present invention relates to the field of a bispecific antibody having specificity for a mutant CALR protein and its utilization in therapeutic and diagnostic applications.

Background Art

[0002]

In a part of patients with Philadelphia-negative myeloproliferative neoplasms (MPNs), nucleotide deletion or insertion is found in exon 9 of the calreticulin (CALR) gene (Non Patent Literatures 1 and 2). It has been already revealed that the mutant CALR protein produced by a mutant CALR gene has oncogenicity independently causing a myeloproliferative neoplasm (MPN) by constitutively activating a thrombopoietin (TPO) receptor (Non Patent Literatures 3 to 6).

[0003]

The CALR gene mutation found in MPN patients is a frameshift mutation which is always localized in the last exon, and a shift in the amino acid reading frame due to the frameshift mutation is always +1. From these

findings, a sequence not present in the wild type is present at the C-terminus of the mutant CALR protein, and particularly, 44 amino acids at the C-terminus are common to almost all mutant CALR proteins. As an example thereof, Figure 1 [a] shows a comparison of the C-terminal sequences of 52-nucleotide deletion type (Del 52), which is the most frequent mutation among CALR gene mutations found in MPN patients, and 5-nucleotide insertion type (Ins 5), which is the next most frequent CALR mutation, with the corresponding region of the wild-type CALR protein.

Since the mutant CALR protein causing an MPN is expressed in tumor cells, it has been suggested that a sequence specific to the mutant CALR protein caused by the frameshift mutation may become a diagnostic marker or a therapeutic target as a neoantigen (Patent Literatures 1 and 2).

[0004]

However, it turned out that an antibody that simply recognizes a sequence specific to the mutant CALR protein (neoantigen) generated by the frameshift mutation cannot accurately detect the mutant CALR protein in a cell extract or on a cell surface. As a result of functional analysis of the mutant CALR proteins, many of the mutant CALR proteins expressed in tumor cells are cleaved in sequences specific to the mutant CALR proteins and that cleaved mutant CALR proteins, in which most of the sequences inferred as neoantigens have been lost, are

largely expressed. Accordingly, when an antibody which specifically recognizes a very short amino acid sequence located at the N-terminal side of the cleavage site in the neoantigen was made (Figure 1 [b]), it was revealed that an excellent MPN therapeutic effect is obtained. From these findings, it was suggested that, in diagnosis or treatment of MPN patients having a CALR gene mutation, pharmaceutical products that achieve higher-performance can be developed by detecting or targeting mutant CALR proteins including not only the full-length (uncleaved) but also cleaved proteins.

[0005]

Furthermore, in recent years, an antibody capable of specifically binding to two antigens for the purpose of enhancing anticancer activity has been actively studied as a more effective therapeutic agent with specific antitumor effects by taking advantage of the properties of the antibody. Much attention has been paid to a bispecific antibody in which one site can bind to a surface antigen on a target cell and the other site can bind to a surface antigen on an effector cell. As the effector cell, an immune effector cell such as a T cell is often used, and the bispecific antibody aims to bring about a transient interaction between the target cell and the immune effector cell. Among T cell surface antigens, for example, an antibody having a binding site for a CD3 epsilon subunit derived from a T-cell receptor (TCR) protein complex is capable of causing lysis of the target

cell primarily by the T cell, that is, T cell-redirected killing. Thus, since the bispecific antibody specifically binds to a target cell and a T cell and causes the T cell to directly act on the target cell, studies are in progress to target substances abnormally expressed in cancer cells or substances altered during oncogenic transformation.

[0006]

For example, Patent Literature 3 describes a bispecific antibody with the first specificity for human CD19 and the second specificity for a human CD3 antigen, and Patent Literature 4 describes a bispecific antibody related to CALR and immune cells.

Citation List

Patent Literature

[0007]

Patent Literature 1: WO 2015/036599

Patent Literature 2: WO 2016/087514

Patent Literature 3: WO 1999/054440

Patent Literature 4: WO 2019/178362

Non Patent Literature

[0008]

Non Patent Literature 1: Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. The New England journal of medicine. 2013; 369: 2379-90.

Non Patent Literature 2: Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. The New England journal of medicine. 2013; 369: 2391-405.

Non Patent Literature 3: Araki M, Yang Y, Masubuchi N, et al. Activation of the thrombopoietin receptor by mutant calreticulin in CALR-mutant myeloproliferative neoplasms. Blood. 2016; 127: 1307-16.

Non Patent Literature 4: Elf S, Abdelfattah NS, Chen E, et al. Mutant Calreticulin Requires Both Its Mutant C-terminus and the Thrombopoietin Receptor for Oncogenic Transformation. Cancer Discov. 2016; 6: 368-81.

Non Patent Literature 5: Marty C, Pecquet C, Nivarthi H, et al. Calreticulin mutants in mice induce an MPL-dependent thrombocytosis with frequent progression to myelofibrosis. Blood. 2016; 127: 1317-24.

Non Patent Literature 6: Vainchenker W, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. Blood. 2017; 129: 667-79.

Summary of Invention

Technical Problem

[0009]

Although mutant CALR proteins are specifically expressed in MPN patient cells, many of the mutant CALR proteins were cleaved mutant CALR proteins. Therefore, the problem of the present invention is to provide a pharmaceutical composition containing an antibody or

functional fragment thereof which is capable of accurately detecting the full-length and cleaved mutant CALR proteins and can be therapeutically applied.

Solution to Problem

[0010]

By using an antibody which specifically recognizes a very short amino acid sequence located at the N-terminal side of the cleavage site, it was verified that the cleaved mutant CALR protein was expressed not only in cultured cells but also in, for example, patient peripheral blood cells, patient platelets, and patient bone marrow cell. When using this anti-cleaved mutant CALR antibody, the detection sensitivity for the mutant CALR protein on a cell surface was dramatically improved. Furthermore, it was revealed that when a bispecific antibody (anti-cleaved mutant CALR-CD3 bispecific antibody) containing a protein which binds to a cleaved mutant CALR protein and CD3 antigen was made, excellent therapeutic effects are obtained against MPNs. It was found that a bispecific antibody based on an antibody to sequences conventionally considered as neoantigens did not provide sufficient therapeutic effects, strongly suggesting that it is important to provide a higher-performance bispecific antibody which specifically binds to the full-length and cleaved mutant CALR protein and a pharmaceutical composition containing the antibody, thus completing the present invention.

[0011]

Accordingly, the present invention provides the following (1) to (22).

(1) A bispecific antibody comprising a first domain which specifically binds to a mutant calreticulin protein and a second domain which specifically binds to a CD3 antigen.

(2) The bispecific antibody according to (1), wherein the first domain competes with an antibody which binds to an epitope of a mutant calreticulin protein of SEQ ID NO: 1.

(3) The bispecific antibody according to (1) or (2), wherein the mutant calreticulin protein comprises an antigen-recognition site in a polypeptide chain consisting of the amino acid sequence of SEQ ID NO: 1 or a polypeptide chain consisting of the amino acid sequence of SEQ ID NO: 1 but having deletion, substitution, or addition of one or several amino acids.

(4) The bispecific antibody according to any one of (1) to (3), wherein the first domain does not bind to a wild-type calreticulin protein but has high affinity to the mutant calreticulin protein.

(5) The bispecific antibody according to any one of (1) to (4), wherein the first domain comprises a high affinity to the mutant calreticulin protein after cleavage.

(6) The bispecific antibody according to any one of (1) to (5), wherein a binding of the first domain with the mutant calreticulin protein has a K_D of 10^{-7} M or less.

(7) The bispecific antibody according to any one of (1) to (6), wherein a CDR of the first domain comprises (a) an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 10, SEQ ID NO: 11 or 126, and SEQ ID NO: 12 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 13, SEQ ID NO: 14 or 127, and SEQ ID NO: 15 (VLCDR); (b) an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 16 to 18 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 19 to 21 (VLCDR); or (c) an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 22 to 24 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 25 to 27 (VLCDR).

(8) The bispecific antibody according to any one of (1) to (7), wherein the first domain comprises (d) a VH region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 2, 90, 91, 92, 93, 94, 133, 134, 135, and 136, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 3, 95, 96, 97, 98, 99, 137, 138, 139, 140, and 141; (e) a VH region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 4, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 5; or

(f) a VH region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 6, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 7.

(9) The bispecific antibody according to any one of (1) to (8), wherein a CDR of the second domain comprises an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 28, SEQ ID NO: 29 or 128, and SEQ ID NO: 30, 129 or 130 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 31 or 131, SEQ ID NO: 32 or 132, and SEQ ID NO: 33 (VLCDR).

(10) The bispecific antibody according to any one of (1) to (9), wherein the second domain comprises a VH region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 8, 204, 205, and 206, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 9, 207, and 208.

(11) The bispecific antibody according to any one of (1) to (10), wherein a dimerization module is a hetero-H chain having one or more knob-into-hole structures, wherein an amino acid(s) at one or more positions selected from the group consisting of 349, 354, 366, 368, and 407 in a constant region of the H chain are replaced by Y349C, T366S, L368A, Y407V (hole or cavity)S354C, or

T366W (knob or protrusion), the bispecific antibody comprising an Fc region of SEQ ID NO:209 or 210.

(12) The bispecific antibody according to any one of (1) to (11), comprising a mutated Fc region of SEQ ID NO: 211 with substitution of an amino acid(s) at one or more positions selected from the group consisting of 234 and 235 in a H-chain Fc region to each introduce L234A or L235A mutation.

(13) The bispecific antibody according to any one of (1) to (12), which is a human antibody, a humanized antibody, a chimeric antibody of an animal-derived antibody and a human antibody, or a synthetic antibody.

(14) A pharmaceutical composition comprising the bispecific antibody according to any one of (1) to (13) or a functional fragment thereof.

(15) The pharmaceutical composition according to (14), for treatment or diagnosis of a myeloproliferative neoplasm.

(16) A method for detecting a mutant calreticulin protein, comprising detecting the following polypeptide (A) or (B) in a biological sample using the bispecific antibody according to any one of (1) to (13) or a functional fragment thereof:

(A) a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1; or

(B) a polypeptide consisting of an amino acid sequence of SEQ ID NO: 1 but having deletion, substitution, or addition of one or several amino acids.

(17) The bispecific antibody according to any one of (1) to (13) or a functional fragment thereof, for use in detection of a mutant calreticulin protein.

(18) A diagnostic method for a myeloproliferative neoplasm, comprising detecting the following polypeptide (A) or (B) in a biological sample using the bispecific antibody according to any one of (1) to (13) or a functional fragment thereof:

(A) a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1; or

(B) a polypeptide consisting of an amino acid sequence of SEQ ID NO: 1 but having deletion, substitution, or addition of one or several amino acids.

(19) The bispecific antibody according to any one of (1) to (13) or a functional fragment thereof, for use in treatment or diagnosis of a myeloproliferative neoplasm.

(20) Use of the bispecific antibody according to any one of (1) to (13) or a functional fragment thereof, for producing a medicament for treatment or diagnosis of a myeloproliferative neoplasm.

(21) A method for prevention or treatment of a tumor, comprising administering the bispecific antibody according to any one of (1) to (13) or a functional fragment thereof.

(22) The method according to (21), wherein the tumor is a hematologic tumor, and preferably a myeloproliferative neoplasm, myeloid leukemia, thrombocytosis, polycythemia, primary myelofibrosis, or secondary myelofibrosis.

Advantageous Effects of Invention

[0012]

The bispecific antibody of the present invention has an antigen-recognition site (epitope) in the polypeptide chain (A) or (B), and by using this antibody, it is possible to detect a cleaved mutant CALR protein caused by cleavage of a mutant CALR protein and to dramatically improve the detection sensitivity for the mutant CALR protein on a cell surface. The development of anti-cleaved mutant CALR-CD3 bispecific antibodies having the first specificity for binding to the epitope of the cleaved mutant CALR protein and the second specificity for binding to CD3 antigen will provide an antibody having strong cytotoxic activity specific to mutant CALR positive cells. Therefore, the bispecific antibody of the present invention can be used to specifically prevent or treat diseases with expression of a mutant CALR.

Brief Description of Drawings

[0013]

[Figure 1] Figure 1 is a diagram showing the characteristics of mutant CALR proteins. [a] The CALR gene mutation responsible for the development in MPN patients is +1 frameshift mutation, and an amino acid sequence common to mutant proteins is found in the C-terminus of the mutant CALR proteins produced as the results of the mutation. SP: signal sequence, N: N

domain, and P: P domain. The arrow indicates where the amino acid sequences different from the wild-type (WT) sequence start. [b] An antibody which recognizes a mutant-specific sequence which is N-terminal to the cleavage site of the protein occurring within the mutant CALR protein-specific sequence is shown (black).

[Figure 2] Figure 2 shows that all clone B3, C6, and G1 antibodies recognize the full-length and cleaved mutant CALR proteins. In detail, the diagram shows the results of immunoblot analysis of proteins secreted from UT-7/TPO cells using the clone B3, C6, and G1 antibodies. Both the full-length Del 52 type and the Ins 5 type each with a FLAG-tag added to the downstream of the N-terminal signal sequence and the cleaved CALR proteins having lower molecular weights are also detected by any of the B3, C6, and G1 antibodies, as well as the anti-FLAG antibody.

[Figure 3] Figure 3 shows the results of flow cytometry analysis of [a] UT-7/TPO vec cells expressing no mutant CALR, [b] cells expressing Ins 5 mutant CALR (UT-7/TPO CALR Ins 5 cells), or [c] cells expressing Del 52 mutant CALR (UT-7/TPO CALR Del 52 cells) using an anti-cleaved mutant CALR-CD3 bispecific antibody made using a VH chain of SEQ ID NO: 2, a VL chain of SEQ ID NO: 3, a VH chain of SEQ ID NO: 8, and a VL chain of SEQ ID NO: 9.

[Figure 4] Figure 4 shows the results of flow cytometry analysis of CD3 expressing cells (T-LAK cells) using the

anti-cleaved mutant CALR-CD3 bispecific antibody described in Figure 3.

[Figure 5] Figure 5 shows the results of the evaluation, by the surface plasmon resonance analysis method, of binding strength to the anti-cleaved mutant CALR-CD3 bispecific antibody described in Figure 3 and antigen used to make the antibody.

[Figure 6] Figure 6 is a schematic diagram showing an anti-cleaved CALR-CD3 bispecific antibody.

Description of Embodiments

[0014]

The present invention is directed to a bispecific antibody which recognizes only 13 amino acids (SEQ ID NO: 1: RRMMRTKMRMRRM) in the C-terminal polypeptide (Figure 1[a]) which remains after cleavage in a sequence specific to a mutant CALR protein, used in methods for detecting and diagnosing mutant CALR proteins related to an MPN and for prevention or treatment of an MPN, and the present inventors have found for the first time that the C-terminal side of the mutant CALR protein is cleaved, resulting in a cleaved mutant CALR protein, and that most of the common region of the mutant CALR protein is lost from the mutant CALR protein. Furthermore, the present inventors have found that a bispecific antibody with a first domain which binds to the cleaved mutant CALR protein and a second domain which binds to the CD3

antigen can be effectively used to treat, prevent, detect, and diagnose tumors.

In other words, the present invention provides a bispecific antibody which specifically recognizes the cleaved mutant CALR protein and the CD3 antigen, and a pharmaceutical composition containing the bispecific antibody, especially a pharmaceutical composition for treatment or prevention of an MPN.

[0015]

As used herein, "bispecific antibody" refers to a monoclonal antibody having at least two antigen-binding sites capable of binding to different antigens. Specifically, the "bispecific antibody" means proteins having the ability to recognize two different antigens, which includes, for example, at least one first antigen-binding site (first domain) formed from the variable region of the heavy chain of the first antibody and the variable region of the light chain of the first antibody, and at least one second antigen-binding site (second domain) formed from the variable region of the heavy chain of the second antibody and the variable region of the light chain of the second antibody.

[0016]

The form of the bispecific antibody is not particularly limited and may be any form known in the art, or may be any form as long as it retains specificity for the two antigens. The form of the bispecific antibody is broadly classified into IgG-like type and low

molecular weight type. An IgG-like type is a form that retains the Fc region. Examples of the forms of IgG-like antibodies include, but are not limited to, CrossMab, DAF (two-in-one), DAF (four-in-one), DutaMab, DT-IgG, knobs-into-holes, knobs-into-holes common LC, SEEDbody, Triomab, $\kappa\lambda$ -body, DVD-Ig, IgG-scFv, and DuoBody. Since the IgG-like antibodies retain the Fc region, they are expected to, for example, exhibit effector functions such as ADCC and CDC, facilitate purification, improve stability, and extend the half-life in the blood. Meanwhile, the low molecular weight type usually has an Fv region composed of a heavy chain variable region and a light chain variable region as its basic component. Examples of the forms of the low molecular weight antibodies include, but are not limited to, Diabody (Db), BiTE, DART, TandAb, scDb, triple body, mini antibody, minibody, scFv, tandem scFv, and F(ab')₂. The low molecular weight type is expected to have improved tissue permeability and high productivity due to its size. In addition, modified antibodies are also included, such as those in which amino acid sequence is deleted, substituted, or added while retaining the ability to bind to an antigen, those in which part or all of the sugar chain is deleted or added, those in which a linker or the like is added, those fused with other proteins, and antibody-drug conjugates (ADC) in which an antibody is bound to a low molecular weight pharmaceutical via a linker.

[0017]

The bispecific antibody of the present invention has a first domain which specifically binds to a mutant calreticulin protein and a second domain which specifically binds to a CD3 antigen.

[0018]

The first domain competes with an antibody which binds to an epitope of a mutant calreticulin protein of SEQ ID NO: 1. Here, the mutant calreticulin protein preferably has an antigen-recognition site in a polypeptide chain consisting of the amino acid sequence of SEQ ID NO: 1 or a polypeptide chain consisting of an amino acid sequence of SEQ ID NO: 1 but having deletion, substitution, or addition of one or several amino acids.

[0019]

The first domain does not bind to a wild-type calreticulin protein but has a high affinity to the mutant calreticulin protein, and the first domain has a high affinity to the mutant calreticulin protein especially after cleavage.

[0020]

Examples of cleaved CALR proteins include (A) a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1 and (B) a polypeptide consisting of an amino acid sequence of SEQ ID NO: 1 but having deletion, substitution, or addition of one or several amino acids. In (B), an amino acid sequence in which 1 to 4 amino acids are deleted, substituted, or added is preferred,

and an amino acid sequence in which 1 to 3 amino acids are deleted, substituted, or added is more preferred. More specifically, an amino acid sequence in which 3 amino acids are deleted from the C-terminal side of SEQ ID NO: 1 is more preferred. The identity of the amino acid sequence of the polypeptide (B) and the amino acid sequence of SEQ ID NO: 1 is preferably 80% or more, more preferably 85% or more, and even more preferably 90% or more.

[0021]

The first domain may be any antibody as long as it has an antigen-recognition site (epitope) in the polypeptide chain (A) or (B), and may bind only to a cleaved mutant CALR protein or may be bind to both the cleaved mutant CALR protein and a full-length mutant CALR protein. The antibody is preferably an antibody specific to "mutant CALR protein" which binds to both the cleaved mutant CALR protein and the full-length mutant CALR protein.

[0022]

Such first domains are preferably heavy-chain and light-chain variable regions of anti-mutant CALR antibodies (clone B3, C6, and G1 antibodies) that the present inventors have found, as well as heavy-chain complementarity determining regions (CDR-H1, CDR-H2, and CDR-H3) and light-chain complementarity determining regions (CDR-L1, CDR-L2, and CDR-L3) or mutants thereof.

[0023]

The second domain may be a domain which specifically binds to a CD3 antigen and is preferably heavy-chain and light-chain variable regions derived from existing anti-CD3 antibodies, as well as heavy-chain complementarity determining regions (CDR-H1, CDR-H2, and CDR-H3) and light-chain complementarity determining regions (CDR-L1, CDR-L2, and CDR-L3), derived from an already-known anti-CD3 antibody, or mutants thereof. Examples of the existing anti-CD3 antibodies include OKT3.

[0024]

As preferred examples of the first and second domains, Tables 1-1 to 1-4 show amino acid sequences of heavy-chain and light-chain variable regions of anti-mutant CALR antibodies (clone B3, C6, and G1 antibodies) and mutants thereof and anti-CD3 antibodies (OKT3) and mutants thereof, as well as amino acid sequences of heavy-chain complementarity determining regions (CDR-H1, CDR-H2, and CDR-H3) and light-chain complementarity determining regions (CDR-L1, CDR-L2, and CDR-L3).

[0025]

[Table 1-1]

SEQ ID NO	Name	Amino acid sequence
2	B3 VH	QVQLQQSGAELVKPGSSVKISCKASGYTFTRNFI HWIKQQPGNGLEWIGWIFPGDGDTEYNQKFNG KATLTADKSSSTAYMQLSSLTSEDSAVYFCARGN YNYEYFDYWGQGVMTVSS
3	B3 VL	DIQMTQSPASLSASLGETVSIIECLASEDIYSYLA WYQQKPGKSPQLLIFAANRLQDGVPSRFSGSGS GTQFSLKISGMQPEDEGDYFCLQGSKFPYTFGP GTKLELN
4	C6 VH	QVQLQQSGAELVKPGSSVKISCKASGYTFTSYF VHWIKQQPGDGLEWIGWIYPGDGDTEYNHKN GKSTLTADRSSSTAYMQLSSLTSEDSAVYFCARG NYYDGREVMDAWGQGASVTVSS
5	C6 VL	DVVLQTPTLSATIGQSVSISCRSSQSLLDSDGE TYLNWLLQRPQGSPQLLIYSVSNLESGVPSRFS GSGSETDFTLKISGMEADLGVIYCMQATHGP YTFGAGTKLELK
6	G1 VH	QVQLQQSGAELVKPGSSVKISCKASGYTFTSYF VHWIKQQPGDGLEWIGWIYPGDGDTEYNQKFN GKATLTADRSSSTAYMQLSSLTSEDSAVYFCARG NYYDGREVMDAWGQGASVTVSS
7	G1 VL	DVVLQTPTLSATIGQSVSISCRSSQSLLDSDGE TYLNWLLQRPQGCPQLLIYSVSNLESGVPSRFS GSGSETDFTLKISGVEADLGVIYCMQGTHGP YTFGAGTKLELK
8	hOKT3 VH	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRY TMHWVRQAPGKGLEWIGYINPSRGYTNYNQK VKDRFTISRDNKNTAFLQMDSLRLPEDTGVYFC ARYYDDHYSLDYWGQGTPVTVSS
204	hOKT3 VH-2	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRY TMHWVRQAPGKGLEWIGYINPSRGYTNYNQK VKDRFTISRDNKNTAFLQMDSLRLPEDTGVYFC ARYYNDHYSLDYWGQGTPVTVSS
205	hOKT3 VH-3	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRY TMHWVRQAPGKGLEWIGYINPSRGYTNYNQK VKDRFTISRDNKNTAFLQMDSLRLPEDTGVYFC ARYYDDHFSLDYWGQGTPVTVSS
206	hOKT3 VH-4	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRY TMHWVRQAPGKGLEWIGYINPSMGATNYNQK VKDRFTISRDNKNTAFLQMDSLRLPEDTGVYFC ARYYDDHYSLDYWGQGTPVTVSS

[Table 1-2]

SEQ ID NO	Name	Amino acid sequence
9	hOKT3 VL	DIQMTQSPSSLSASVGDRVTITCSASSSVSYMN WYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGS GTDYFTFTISLQPEDATYYCQQWSSNPFTFGQG TKLQITR
207	hOKT3 VL-2	DIQMTQSPSSLSASVGDRVTITCSASSSVAYMN WYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGS GTDYFTFTISLQPEDATYYCQQWSSNPFTFGQG TKLQITR
208	hOKT3 VL-3	DIQMTQSPSSLSASVGDRVTITCSASSSVSYMN WYQQTPGKAPKRWIYNTSKLASGVPSRFSGSGS GTDYFTFTISLQPEDATYYCQQWSSNPFTFGQG TKLQITR
10	B3 CDR-H1	GYTFTRNFIH
11	B3 CDR-H2	WIFPGDGDTEYNQKFNG
126	B3 CDR-H2-2	WIFPGDADTEYNQKFSG
12	B3 CDR-H3	GNYNIEYFDY
13	B3 CDR-L1	LASEDIYSYLA
14	B3 CDR-L2	AANRLQD
127	B3 CDR-L2-2	AANRLQS
15	B3 CDR-L3	LQGSKFPLYT
16	C6 CDR-H1	GYTFTSYFVH
17	C6 CDR-H2	WIYPGDGDTEYNHKKFNG
18	C6 CDR-H3	GNYYDGREVMMDA
19	C6 CDR-L1	RSSQSLLDSDGETYLN

[0027]

[Table 1-3]

SEQ ID NO	Name	Amino acid sequence
20	C6 CDR-L2	SVSNLES
21	C6 CDR-L3	MQATHGPYT
22	G1 CDR-H1	GYTFTSYFVH
23	G1 CDR-H2	WIYPGDGDTEYNQKFNG
24	G1 CDR-H3	GNYYDGREVMMDA
25	G1 CDR-L1	RSSQSLLDSDGETYLN
26	G1 CDR-L2	SVSNLES
27	G1 CDR-L3	MQGTHGPYT
28	OKT3 CDR-H1	RYTMH
29	OKT3 CDR-H2	YINPSRGYTNYNQKVKD
128	OKT3 CDR-H2-2	YINPSMGATNYNQKVKD
30	OKT3 CDR-H3	YYDDHYSLDY
129	OKT3 CDR-H3-2	YYNDHYSLDY
130	OKT3 CDR-H3-3	YYDDHFSLDY
31	OKT3 CDR-L1	SASSSVSYMN
131	OKT3 CDR-L1-2	SASSSVAYMN

[0028]

[Table 1-4]

SEQ ID NO	Name	Amino acid sequence
32	OKT3 CDR-L2	DTSKLAS
132	OKT3 CDR-L2-2	NTSKLAS
33	OKT3 CDR-L3	QQWSSNPFT

[0029]

These regions can be made as recombinant antibodies by genetic engineering techniques based on sequence information of antibodies obtained by immunizing animals with a part or the full length of the cleaved mutant CALR protein or by sensitizing lymphocytes in vitro and then fusing it with myeloma, and antibodies obtained, for example, from antibody libraries such as phage display. The sequence information related to anti-CD3 antibodies can be obtained from existing information. In other words, heavy-chain and light-chain genes of the antibodies are synthesized from the obtained genetic information, inserted into a vector (e.g., plasmid), and then transfected into host cells (e.g., CHO cells, HEK cells, etc.) to culture the host cells, thereby collecting the recombinant antibodies from the culture. In the synthesis of heavy-chain and light-chain genes of the antibodies, it is preferable to optimize codons.

[0030]

Examples of preferred bispecific antibodies of the present invention include bispecific antibodies in which the CDR of the first domain has (a) an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 10, SEQ ID NO: 11 or 126, and SEQ

ID NO: 12 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 13, SEQ ID NO: 14 or 127, and SEQ ID NO: 15 (VLCDR); (b) an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 16 to 18 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 19 to 21 (VLCDR); or (c) an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 22 to 24 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 25 to 27 (VLCDR).

[0031]

Furthermore, examples thereof include bispecific antibodies in which the first domain has (d) a VH region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 2, 90, 91, 92, 93, 94, 133, 134, 135, and 136, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 3, 95, 96, 97, 98, 99, 137, 138, 139, 140, and 141; (e) a VH region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 4, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 5; or (f) a VH region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 6, and

a VL region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 7.

[0032]

A bispecific antibody is preferred in which the CDR of the second domain has an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 28, SEQ ID NO: 29 or 128, and SEQ ID NO: 30, 129 or 130 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences set forth in SEQ ID NO: 31 or 131, SEQ ID NO: 32 or 132, and SEQ ID NO: 33 (VLCDR).

Furthermore, a bispecific antibody is preferred in which the second domain has a VH region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 8, 204, 205, and 206, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 9, SEQ ID NO: 207, and 208.

[0033]

Examples of more preferred bispecific antibodies of the present invention include bispecific antibodies in which the CDR of the first domain has (a) an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 10, SEQ ID NO: 11 or 126, and SEQ ID NO: 12 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 13, SEQ ID NO: 14 or 127, and SEQ ID NO: 15 (VLCDR); (b) an amino acid sequence having 80% or more homology to

the amino acid sequences of SEQ ID NO: 16 to 18 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 19 to 21 (VLCDR); or (c) an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 22 to 24 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 25 to 27 (VLCDR), and in which the CDR of the second domain has an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 28, SEQ ID NO: 29 or 128, and SEQ ID NO: 30, SEQ ID NO: 129 or 130 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 31 or 131, SEQ ID NO: 32 or 132, and SEQ ID NO: 33 (VLCDR).

Examples of further preferred bispecific antibodies include bispecific antibodies in which the first domain has (d) a VH region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 2, 90, 91, 92, 93, 94, 133, 134, 135, and 136, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 3, 95, 96, 97, 98, 99, 137, 138, 139, 140, and 141; (e) a VH region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 4, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 5; or (f) a VH region having an amino acid sequence having 80% or more homology to the amino acid sequence of

SEQ ID NO: 6, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 7, and in which the second domain has a VH region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 8, SEQ ID NO: 204, SEQ ID NO: 205, and 206, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 9, SEQ ID NO: 207, and 208.

[0034]

As described above, the form of the bispecific antibody of the present invention may be any form as long as it retains specificity for the two antigens, and may be an IgG-like type or a low molecular weight type. Specifically, it may have an Fc region, a hetero-H chain, or the like.

[0035]

Specific examples of the bispecific antibody include antibodies selected from the group consisting of the following (I) to (XXVII).

(I) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain containing

the amino acid sequences of SEQ ID NO: 28, 29, and 30 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VH-A, VL-A, VH-B, and VL-B from the N-terminal side; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 1: SEQ ID NO: 34).

(II) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VL-A, VH-A, VH-B, and VL-B from the N-terminal side.

(III) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12

(VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VH-B, VL-B, VH-A, and VL-A from the N-terminal side; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 3: SEQ ID NO: 36).

[0036]

(IV) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VH-

B, VL-B, VL-A, and VH-A from the N-terminal side; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 4: SEQ ID NO: 37).

(V) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consist of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VH-B, VL-A, VH-A, and VL-B from the N-terminal side.

(VI) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30

(VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VL-B, VH-A, VL-A, and VH-B from the N-terminal side; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 6: SEQ ID NO: 39).

[0037]

(VII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VH-A, VL-B, VH-B, and VL-A from the N-terminal side.

(VIII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12

(VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VL-A, VH-B, VL-B, and VH-A from the N-terminal side.

(IX) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 (VL-B), wherein the domains are fused via peptide linkers in the order of VH-A, VL-A, VH-B, and VL-B from the N-terminal side (Antibody 9: SEQ ID NO: 42).

(X) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12

(VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 (VL-B), wherein the domains are fused via peptide linkers in the order of VL-A, VH-A, VH-B, and VL-B from the N-terminal side.

[0038]

(XI) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 (VL-B), wherein the domains are fused via peptide linkers in the order of VH-B, VL-B, VH-A, and VL-A from the N-terminal side (Antibody 11: SEQ ID NO: 44).

(XII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12

(VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 (VL-B), wherein the domains are fused via peptide linkers in the order of VH-B, VL-B, VL-A, and VH-A from the N-terminal side.

(XIII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is an IgG antibody which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, the single-chain Fv being fused to the heavy chain C-terminus of the IgG antibody via a peptide linker, wherein one Fc domain contains a knob and the other Fc domain contains a hole in accordance with the knob-into-hole method, resulting in hetero-association of these antibodies; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 13: SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48).

[0039]

(XIV) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is an IgG antibody which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, the single-chain Fv being fused to the heavy chain C-terminus of the IgG antibody via a peptide linker; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor. (Antibody 14: SEQ ID NO: 48 and SEQ ID NO: 49).

(XV) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is an IgG antibody which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33 and the single-chain Fv is fused to the Fc via a peptide linker

to form scFv-Fc, wherein one Fc domain contains a knob and the other Fc domain contains a hole in accordance with the knob-into-hole method, resulting in hetero-association of the IgG antibody and the scFv-Fc; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 15: SEQ ID NO: 46, SEQ ID NO: 48, and SEQ ID NO: 50).

(XVI) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, wherein the single-chain Fv which specifically binds to the CD3 antigen is fused to the heavy chain C-terminus of the Fab fragment which specifically binds to the mutant CALR protein via a peptide linker (Antibody 16: SEQ ID NO: 48 and SEQ ID NO: 51).

[0040]

(XVII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a

VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, wherein the Fc is further fused to a polypeptide in which the single-chain Fv which specifically binds to the CD3 antigen is fused to the heavy chain C-terminus of the Fab fragment which specifically binds to the mutant CALR protein via a peptide linker; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 17: SEQ ID NO: 48 and SEQ ID NO: 52).

(XVIII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, wherein a CH3 domain is further fused via a peptide linker to a polypeptide in which the single-chain Fv which specifically binds to the CD3 antigen is fused to the

heavy chain C-terminus of the Fab fragment which specifically binds to the mutant CALR protein via a peptide linker (Antibody 18: SEQ ID NO: 48 and SEQ ID NO: 53).

(XIX) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, wherein the single-chain Fv which specifically binds to the CD3 antigen is fused to the heavy chain C-terminus of the Fab fragment which specifically binds to the mutant CALR protein via a peptide linker, and the heavy chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is further fused to the C-terminus of the single-chain Fv via a peptide linker (Antibody 19: SEQ ID NO: 48 and SEQ ID NO: 54).

(XX) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds

to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, wherein the heavy chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker (Antibody 20: SEQ ID NO: 48 and SEQ ID NO: 55).

[0041]

(XXI) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is an IgG antibody which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, wherein the heavy chain N-terminus of the IgG which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker (SEQ ID NO: 48 and SEQ ID NO: 56).

(XXII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain containing the

amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, wherein a CH3 domain is further fused via peptide linkers to a polypeptide in which the heavy chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker (Antibody 22: SEQ ID NO: 48 and SEQ ID NO: 57).

(XXIII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, wherein the light chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker (Antibody 23: SEQ ID NO: 58 and SEQ ID NO: 59).

(XXIV) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is an IgG antibody which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, wherein the light chain N-terminus of the IgG which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker (Antibody 24: SEQ ID NO: 59 and SEQ ID NO: 60).

(XXV) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, wherein the bispecific antibody contains a polypeptide in which the light chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to

the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker and a polypeptide in which a CH3 domain is further fused to the heavy chain C-terminus of the Fab fragment which specifically binds to the mutant CALR protein via a peptide linker (Antibody 25: SEQ ID NO: 59 and SEQ ID NO: 61).

(XXVI) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, wherein the bispecific antibody contains a polypeptide in which the heavy chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker and a polypeptide in which the light chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker (Antibody 26: SEQ ID NO: 55 and SEQ ID NO: 59).

(XXVII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, 11, and 12 and a VL domain containing the amino acid sequences of SEQ ID NO: 13, 14, and 15, and a domain which specifically binds to a CD3 antigen is a single-chain Fv which consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, 29, and 30 and a VL domain containing the amino acid sequences of SEQ ID NO: 31, 32, and 33, wherein the bispecific antibody contains a polypeptide in which the single-chain Fv which specifically binds to the CD3 antigen is fused to the heavy chain C-terminus of the Fab fragment which specifically binds to the mutant CALR protein via a peptide linker and a polypeptide in which the light chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker (Antibody 27: SEQ ID NO: 51 and SEQ ID NO: 59).

[0042]

[Table 2]

SEQ ID NO	Amino acid sequence
3 4	QVQLQQSGAELVKPGSSVKISCKASGYTFTTRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQGMVTVSSGGG GSGGGGSGGGGSDIQMTQSPASLSASLGETVSIIECLASEDIYSYLAWYQQKPGKSPQLLIF AANRLQDGVPSRFSGSGSGTQFSLKISGMQPEDEGDYFCLQGSKFPYTFGPGTKLELNGGG GSQVQLVQSGGGVVPGRSLRLSCKASGYTFTTRYTMHWVRQAPGKGLEWIGYINPSRGYTN YNQKVKDRFTISRDNKNTAFLQMDSLRPEDTGYYFCARYYDDHYSLDYWGGGTPVTVSSG GGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTGKAPKRWI YDTSKLAGVPSRFSGSGSGTDYFTTISLQPEDIAITYYCQQWSSNPFTFGQGTKLQITRD KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF FLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
3 5	DIQMTQSPASLSASLGETVSIIECLASEDIYSYLAWYQQKPGKSPQLLIFAANRLQDGVPSR FSGSGSGTQFSLKISGMQPEDEGDYFCLQGSKFPYTFGPGTKLELNGGGGSGGGGSGGGG QVQLQQSGAELVKPGSSVKISCKASGYTFTTRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQGMVTVSSGGG GSQVQLVQSGGGVVPGRSLRLSCKASGYTFTTRYTMHWVRQAPGKGLEWIGYINPSRGYTN YNQKVKDRFTISRDNKNTAFLQMDSLRPEDTGYYFCARYYDDHYSLDYWGGGTPVTVSSG GGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTGKAPKRWI YDTSKLAGVPSRFSGSGSGTDYFTTISLQPEDIAITYYCQQWSSNPFTFGQGTKLQITRL EVLFGGPKDTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
3 6	QVQLVQSGGGVVPGRSLRLSCKASGYTFTTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN QKVKDRFTISRDNKNTAFLQMDSLRPEDTGYYFCARYYDDHYSLDYWGGGTPVTVSSGGG GSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTGKAPKRWIYD TSKLAGVPSRFSGSGSGTDYFTTISLQPEDIAITYYCQQWSSNPFTFGQGTKLQITRGGG GSQVQLQQSGAELVKPGSSVKISCKASGYTFTTRNFIHWIKQQPGNGLEWIGWIFPGDGDTE YNQKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQGMVTVSSG GGGSGGGGSGGGGSDIQMTQSPASLSASLGETVSIIECLASEDIYSYLAWYQQKPGKSPQLL IFAANRLQDGVPSRFSGSGSGTQFSLKISGMQPEDEGDYFCLQGSKFPYTFGPGTKLELND KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF FLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[0043]

[Table 3]

SEQ ID NO	Amino acid sequence
3 7	QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN QKVKDRFTISRDN SKNTAFLQMDSLRPEDTGVYFCARYYDDHYSLDYWGQGTPTVTVSSGGG GSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTPGKAPKRWIYD TSKLASGVPSRFSGSGSGTDYFTTISLQPEDIAITYYCQQWSSNPFTFGQGTKLQITRGGG GSDIQMTQSPASLSASLGETVSI ECLASEDIYSYLAWYQQKPGKSPQLLIFAANRLQDGV SRFSGSGSGTQFSLKISGMQPEDEGDYFCLQGSKFYPTFGPGTKLELNGGGSGGGSGGG GSQVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTE YNQKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGQGVMTVSSD KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF FLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK
3 8	QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN QKVKDRFTISRDN SKNTAFLQMDSLRPEDTGVYFCARYYDDHYSLDYWGQGTPTVTVSSGGG GSDIQMTQSPASLSASLGETVSI ECLASEDIYSYLAWYQQKPGKSPQLLIFAANRLQDGV SRFSGSGSGTQFSLKISGMQPEDEGDYFCLQGSKFYPTFGPGTKLELNGGGSGGGSGGG GSGGGGSGVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPG DGDTEYNQKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGQGVMT TVSSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSK LASGVPSRFSGSGSGTDYFTTISLQPEDIAITYYCQQWSSNPFTFGQGTKLQITRLEVLFP GPDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK
3 9	DIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPSRF SGSGSGTDYFTTISLQPEDIAITYYCQQWSSNPFTFGQGTKLQITRGGGGSGVQLQQSGAE LVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYNQKFNGKATLT ADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGQGVMTVSSGGGGSGGGSGGG GSGGGGSDIQMTQSPASLSASLGETVSI ECLASEDIYSYLAWYQQKPGKSPQLLIFAANRL QDGVPSRFSGSGSGTQFSLKISGMQPEDEGDYFCLQGSKFYPTFGPGTKLELNGGGSGVQ LVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQK KDRFTISRDN SKNTAFLQMDSLRPEDTGVYFCARYYDDHYSLDYWGQGTPTVTVSSDKTHTC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSK LTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK

[0044]

[Table 4]

SEQ ID NO	Amino acid sequence
4 0	QVQLQQSGAELVKPGSSVKISCKASGYTFRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGQGVMTVSSGGG GSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTGKAPKRWIYDTSKLASGVPS RFSGSGSGTDYFTFTISSLPEDIATYYCQQWSSNPFTFGQGTKLQITRGGGSGGGSGGG GSGGGGSQVQLVQSGGGVVPGRSLRLSCKASGYTFRYTMHWVRQAPGKGLEWIGYINPS RGYTNYNQKVKDRFTISRDNSENKTAFLQMDSLRPEDTGYYFCARYYDDHYSLDYWGQGT TVSSGGGGSDIQMTQSPASLSASLGETVSI ECLASEDIYSYLAWYQQKPGKSPQLLIFAAN RLQDGVPSRFSGSGSGTDYFTFTISSLPEDIATYYCQQWSSNPFTFGQGTKLQITRGGG PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLIHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
4 1	DIQMTQSPASLSASLGETVSI ECLASEDIYSYLAWYQQKPGKSPQLLIFAANRLQDGVPSR FSGSGSGTDYFTFTISSLPEDIATYYCQQWSSNPFTFGQGTKLQITRGGGSGGGSGGG VVQPGRLSLRLSCKASGYTFRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKVKDRFTIS RDNSENKTAFLQMDSLRPEDTGYYFCARYYDDHYSLDYWGQGTPTVTVSSGGGGSGGGSGGG GSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTGKAPKRWIYDTSKLA SGVPSRFSGSGSGTDYFTFTISSLPEDIATYYCQQWSSNPFTFGQGTKLQITRGGGSGVQ LQQSGAELVKPGSSVKISCKASGYTFRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYNQKF NGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGQGVMTVSSLEVL GPDKTHTCPTCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLIHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
4 2	QVQLQQSGAELVKPGSSVKISCKASGYTFRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGQGVMTVSSGGG GSGGGGSGGGGSDIQMTQSPASLSASLGETVSI ECLASEDIYSYLAWYQQKPGKSPQLLIF AANRLQDGVPSRFSGSGSGTDYFTFTISSLPEDIATYYCQQWSSNPFTFGQGTKLQITRH GSQVQLVQSGGGVVPGRSLRLSCKASGYTFRYTMHWVRQAPGKGLEWIGYINPSRGYTN YNQKVKDRFTISRDNSENKTAFLQMDSLRPEDTGYYFCARYYDDHYSLDYWGQGTPTVTVSSG GGGSGGGSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTGKAPKRWI YDTSKLASGVPSRFSGSGSGTDYFTFTISSLPEDIATYYCQQWSSNPFTFGQGTKLQITRH HHHHH
4 3	DIQMTQSPASLSASLGETVSI ECLASEDIYSYLAWYQQKPGKSPQLLIFAANRLQDGVPSR FSGSGSGTDYFTFTISSLPEDIATYYCQQWSSNPFTFGQGTKLQITRGGGSGGGSGGGG QVQLQQSGAELVKPGSSVKISCKASGYTFRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGQGVMTVSSGGG GSQVQLVQSGGGVVPGRSLRLSCKASGYTFRYTMHWVRQAPGKGLEWIGYINPSRGYTN YNQKVKDRFTISRDNSENKTAFLQMDSLRPEDTGYYFCARYYDDHYSLDYWGQGTPTVTVSSG GGGSGGGSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTGKAPKRWI YDTSKLASGVPSRFSGSGSGTDYFTFTISSLPEDIATYYCQQWSSNPFTFGQGTKLQITRH HHHHH

[Table 5]

SEQ ID NO	Amino acid sequence
4 4	QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN QKVKDRFTISRDN SKNTAFLQMDSLRPEDTGVYFCARYYDDHYSLDYWGGQTPVTVSSGGG GSGGGGSGGGGSDIQMTQSPSSLASVGDRTITCSASSSVSYMNWYQQTGKAPKRWIYD TSKLASGVPSRFSGSGSGTDYFTFTISSLPEDIAITYYCQQWSSNPFTFGQGTKLQITRGGG GSQVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTE YNQKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQGMVTVSSG GGGSGGGGSGGGGSDIQMTQSPASLSASLGETVSI ECLASEDIYSYLAWYQQKPKSPQLL IFAANRLQDGVPSRFSGSGSGTDYFTFTISSLPEDIAITYYCQQWSSNPFTFGQGTKLQITR HHHHH
4 5	QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN QKVKDRFTISRDN SKNTAFLQMDSLRPEDTGVYFCARYYDDHYSLDYWGGQTPVTVSSGGG GSGGGGSGGGGSDIQMTQSPSSLASVGDRTITCSASSSVSYMNWYQQTGKAPKRWIYD TSKLASGVPSRFSGSGSGTDYFTFTISSLPEDIAITYYCQQWSSNPFTFGQGTKLQITRGGG GSDIQMTQSPASLSASLGETVSI ECLASEDIYSYLAWYQQKPKSPQLLIFAANRLQDGVPS RFSGSGSGTDYFTFTISSLPEDIAITYYCQQWSSNPFTFGQGTKLELNGGGGSGGGGSGGGG GSQVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTE YNQKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQGMVTVSSH HHHHH
4 6	QVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQGMVTVSSAST KGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKHTHTCPPCPAPEAAGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SYMHEALHNHYTQKSLSLSPGK
4 7	QVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQGMVTVSSAST KGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKHTHTCPPCPAPEAAGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSREEMTKNQVS LSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SYMHEALHNHYTQKSLSLSPGKGGGGSQVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTM HWVRQAPGKGLEWIGYINPSRGYTNYNQKVKDRFTISRDN SKNTAFLQMDSLRPEDTGVYF CARYYDDHYSLDYWGGQTPVTVSSGGGSGGGGSGGGGSDIQMTQSPSSLASVGDRTIT CSASSSVSYMNWYQQTGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYFTFTISSLPEDIA ITYYCQQWSSNPFTFGQGTKLQITR
4 8	DIQMTQSPASLSASLGETVSI ECLASEDIYSYLAWYQQKPKSPQLLIFAANRLQDGVPSR FSGSGSGTDYFTFTISSLPEDIAITYYCQQWSSNPFTFGQGTKLELNRVAAPSVFIFPPSD EQLKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[Table 6]

4 9	QVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQVMVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SVMHEALHNHYTQKSLSLSPGKGGGGSQVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTM HWVRQAPGKLEWIGYINPSRGYTNYNQKVKDRFTISRDNKNTAFLQMDSLRPEDTGVYF CARYYDDHYSLDYWGGQGPVTVSSGGGGSGGGSGGGSDIQMTQSPSSLSASVGDRTIT CSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYFTFTISSLQPEDIA TYYCQQWSSNPFTFGQGTKLQITR
5 0	QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGKLEWIGYINPSRGYTNYN QKVKDRFTISRDNKNTAFLQMDSLRPEDTGVYFCARYYDDHYSLDYWGGQGPVTVSSGGG SGGGGGSGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTPGKAPKRWIYD TSKLASGVPSRFSGSGSGTDYFTFTISSLQPEDIA TYYCQQWSSNPFTFGQGTKLQITREP KSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVCTLPSSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
5 1	QVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQVMVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCGGGGSQVQLVQSGGGVVPGRS LRLSCKASGYTFTRYTMHWVRQAPGKLEWIGYINPSRGYTNYNQKVKDRFTISRDNKNT AFLQMDSLRPEDTGVYFCARYYDDHYSLDYWGGQGPVTVSSGGGGSGGGSGGGSDIQMT QSPSSLSASVGDRTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGS GTDYFTFTISSLQPEDIA TYYCQQWSSNPFTFGQGTKLQITRHHHHHH
5 2	QVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQVMVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCGGGGSQVQLVQSGGGVVPGRS LRLSCKASGYTFTRYTMHWVRQAPGKLEWIGYINPSRGYTNYNQKVKDRFTISRDNKNT AFLQMDSLRPEDTGVYFCARYYDDHYSLDYWGGQGPVTVSSGGGGSGGGSGGGSDIQMT QSPSSLSASVGDRTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGS GTDYFTFTISSLQPEDIA TYYCQQWSSNPFTFGQGTKLQITRDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SCSVMHEALHNHYTQKSLSLSPGK

[0047]

[Table 7]

SEQ ID NO	Amino acid sequence
5 3	QVQLQQSGAELVKPGSSVKISCKASGYTFRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQVMVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCGGGGSQVQLVQSGGGVVPGRS LRLSCKASGYTFRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQVKDRFTISRDN SKNT AFLQMDSLRPEDTGYYFCARYYDDHYSLDYWGGQTPVTVSSGGGSGGGGSGGGGSDIQT QSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGS GTDYFTFTISSLPEDIAATYYCQQWSSNPFTFGQGTKLQITRAAAGGGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
5 4	QVQLQQSGAELVKPGSSVKISCKASGYTFRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQVMVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCGGGGSQVQLVQSGGGVVPGRS LRLSCKASGYTFRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQVKDRFTISRDN SKNT AFLQMDSLRPEDTGYYFCARYYDDHYSLDYWGGQTPVTVSSGGGSGGGGSGGGGSDIQT QSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGS GTDYFTFTISSLPEDIAATYYCQQWSSNPFTFGQGTKLQITRGGGGSQVQLQQSGAELVKPG SSVKISCKASGYTFRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYNQKFNGKATLTADKSS STAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQVMVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKRVEPKSCHHHHHH
5 5	QVQLVQSGGGVVPGRSLRLSCKASGYTFRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN QKVKDRFTISRDN SKNTAFLQMDSLRPEDTGYYFCARYYDDHYSLDYWGGQTPVTVSSGGG GSGGGGSGGGGSDIQTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYD TSKLASGVPSRFSGSGSGTDYFTFTISSLPEDIAATYYCQQWSSNPFTFGQGTKLQITRGGG GSQVQLQQSGAELVKPGSSVKISCKASGYTFRNFIHWIKQQPGNGLEWIGWIFPGDGDTE YNQKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQVMVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCHHHHHH
5 6	QVQLVQSGGGVVPGRSLRLSCKASGYTFRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN QKVKDRFTISRDN SKNTAFLQMDSLRPEDTGYYFCARYYDDHYSLDYWGGQTPVTVSSGGG GSGGGGSGGGGSDIQTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYD TSKLASGVPSRFSGSGSGTDYFTFTISSLPEDIAATYYCQQWSSNPFTFGQGTKLQITRGGG GSQVQLQQSGAELVKPGSSVKISCKASGYTFRNFIHWIKQQPGNGLEWIGWIFPGDGDTE YNQKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWGGQVMVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKHTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FCSCVMHEALHNHYTQKSLSLSPGK

[Table 8]

SEQ ID NO	Amino acid sequence
5 7	QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN QKVKDRFTISRDN SKNTAFLQMDSLRPEDTGVYFCARYYDDHYSLDYWGQGTPTVTVSSGGG GSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTPGKAPKRWIYD TSKLASGVPSRFSGSGSGTDYFTTISLQPEDIAITYYCQQWSSNPFTFGQGTKLQITRGGG GSQVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTE YNQKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWQGQVMVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCAAAGGGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMEALHNHYTQKSLSLSPGK
5 8	QVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWQGQVMVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCHHHHHH
5 9	QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYN QKVKDRFTISRDN SKNTAFLQMDSLRPEDTGVYFCARYYDDHYSLDYWGQGTPTVTVSSGGG GSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTPGKAPKRWIYD TSKLASGVPSRFSGSGSGTDYFTTISLQPEDIAITYYCQQWSSNPFTFGQGTKLQITRGGG GSDIQMTQSPASLSASLGETVSI ECLASEDIYSYLAWYQQKPGKSPQLLIFAANRLQDQVP SRFSGSGSGTQFSLKISGMQPEDEGDYFCLQGSKFYTFPGGTKLELNRTVAAPSVFIFPP SDEQLKSGTASVVCILNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSDSTYSLSSTLT SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
6 0	QVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWQGQVMVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMEALHNHYTQKSLSLSPGK
6 1	QVQLQQSGAELVKPGSSVKISCKASGYTFTRNFIHWIKQQPGNGLEWIGWIFPGDGDTEYN QKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGNYNYEYFDYWQGQVMVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCAAAGGGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMEALHNHYTQKSLSLSPGK

[0049]

The bispecific antibody is preferably an antibody further selected from the group consisting of the following (XXVIII) to (LIV).

(XXVIII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain of SEQ ID NO: 2 (VH-A) and a VL domain of SEQ ID NO: 3 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 (VH-B) and a VL domain of SEQ ID NO: 9 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VH-A, VL-A, VH-B, and VL-B from the N-terminal side; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 1: SEQ ID NO: 34).

(XXIX) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain of SEQ ID NO: 2 (VH-A) and a VL domain of SEQ ID NO: 3 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 (VH-B) and a VL domain of SEQ ID NO: 9 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VL-A, VH-A, VH-B, and VL-B from the N-terminal side.

(XXX) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain of SEQ ID NO: 2 (VH-A) and a VL domain of SEQ ID NO: 3 (VL-A), and

a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 (VH-B) and a VL domain of SEQ ID NO: 9 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VH-B, VL-B, VH-A, and VL-A from the N-terminal side; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 3: SEQ ID NO: 36).

[0050]

(XXXI) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain of SEQ ID NO: 2 (VH-A) and a VL domain of SEQ ID NO: 3 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 (VH-B) and a VL domain of SEQ ID NO: 9 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VH-B, VL-B, VL-A, and VH-A from the N-terminal side; in some cases, a bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce binding to an Fc receptor (Antibody 4: SEQ ID NO: 37).

(XXXII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain of SEQ ID

NO: 2 (VH-A) and a VL domain of SEQ ID NO: 3 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 (VH-B) and a VL domain of SEQ ID NO: 9 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VH-B, VL-A, VH-A, and VL-B from the N-terminal side.

[0051]

(XXXIII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain of SEQ ID NO: 2 (VH-A) and a VL domain of SEQ ID NO: 3 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 (VH-B) and a VL domain of SEQ ID NO: 9 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VL-B, VH-A, VL-A, and VH-B from the N-terminal side; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 6: SEQ ID NO: 39).

(XXXIV) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain of SEQ ID NO: 2 (VH-A) and a VL domain of SEQ ID NO: 3 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID

NO: 8 (VH-B) and a VL domain of SEQ ID NO: 9 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VH-A, VL-B, VH-B, and VL-A from the N-terminal side.

(XXXV) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain of SEQ ID NO: 2 (VH-A) and a VL domain of SEQ ID NO: 3 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 (VH-B) and a VL domain of SEQ ID NO: 9 (VL-B), wherein the Fc is further fused to a polypeptide in which the domains are fused via peptide linkers in the order of VL-A, VH-B, VL-B, and VH-A from the N-terminal side.

(XXXVI) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain of SEQ ID NO: 2 (VH-A) and a VL domain of SEQ ID NO: 3 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 (VH-B) and a VL domain of SEQ ID NO: 9 (VL-B), wherein the domains are fused via peptide linkers in the order of VH-A, VL-A, VH-B, and VL-B from the N-terminal side (Antibody 9: SEQ ID NO: 42).

(XXXVII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a

single-chain Fv which consists of a VH domain of SEQ ID NO: 2 (VH-A) and a VL domain of SEQ ID NO: 3 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 (VH-B) and a VL domain of SEQ ID NO: 9 (VL-B), wherein the domains are fused via peptide linkers in the order of VL-A, VH-A, VH-B, and VL-B from the N-terminal side.

[0052]

(XXXVIII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain of SEQ ID NO: 2 (VH-A) and a VL domain of SEQ ID NO: 3 (VL-A), and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 (VH-B) and a VL domain of SEQ ID NO: 9 (VL-B), wherein the domains are fused via peptide linkers in the order of VH-B, VL-B, VH-A, and VL-A from the N-terminal side (Antibody 11: SEQ ID NO: 44).

(XXXIX) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a single-chain Fv which consists of a VH domain of SEQ ID NO: 2 (VH-A) and a VL domain of SEQ ID NO: 3 (VL-A), and a domain which specifically binds to the CD3 antigen is a VH domain of SEQ ID NO: 8 (VH-B) and a VL domain of SEQ ID NO: 9 (VL-B), wherein the domains are fused via peptide linkers in the order of VH-B, VL-B, VL-A, and VH-A from the N-terminal side.

(XL) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is an IgG antibody which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein the single-chain Fv is fused to the heavy chain C-terminus of the IgG antibody via a peptide linker, wherein one Fc domain contains a knob and the other Fc domain contains a hole in accordance with the knob-into-hole method, resulting in hetero-association of these antibodies; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 13: SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48).

[0053]

(XLI) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is an IgG antibody which consists of a VH domain of SEQ ID NO:2 and a VL domain of SEQ ID NO:3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of VH domain of SEQ ID NO:8 and a VL domain of SEQ ID NO: 9, wherein the single-chain Fv is fused to the heavy chain C-terminus of the IgG antibody via a peptide linker; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding

to an Fc receptor (Antibody 14: SEQ ID NO: 48 and SEQ ID NO: 49).

(XLII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is an IgG antibody which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein the single-chain Fv is fused to the Fc via a peptide linker to form scFV-Fc, wherein one Fc domain contains a knob and the other Fc domain contains a hole in accordance with the knob-into-hole method, resulting in hetero-association of the IgG antibody and the scFv-Fc; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 15: SEQ ID NO: 46, SEQ ID NO: 48, and SEQ ID NO: 50).

(XLIII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein the single-chain Fv which specifically binds to the CD3 antigen is fused to the heavy chain C-terminus of the Fab fragment which specifically binds to the mutant CALR protein via a

peptide linker (Antibody 16: SEQ ID NO: 48 and SEQ ID NO: 51).

(XLIV) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein the Fc is further fused to a polypeptide in which the single-chain Fv which specifically binds to the CD3 antigen is fused to the heavy chain C-terminus of the Fab fragment which specifically binds to the mutant CALR protein via a peptide linker; in some cases, the bispecific antibody whose Fc domain is an Fc domain containing one or more amino acid substitutions which reduce the binding to an Fc receptor (Antibody 17: SEQ ID NO: 48 and SEQ ID NO: 52).

(XLV) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein a CH3 domain is fused further via a peptide linker to a polypeptide in which the single-chain Fv which specifically binds to the CD3 antigen is fused to the heavy chain C-terminus of the Fab

fragment which specifically binds to the mutant CALR protein via a peptide linker (Antibody 18: SEQ ID NO: 48 and SEQ ID NO: 53).

(XLVI) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein the single-chain Fv which specifically binds to the CD3 antigen is fused to the heavy chain C-terminus of the Fab fragment which specifically binds to the mutant CALR protein via a peptide linker, and the heavy chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is further fused to the C-terminus of the single-chain Fv via a peptide linker (Antibody 19: SEQ ID NO: 48 and SEQ ID NO: 54).

(XLVII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein the heavy chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3

antigen via a peptide linker (Antibody 20: SEQ ID NO: 48 and SEQ ID NO: 55).

[0054]

(XLVIII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is an IgG antibody which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein the heavy chain N-terminus of the IgG which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker (SEQ ID NO: 48, SEQ ID NO: 56).

(XLIX) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein a CH3 domain is fused further via a peptide linker to a polypeptide in which the heavy chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker (Antibody 22: SEQ ID NO: 48 and SEQ ID NO: 57).

(L) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein the light chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker (Antibody 23: SEQ ID NO: 58 and SEQ ID NO: 59).

(LI) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is an IgG antibody which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein the light chain N-terminus of the IgG which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker (Antibody 24: SEQ ID NO: 59 and SEQ ID NO: 60).

[0055]

(LII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain of SEQ ID NO: 2

and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein the bispecific antibody contains a polypeptide in which the light chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker and a polypeptide in which a CH3 domain is further fused to the heavy chain C-terminus of the Fab fragment which specifically binds to the mutant CALR protein via a peptide linker (Antibody 25: SEQ ID NO: 59 and SEQ ID NO: 61).

(LIIII) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein the bispecific antibody contains a polypeptide in which the heavy chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker and a polypeptide in which the light chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically

binds to the CD3 antigen via a peptide linker (Antibody 26: SEQ ID NO: 55 and SEQ ID NO: 59).

(LIV) A bispecific antibody, wherein a domain which specifically binds to the mutant CALR protein is a Fab fragment which consists of a VH domain of SEQ ID NO: 2 and a VL domain of SEQ ID NO: 3, and a domain which specifically binds to the CD3 antigen is a single-chain Fv which consists of a VH domain of SEQ ID NO: 8 and a VL domain of SEQ ID NO: 9, wherein the bispecific antibody contains a polypeptide in which the single-chain Fv which specifically binds to the CD3 antigen is fused to the heavy chain C-terminus of the Fab fragment which specifically binds to the mutant CALR protein via a peptide linker and a polypeptide in which the light chain N-terminus of the Fab fragment which specifically binds to the mutant CALR protein is fused to the C-terminus of the single-chain Fv which specifically binds to the CD3 antigen via a peptide linker (Antibody 27: SEQ ID NO: 51 and SEQ ID NO: 59).

[0056]

[Table 9]

Amino acid sequence	Nucleotide sequence
SEQ ID NO 3 4	SEQ ID NO 6 2
SEQ ID NO 3 5	SEQ ID NO 6 3
SEQ ID NO 3 6	SEQ ID NO 6 4
SEQ ID NO 3 7	SEQ ID NO 6 5
SEQ ID NO 3 8	SEQ ID NO 6 6
SEQ ID NO 3 9	SEQ ID NO 6 7
SEQ ID NO 4 0	SEQ ID NO 6 8
SEQ ID NO 4 1	SEQ ID NO 6 9
SEQ ID NO 4 2	SEQ ID NO 7 0
SEQ ID NO 4 3	SEQ ID NO 7 1
SEQ ID NO 4 4	SEQ ID NO 7 2
SEQ ID NO 4 5	SEQ ID NO 7 3
SEQ ID NO 4 6	SEQ ID NO 7 4
SEQ ID NO 4 7	SEQ ID NO 7 5
SEQ ID NO 4 8	SEQ ID NO 7 6
SEQ ID NO 4 9	SEQ ID NO 7 7
SEQ ID NO 5 0	SEQ ID NO 7 8
SEQ ID NO 5 1	SEQ ID NO 7 9
SEQ ID NO 5 2	SEQ ID NO 8 0
SEQ ID NO 5 3	SEQ ID NO 8 1
SEQ ID NO 5 4	SEQ ID NO 8 2
SEQ ID NO 5 5	SEQ ID NO 8 3
SEQ ID NO 5 6	SEQ ID NO 8 4
SEQ ID NO 5 7	SEQ ID NO 8 5
SEQ ID NO 5 8	SEQ ID NO 8 6
SEQ ID NO 5 9	SEQ ID NO 8 7
SEQ ID NO 6 0	SEQ ID NO 8 8
SEQ ID NO 6 1	SEQ ID NO 8 9

[0057]

Preferred specific examples of the bispecific antibodies of the present invention include those having each of the (I) to (XXVII) sequences or each of the (XXVIII) to (LIV) sequences, and amino acid sequences in the regions other than these regions are not particularly restricted. The antibody of the present invention may be a humanized antibody and a non-human mammalian antibody. More specifically, the antibody may be a chimeric antibody composed of the variable regions of the heavy

chain and light chain of an antibody of a non-human mammal, for example, a rat, and the constant regions of the heavy chain and light chain of a human antibody, and such an antibody can be obtained by ligating a DNA encoding the variable region of a rat antibody with a DNA encoding the constant region of a human antibody, incorporating this into an expression vector and transfecting the vector into a host for production. As an example of the humanized antibody, VH 1 to 8 and VL 1 to 7 are shown below (Tables 10-1 to 10-2 and Table 11).

[0058]

[Table 10-1]

SEQ ID NO	Name	Amino acid sequence
90	VH1	QVQLVQSGAEVKKPGASVKVSCKASGYTFTRNFIHWVRQAP GQGLEWMGWIFPGDGDTEYNQKFNGRVTMTRDTSISTAYME LSRLRSDDTAVYYCARGNYYEYFDYWGQGTMTVTVSS
91	VH2	QVQLQESGAEVKKPGASVKVSCKASGYTFTRNFIHWVRQAP GQRLEWMGWIFPGDGDTEYNQKFNGRVTITRDTSASTAYME LSSLRSED TAVYYCARGNYYEYFDYWGQGTMTVTVSS
92	VH3	QVQLVQSGAEVKKPGASVKISCKASGYTFTRNFIHWVRQAPG QSLEWVGWIFPGDGDTEYNQKFNGRITITRDTSANTAYMELS SLKSED TAVYYCARGNYYEYFDYWGQGTMTVTVSS
93	VH4	QMQLVQSGAEVKKGTSSVKVSCKASGYTFTRNFIHWVRQAP GQALEWMGWIFPGDGDTEYNQKFNGRVTITRDRSMSTAYME LSSLRSED TAVYYCARGNYYEYFDYWGQGTMTVTVSS
133	VH4-2	QMQLVQSGAEVKKGTSSVKVSCKASGYTFTRNFIHWVRQAP GQALEWMGWIFPGDADTEYNQKFSGRVTITRDRSMSTAYME LSSLRSED TAVYYCARGNYYEYFDYWGQGTMTVTVSS
94	VH5	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTRNFIHWVRQAP GQGLEWMGWIFPGDGDTEYNQKFNGRVTITADKSTSTAYMQ LSSLTSED TAVYFCARGNYYEYFDYWGQGVMTVTVSS
134	VH6	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTRNFIHWIRQAPG QGLEWIGWIFPGDADTEYNQKFSGRATITADKSTSTAYMELSS LRSED TAVYYCARGNYYEYFDYWGQGTMTVTVSS
135	VH7	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTRNFIHWIRQAPG QGLEWIGWIFPGDADTEYNQKFSGRVTITADKSTSTAYMELSS LRSED TAVYYCARGNYYEYFDYWGQGTMTVTVSS
136	VH8	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTRNFIHWIRQAPG QGLEWIGWIFPGDGDTEYNQKFNGRATITADKSTSTAYMELSS LRSED TAVYYCARGNYYEYFDYWGQGTMTVTVSS
95	VL1	DIQMTQSPSFVSASVGDRVITITCLASEDIYSYLAWYQQKPGKA PKLLIFAANRLQDGVPSRFSGGSGTGFTLTISLQPEDFATYF CLQGSKFPYTFGQGTKLEIN
137	VL1-2	DIQMTQSPSFVSASVGDRVITITCLASEDIYSYLAWYQQKPGKA PKLLIFAANRLQSGVPSRFSGGSGTGFTLTISLQPEDFATYFC LQGSKFPYTFGQGTKLEIN
138	VL1-3	DIQMTQSPSFVSASVGDRVITITCLASEDIYSYLAWYQQKPGKA PKLLIFAANRLQSGVPSRFSGGSGTGFTLTISLQPEDFATYFC LQGSKFPYTFGQGTKLEIK
139	VL1-4	DIQMTQSPSFVSASVGDRVITITCLASEDIYSYLAWYQQKPGKA PKLLIFAANRLQDGVPSRFSGGSGTGFTLTISLQPEDFATYF CLQGSKFPYTFGQGTKLEIK
96	VL2	DIQLTQSPAFLSASLGDRVITITCLASEDIYSYLAWYQQKPGKA PKLLISAANRLQDGVPSRFSGSGSGTEFTLTISLQPEDFATYSC LQGSKFPYTFGQGTKLEIK
97	VL3	DIQLTQSPSSLSASVGDRVITITCLASEDIYSYLAWYQQKPGKG PKLLIYAANRLQDGVPSRFSGSGSGTDFSLTISLQPEDLATYY CLQGSKFPYTFGGGTKVEIK
98	VL4	DIQMTQSPSSLSASVGDVTITACLAASEDIYSYLAWYQQKPGK APKLLIYAANRLQDGVPSRFSGSGSFTDFLTINSLQPD SATY YCLQGSKFPYTFGGGTKVDIK

[Table 10-2]

SEQ ID NO	Name	Amino acid sequence
99	VL5	DIQMTQSPSSVSASVGDSVTIECLASEDIYSYLAWEYQQKPGKSPKLLIFAAANRLQDGVPSRFSGSGSGTQFTLKISLQPEDFAEYFCLQASKFPYTFGPGTKLELN
140	VL6	DIQMTQSPSSLSASVGDRVTITCLASEDIYSYLAWEYQQKPGKSPKLLIFAAANRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQGSKFPYTFGPGTKVDIK
141	VL7	DIQMTQSPSSLSASVGDRVTITCLASEDIYSYLAWEYQQKPGKSPKLLIFAAANRLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQGSKFPYTFGPGTKVDIK

[0060]

[Table 11]

Amino acid sequence	Nucleotide sequence
SEQ ID NO 90	SEQ ID NO 100
SEQ ID NO 91	SEQ ID NO 101
SEQ ID NO 92	SEQ ID NO 102
SEQ ID NO 93	SEQ ID NO 103
SEQ ID NO 94	SEQ ID NO 104
SEQ ID NO 95	SEQ ID NO 105
SEQ ID NO 96	SEQ ID NO 106
SEQ ID NO 97	SEQ ID NO 107
SEQ ID NO 98	SEQ ID NO 108
SEQ ID NO 99	SEQ ID NO 109
SEQ ID NO 133	SEQ ID NO 142
SEQ ID NO 134	SEQ ID NO 143
SEQ ID NO 135	SEQ ID NO 144
SEQ ID NO 136	SEQ ID NO 145
SEQ ID NO 137	SEQ ID NO 146
SEQ ID NO 138	SEQ ID NO 147
SEQ ID NO 139	SEQ ID NO 148
SEQ ID NO 140	SEQ ID NO 149
SEQ ID NO 141	SEQ ID NO 150

[0061]

Specific examples of bispecific antibodies using humanized antibodies include, but are not limited to, the following sequences (Table 12).

[0062]

[Table 12]

	SEQ ID NO 116	SEQ ID NO 117	SEQ ID NO 118	SEQ ID NO 119	SEQ ID NO 120
SEQ ID NO 121	Antibody 31	Antibody 32	Antibody 33	Antibody 34	Antibody 35
SEQ ID NO 122	Antibody 36	Antibody 37	Antibody 38	Antibody 39	Antibody 40
SEQ ID NO 123	Antibody 41	Antibody 42	Antibody 43	Antibody 44	Antibody 45
SEQ ID NO 124	Antibody 46	Antibody 47	Antibody 48	Antibody 49	Antibody 50
SEQ ID NO 125	Antibody 51	Antibody 52	Antibody 53	Antibody 54	Antibody 55

[0063]

Furthermore, specific examples of the humanized bispecific antibodies include the following antibodies (Tables 13-1 to 13-2).

[A] A bispecific antibody having Structure 1 in Figure 6, wherein a domain which specifically binds to the mutant CALR protein consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, SEQ ID NO: 11 or 126, and SEQ ID NO: 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, SEQ ID NO: 14 or 127, and SEQ ID NO: 15 (VL-A), and a domain which specifically binds to the CD3 antigen consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, SEQ ID NO: 29 or 128, and SEQ ID NO: 30, 129 or 130 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31 or 131, SEQ ID NO: 32 or 132, and SEQ ID NO: 33 (VL-B) (Antibodies 56 to 99).

[B] A bispecific antibody having Structure 2 in Figure 6, wherein a domain which specifically binds to

the mutant CALR protein consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, SEQ ID NO: 11 or 126, and SEQ ID NO: 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, SEQ ID NO: 14 or 127, and SEQ ID NO: 15 (VL-A), and a domain which specifically binds to the CD3 antigen consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, SEQ ID NO: 29 or 128, and SEQ ID NO: 30, 129 or 130 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31 or 131, SEQ ID NO: 32 or 132, and SEQ ID NO: 33 (VL-B) (Antibodies 100 to 104).

[C] A bispecific antibody having Structure 3 in Figure 6, wherein a domain which specifically binds to the mutant CALR protein consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, SEQ ID NO: 11 or 126, and SEQ ID NO: 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, SEQ ID NO: 14 or 127, and SEQ ID NO: 15 (VL-A), and a domain which specifically binds to the CD3 antigen consisting of a VH domain containing the amino acid sequences of SEQ ID NO: 28, SEQ ID NO: 29 or 128, and SEQ ID NO: 30, 129 or 130 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31 or 131, SEQ ID NO: 32 or 132, and SEQ ID NO: 33 (VL-B) (Antibody 105).

[D] A bispecific antibody having Structure 4 in Figure 6, wherein a domain which specifically binds to the mutant CALR protein consists of a VH domain

containing the amino acid sequences set forth in SEQ ID NO: 10, SEQ ID NO: 11 or 126, and SEQ ID NO: 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, SEQ ID NO: 14 or 127, and SEQ ID NO: 15 (VL-A), and a domain which specifically binds to the CD3 antigen consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, SEQ ID NO: 29 or 128, and SEQ ID NO: 30, 129 or 130 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31 or 131, SEQ ID NO: 32 or 132, and SEQ ID NO: 33 (VL-B) (Antibody 106).

[E] A bispecific antibody having Structure 5 in Figure 6, wherein a domain which specifically binds to the mutant CALR protein consist of a VH domain containing the amino acid sequences of SEQ ID NO: 10, SEQ ID NO: 11 or 126, and SEQ ID NO: 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, SEQ ID NO: 14 or 127, and SEQ ID NO: 15 (VL-A), and a domain which specifically binds to the CD3 antigen consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, SEQ ID NO: 29 or 128, and SEQ ID NO: 30, 129 or 130 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31 or 131, SEQ ID NO: 32 or 132, and SEQ ID NO: 33 (VL-B) (Antibodies 107 and 109 to 111).

[F] A bispecific antibody having Structure 6 in Figure 6, wherein a domain which specifically binds to the mutant CALR protein consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, SEQ ID NO: 11 or 126, and SEQ ID NO: 12 (VH-A) and a VL

domain containing the amino acid sequences of SEQ ID NO: 13, SEQ ID NO: 14 or 127, and SEQ ID NO: 15 (VL-A), and a domain which specifically binds to the CD3 antigen consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, SEQ ID NO: 29 or 128, and SEQ ID NO: 30, 129 or 130 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31 or 131, SEQ ID NO: 32 or 132, and SEQ ID NO: 33 (VL-B) (Antibodies 112 and 113).

[G] A bispecific antibody having Structure 7 in Figure 6, wherein a domain which specifically binds to the mutant CALR protein consists of a VH domain containing the amino acid sequences of SEQ ID NO: 10, SEQ ID NO: 11 or 126, and SEQ ID NO: 12 (VH-A) and a VL domain containing the amino acid sequences of SEQ ID NO: 13, SEQ ID NO: 14 or 127, and SEQ ID NO: 15 (VL-A), and a domain which specifically binds to the CD3 antigen consists of a VH domain containing the amino acid sequences of SEQ ID NO: 28, SEQ ID NO: 29 or 128, and SEQ ID NO: 30, 129 or 130 (VH-B) and a VL domain containing the amino acid sequences of SEQ ID NO: 31 or 131, SEQ ID NO: 32 or 132, and SEQ ID NO: 33 (VL-B) (Antibody 114).

[0064]

[Table 13-1]

Name	Structure	Amino acid sequence 1	Amino acid sequence 2	Amino acid sequence 3
Antibody 56	Structure 1	SEQ ID NO: 151	SEQ ID NO: 121	
Antibody 57	Structure 1	SEQ ID NO: 152	SEQ ID NO: 121	
Antibody 58	Structure 1	SEQ ID NO: 153	SEQ ID NO: 121	
Antibody 59	Structure 1	SEQ ID NO: 154	SEQ ID NO: 121	
Antibody 60	Structure 1	SEQ ID NO: 155	SEQ ID NO: 121	
Antibody 61	Structure 1	SEQ ID NO: 156	SEQ ID NO: 121	
Antibody 62	Structure 1	SEQ ID NO: 157	SEQ ID NO: 121	
Antibody 63	Structure 1	SEQ ID NO: 158	SEQ ID NO: 121	
Antibody 64	Structure 1	SEQ ID NO: 159	SEQ ID NO: 121	
Antibody 65	Structure 1	SEQ ID NO: 160	SEQ ID NO: 121	
Antibody 66	Structure 1	SEQ ID NO: 161	SEQ ID NO: 121	
Antibody 67	Structure 1	SEQ ID NO: 162	SEQ ID NO: 121	
Antibody 68	Structure 1	SEQ ID NO: 157	SEQ ID NO: 165	
Antibody 69	Structure 1	SEQ ID NO: 158	SEQ ID NO: 165	
Antibody 70	Structure 1	SEQ ID NO: 159	SEQ ID NO: 165	
Antibody 71	Structure 1	SEQ ID NO: 160	SEQ ID NO: 165	
Antibody 72	Structure 1	SEQ ID NO: 161	SEQ ID NO: 165	
Antibody 73	Structure 1	SEQ ID NO: 162	SEQ ID NO: 165	
Antibody 74	Structure 1	SEQ ID NO: 157	SEQ ID NO: 167	
Antibody 75	Structure 1	SEQ ID NO: 158	SEQ ID NO: 167	
Antibody 76	Structure 1	SEQ ID NO: 159	SEQ ID NO: 167	
Antibody 77	Structure 1	SEQ ID NO: 160	SEQ ID NO: 167	
Antibody 78	Structure 1	SEQ ID NO: 161	SEQ ID NO: 167	
Antibody 79	Structure 1	SEQ ID NO: 162	SEQ ID NO: 166	
Antibody 80	Structure 1	SEQ ID NO: 170	SEQ ID NO: 197	
Antibody 81	Structure 1	SEQ ID NO: 175	SEQ ID NO: 197	
Antibody 82	Structure 1	SEQ ID NO: 170	SEQ ID NO: 198	
Antibody 83	Structure 1	SEQ ID NO: 171	SEQ ID NO: 198	
Antibody 84	Structure 1	SEQ ID NO: 172	SEQ ID NO: 198	
Antibody 85	Structure 1	SEQ ID NO: 173	SEQ ID NO: 198	
Antibody 86	Structure 1	SEQ ID NO: 174	SEQ ID NO: 198	

[0065]

[Table 13-2]

Antibody 87	Structure 1	SEQ ID NO: 175	SEQ ID NO: 198	
Antibody 88	Structure 1	SEQ ID NO: 176	SEQ ID NO: 198	
Antibody 89	Structure 1	SEQ ID NO: 177	SEQ ID NO: 198	
Antibody 90	Structure 1	SEQ ID NO: 178	SEQ ID NO: 198	
Antibody 91	Structure 1	SEQ ID NO: 179	SEQ ID NO: 198	
Antibody 92	Structure 1	SEQ ID NO: 180	SEQ ID NO: 198	
Antibody 93	Structure 1	SEQ ID NO: 181	SEQ ID NO: 198	
Antibody 94	Structure 1	SEQ ID NO: 182	SEQ ID NO: 197	
Antibody 95	Structure 1	SEQ ID NO: 183	SEQ ID NO: 197	
Antibody 96	Structure 1	SEQ ID NO: 184	SEQ ID NO: 197	
Antibody 97	Structure 1	SEQ ID NO: 185	SEQ ID NO: 197	
Antibody 98	Structure 1	SEQ ID NO: 186	SEQ ID NO: 197	
Antibody 99	Structure 1	SEQ ID NO: 187	SEQ ID NO: 197	
Antibody 100	Structure 2	SEQ ID NO: 163	SEQ ID NO: 168	
Antibody 101	Structure 2	SEQ ID NO: 164	SEQ ID NO: 168	
Antibody 102	Structure 2	SEQ ID NO: 164	SEQ ID NO: 169	
Antibody 103	Structure 2	SEQ ID NO: 189	SEQ ID NO: 199	
Antibody 104	Structure 2	SEQ ID NO: 190	SEQ ID NO: 199	
Antibody 105	Structure 3	SEQ ID NO: 188		
Antibody 106	Structure 4	SEQ ID NO: 194	SEQ ID NO: 197	
Antibody 107	Structure 5	SEQ ID NO: 191	SEQ ID NO: 197	SEQ ID NO: 200
Antibody 109	Structure 5	SEQ ID NO: 193	SEQ ID NO: 198	SEQ ID NO: 200
Antibody 110:	Structure 5	SEQ ID NO: 212	SEQ ID NO: 198	SEQ ID NO: 200
Antibody 111	Structure 5	SEQ ID NO: 191	SEQ ID NO: 198	SEQ ID: NO200
Antibody 112	Structure 6	SEQ ID NO: 195	SEQ ID NO: 203	SEQ ID NO: 202
Antibody 113	Structure 6	SEQ ID NO: 195	SEQ ID NO: 203	SEQ ID NO: 199
Antibody 114	Structure 7	SEQ ID NO: 196	SEQ ID NO: 201	

Another method for obtaining human antibodies is also known. For example, human lymphocytes are sensitized with a desired antigen or a cell expressing a desired antigen in vitro, and the sensitized lymphocytes are fused with human myeloma cells, such as U266, to obtain a desired human antibody having a binding activity to the antigen (See JP-B-1-59878). A desired human antibody can be obtained by immunizing a transgenic animal having all repertoires of human antibody genes with a desired antigen (See WO93/12227, WO92/03918, WO94/02602, WO94/25585, WO96/34096, and WO96/33735). Furthermore, a technique of obtaining a human antibody by panning using a human antibody library is also known. For example, a variable region of a human antibody is expressed as a single-chain antibody (scFv) on the surface of the phage by the phage display method, and the phage binding to the antigen can be selected. By analyzing the gene of the selected phage, the DNA sequence encoding the variable region of the human antibody which binds to the antigen can be determined. If the DNA sequence of the scFv which binds to the antigen is revealed, a human antibody can be obtained by making an appropriate expression vector with the sequence. These methods are already well known, and WO92/01047, WO92/20791, WO93/06213, WO93/11236, WO93/19172, WO95/01438, and WO95/15388 can be referred to.

[0067]

For the bispecific antibodies, bispecific antibody expression vectors can be constructed using, for example, DNA encoding anti-mutant CALR and anti-CD3 antibodies and expression vectors, and the expression vectors can be transfected into CHO cells or HEK293 cells to obtain transformed cells. The culture solution of these transformed cells can be purified by chromatography. The method for making the bispecific antibodies are already well known, and JP2019022497, JP2017137329, and JP2015110628 can be referred to.

[0068]

The class of the antibody is not particularly limited, and antibodies having any isotypes, such as IgG, IgM, IgA, IgD, or IgE, are included. In consideration of, for example, the ease of purification, IgG is preferred.

[0069]

Examples of domain structures include low molecular weight antibodies, such as antibody fragments, and modified antibodies. Specific examples of the antibody fragments include, but are not limited to, Fab, Fab', F(ab')², Fv, scFv, and diabodies. Examples described later show the H chain constant region of human IgG with amino acid mutations at 228, 233-238, 265, 268, 309, 318, and 328-331 which are believed to suppress binding affinity to the Fc receptor and amino acid mutations at 349, 354, 366, 368 and 407 for the hetero H chain, but not exclusively.

[0070]

Among the antibodies of the present invention, the antibody used for detection of the polypeptide (A) or (B) may be any antibody which binds to these polypeptides. Meanwhile, the antibody used for prevention or treatment of an MPN is preferably an antibody which binds to the polypeptide (A) or (B) and has a cytotoxic activity.

[0071]

The antibody which binds to the cleaved mutant CALR protein may be any antibody which binds to a polypeptide chain having the sequence of SEQ ID NO: 1, and is preferably an antibody that competes with at least one of the antibodies or functional fragments thereof for binding to an amino acid sequence portion of SEQ ID NO: 1 in the cleaved mutant CALR protein.

[0072]

The affinity of the mutant CALR-CD3 bispecific antibody to the mutant CALR protein may be a binding constant (K_D) of 10^{-7} M or less and is preferably 10^{-8} M or less. The binding constant is preferably measured by the surface plasmon resonance (SPR) method but can be determined simply by flow cytometry analysis from the concentration dependence of binding to mutant CALR-expressing cells.

[0073]

Here, examples of the biological sample used for the detection include a sample that is a biological sample

collected from a subject and contains the polypeptide (A) or (B). Specifically, examples of the sample include whole blood, plasma, serum, lymphocytes, lymph fluid, platelets, mononuclear cells, granulocytes, body fluids such as saliva and urine, and tissue samples (bone marrow fluid and bone marrow tissue) obtained by bone marrow biopsy.

[0074]

Examples of the method for detecting the polypeptide (A) or (B) include liquid chromatography-mass spectrometry, an immunological method, and a method using a low or medium molecule having the specific binding ability or nucleic acid, or a nucleic acid derivative, and an immunological method is preferred because of its ease and high measurement sensitivity.

[0075]

The immunological method is preferably an immunological method using an antibody which has an antigen-recognition site (epitope) in the polypeptide chain (A) or (B), or a functional fragment thereof. The epitope in the polypeptide chain (A) or (B) may be any sequence present in the polypeptide chain (A) or (B), and is preferably a peptide having three or more consecutive amino acids in the polypeptide chain (A) or (B) and more preferably a peptide having five or more consecutive amino acids in the polypeptide chain (A) or (B).

[0076]

The antibody to be used in the immunological method may be any antibody obtained using a peptide having, for example, three or more consecutive amino acids in the polypeptide chain (A) or (B) as the antigen, and may be a polyclonal antibody, a monoclonal antibody, or a bispecific antibody, and is preferably a bispecific antibody. Here, the monoclonal antibody can be produced by, for example, obtaining antibody-producing cells by sensitization with a peptide having three or more consecutive amino acids in the polypeptide chain (A) or (B), fusing the antibody-producing cells with myeloma cells to prepare hybridomas, selecting a clone producing the target antibody, and proliferating this cell. The bispecific antibody can be produced by constructing a bispecific antibody expression vector using a DNA encoding the antibody and an expression vector, and then proliferating cells transformed with this vector.

[0077]

As the immunological method, for example, any of immunochromatography, enzyme-labeled immunoassay (EIA), radioimmunoassay (RIA), coagulation method, nephelometry, flow cytometric method, tissue immunostaining, and immunoblotting such as western blotting can be used. Here, immunoblotting is a method for transferring a polypeptide onto a membrane and detecting the polypeptide on the membrane with an antibody. In the detection of the antibody, for example, an enzyme label and a fluorescent label are used.

[0078]

If the polypeptide (A) or (B) is detected in a biological sample, the biological sample can be determined to be a biological sample derived from an MPN patient.

[0079]

The agent for diagnosis of MPN of the present invention contains an antibody which has an antigen-recognition site (epitope) in the polypeptide chain (A) or (B). This antibody is preferably an above-described antibody.

The agent for diagnosis of MPN of the present invention may also contain, for example, a buffer and a measurement protocol, in addition to the antibody.

[0080]

The pharmaceutical composition such as the agent for prevention or treatment of MPN of the present invention is only required to contain the antibody and can be produced by formulation together with a pharmaceutically acceptable carrier through, for example, mixing, dissolving, emulsifying, encapsulating, or lyophilizing.

[0081]

The MPNs to be prevented or treated with the pharmaceutical composition of the present invention is preferably an MPN from which mutant CALR is detected.

[0082]

Suitable formulations for oral administration are, for example, liquids prepared by dissolving an effective

amount of the antibody of the present invention in diluents such as water and physiological saline; capsules, granules, powders, or tablets containing an effective amount of the antibody as solids or granules; suspensions prepared by suspending an effective amount of the antibody in an appropriate dispersion medium, and emulsions prepared by dispersing and emulsifying, in an appropriate dispersion medium, a solution prepared by dissolving an effective amount of the antibody.

[0083]

For parenteral administration, the antibody of the present invention can be formulated into dosage forms such as a solution for injection, a suspension, an emulsion, a cream, an ointment, an inhalant, and a suppository together with pharmaceutically acceptable solvents, excipients, binders, stabilizers, dispersants, and the like. In the prescription for injection, the antibody of the present invention can be dissolved in an aqueous solution, preferably a physiologically compatible buffer such as Hanks' solution, Ringer's solution, or physiological saline buffer. Furthermore, the pharmaceutical of the present invention can be in forms such as a suspension, a solution, or an emulsion in an oily or aqueous vehicle. Alternatively, the antibody of the present invention may be produced in a form of powder, and an aqueous solution or a suspension may be prepared using sterile water or the like before use. For administration by inhalation, the antibody of the present

invention is powdered, and a powder mixture can be formed with an appropriate base such as lactose or starch. A suppository prescription can be produced by mixing the antibody of the present invention with a commonly used suppository base such as cocoa butter. Furthermore, the therapeutic agent of the present invention can be prescribed as a sustained release formulation by encapsulating the agent in a polymer matrix or the like.

[0084]

The method for screening for an agent for treatment of MPN of the present invention is characterized by screening for an antibody, a protein, a low or medium molecule, a nucleic acid, or a nucleic acid derivative which recognizes the polypeptide (A) or (B). Specifically, exemplified is the development of an antibody pharmaceutical having an antibody-dependent cytotoxic activity or a complement-dependent cytotoxic activity using an antibody which recognizes the polypeptide (A) or (B). The antibody of the present invention is an example of the antibody pharmaceutical obtained by the screening method of the present invention. Other examples include the development of an antitumor drug that specifically deliver molecules with cytotoxic activity to tumor, for example, a low- or medium molecule, a nucleic acid or nucleic acid derivative, or a protein having a cytocidal effect on tumor cells using the creation of a low- or medium molecule, a nucleic acid or nucleic acid derivative, or a

protein which specifically binds to the polypeptide (A) or (B).

Examples

[0085]

Next, the present invention will be described in more detail with reference to Examples, but the present invention is not limited to these Examples at all.

[0086]

[Test Example 1] Making of antibodies which recognize mutant CALR protein containing cleaved type

A peptide having cysteine added to the C-terminus or N-terminus of the amino acid sequence (RRMMRTKMR) contained in SEQ ID NO: 1 was synthesized and conjugated to a carrier protein, keyhole limpet hemocyanin (KLH), then immunized to 8-week old female WKY/Izm rats. After booster immunization, lymphocytes were collected. The lymphocytes were cell-fused with mouse myeloma SP2 cells by the PEG method, then cultured in a selective medium to obtain hybridomas. The specificity of antibodies in the culture supernatant was screened by the ELISA method using the immunized peptide or purified protein to obtain hybridomas (clones B3, C6, and G1) producing antibodies which bind to mutant CALR proteins containing a cleaved type. Antibodies produced by these hybridomas (clone B3 antibody, C6 antibody, and G1 antibody) were purified and used in tests.

[0087]

[Test Example 2] Making of mutant CALR expressing cells

Human megakaryoblastic leukemia cell line UT-7/TPO cells were transfected with a vector expressing a Del 52 or Ins 5 type mutant CALR protein and a vector used for transfection as a control. The resulting UT-7/TPO CALR Del 52 cells and UT-7/TPO CALR Ins 5 cells neoplastically proliferating, and UT-7/TPO vec cells were each seeded at a density of 6.0×10^5 cells/mL in Opti-MEM medium (Thermo Fisher Scientific K.K.) and cultured in the presence of 5% CO₂ at 37°C for 32 hours. On this occasion, UT-7/TPO vec cells were cultured in a medium containing 10 ng/mL TPO.

[0088]

[Test Example 3] Verification of recognition of mutant CALR proteins

UT-7/TPO cells expressing a Del 52 type or Ins 5 type mutant CALR having a FLAG-tag inserted downstream of the signal sequence at the N-terminus were cultured, and culture supernatants containing the secreted mutant CALR proteins were prepared by centrifugation (1,600 g × 5 mins, 4°C). The culture supernatants were heat-treated in the presence of SDS and a reducing agent, then a gel by SDS polyacrylamide electrophoresis was performed, after that electrically transferred on a polyvinylidene difluoride (PVDF) membrane, and reacted with a TBS-T solution containing 5% skim milk at room temperature for 1 hour, followed by reaction with a 5% BSA/TBS-T solution containing a rat clone B3, C6, or G1 antibody or mouse

anti-DYKDDDDK tag antibody (FUJIFILM Wako Pure Chemical Corporation) at 4°C overnight. After washing with a TBS-T solution, the reaction products were each reacted with a 5% skim milk/TBS-T solution containing a peroxidase-labeled goat anti-rat IgG antibody (Jackson Immuno Research Inc.) or a peroxidase-labeled goat anti-mouse IgG antibody (Jackson Immuno Research Inc.) at room temperature for 1 hour. After washing with a TBS-T solution, the PVDF membranes were reacted with a peroxidase luminescence reagent. The resulting signals were detected using a FUSION image pickup apparatus.

As a result, as shown in Figure 2, it was verified that all of the clone B3, C6, and G1 antibodies recognize the full-length and cleaved mutant CALR proteins.

[0089]

[Test Example 4] Determination of sequence information of antibody of the present invention

To obtain the sequence information of heavy-chain and light-chain antibodies of the present invention, mRNAs were prepared from cells producing the antibodies using a PureLinc RNA Mini kit (Thermo Fisher Scientific K.K.). After cDNAs were synthesized using the resulting mRNAs as templates and reverse transcription primers specific to the heavy chain or the light chain by the rapid amplification of cDNA ends method, the cDNAs were cloned into plasmids. The resulting plasmids were analyzed by sanger sequencing to determine the full-

length sequences of the cDNAs of the heavy chain and light chain.

[0090]

[Example 1] Preparation of bispecific antibodies

Material and test method

Bispecific antibody expression vectors were constructed using DNA encoding bispecific antibodies based on the sequence information of clone B3, C6, and G1 antibodies and anti-CD3 antibodies (Tables 1-1 to 1-4) and expression vectors (pcDNA3.4, Thermo Fisher Scientific K.K.). The expression vectors were transfected into CHO cells or HEK293 cells by using an ExpiFectamine CHO Transfection Kit (Thermo Fisher Scientific K.K.) or an ExpiFectamine 293 Transfection Kit (Thermo Fisher Scientific K.K.), transformed cells were cultured, and then the cells were removed by centrifugation and filtration to collect the culture solution. Purification of the antibodies was performed by a combination of affinity chromatography using nickel-conjugated agarose and gel filtration chromatography, a combination of ProteinA affinity chromatography and gel filtration chromatography, or a combination of affinity chromatography using a CaptureSelect kappa XL resin (Thermo Fisher Scientific K.K.) and gel filtration chromatography. In the same manner as above, based on the sequence information described in Patent Literature 4 and Patent Literature 2, bispecific antibodies (Antibody 28: SEQ ID NO: 110, and SEQ ID NO: 111, Antibody 29: SEQ

ID NO: 112, and SEQ ID NO: 113, Antibody 30: SEQ ID NO: 114, and SEQ ID NO: 115) and B3 chimeric antibodies (SEQ ID NO: 48 and SEQ ID NO: 60) were prepared.

[0091]

[Example 2] Verification of recognition of mutant CALR protein on the cell surface by flow cytometry analysis

Material and test method

UT-7/TPO vec cells transfected with only the vector used for transfection as a control and mutant CALR-expressing UT-7/TPO CALR Ins 5 cells and UT-7/TPO CALR Del 52 cells were cultured using an IMDM medium containing 10% fetal bovine inactivated serum (FBS) in the presence of 5% CO₂ at 37°C. The 5×10^4 cells in the proliferative stage were centrifuged (400 g × 5 mins, 4°C), and then various antibodies were prepared in an FBS/PBS solution at a concentration of 5 µg/mL and reacted on ice for 30 minutes. After the reaction, the cells were washed twice using a MACS buffer, and an anti-human Fc antibody was prepared as a secondary antibody in an FBS/PBS solution at a concentration of 2.5 µg/mL and reacted on ice for 30 minutes. After completion of the reaction, the cells were washed twice by adding a MACS buffer performing centrifugation (400 g × 5 mins, 4°C), and discarding the supernatant. Signals were quantitatively measured by flow cytometry analysis using BD LSRFortessa X-20 (BD Biosciences).

[0092]

The results of flow cytometry analysis of [a] UT-7/TPO vec cells, [b] UT-7/TPO CALR Ins 5 cells, and [c] UT-7/TPO CALR Del 52 cells using representative anti-cleaved mutant CALR-CD3 bispecific antibodies made using a VH chain of SEQ ID NO: 2, a VL chain of SEQ ID NO: 3, a VH chain of SEQ ID NO: 8, and a VL chain of SEQ ID NO: 9 are shown.

As a result, as shown in Figure 3, it was verified that the evaluated bispecific antibodies specifically recognize cells expressing mutant CALR proteins on the cell surface.

[0093]

[Example 3] Verification of recognition of CD3 antigen on the cell surface by flow cytometry analysis

Material and test method

Peripheral blood mononuclear cells (PBMC) isolated from healthy human donors were cultured for two days in plates coated with an anti-CD3 antibody (Takara Bio Inc.) from 4 to 24 hours, and then further cultured in an RPMI-1640 medium containing IL-2 and 10% FBS (Sigma-Aldrich) from 10 to 21 days to induce T-LAK cells. The 5×10^5 T-LAK cells were centrifuged (400 g $\times$ 5 mins, 4°C), and then anti-cleaved mutant CALR-CD3 bispecific antibodies made using a VH chain of SEQ ID NO: 2, a VL chain of SEQ ID NO: 3, a VH chain of SEQ ID NO: 8, and a VL chain of SEQ ID NO: 9 in an FBS/PBS solution were prepared at a concentration of 5 μ g/mL and reacted on ice for 30 minutes. After the reaction, the cells were washed twice

using a MACS buffer, and an anti-human Fc antibody was prepared as a secondary antibody in an FBS/PBS solution at a concentration of 5 µg/mL and reacted on ice for 30 minutes. After completion of the reaction, the cells were washed twice by adding a MACS buffer performing centrifugation (400 g × 5 mins, 4°C), and discarding the supernatant. Signals were quantitatively measured by flow cytometry analysis using BD LSRFortessa X-20 (BD Bioscience).

[0094]

As a result, as shown in Figure 4, it was verified that the bispecific antibodies of the present invention specifically recognize CD3 antigens expressing on the T-LAK cell surface.

[0095]

[Example 4] Evaluation of avidity to antigen by surface plasmon resonance analysis

Material and test method

Sensor chips (GE Healthcare) for surface plasmon resonance analysis were loaded on a surface plasmon resonance analyzer Biacore T200 (GE Healthcare), and then equilibrated with an HBS-EP buffer (GE Healthcare). Anti-cleaved mutant CALR-CD3 bispecific antibodies made using a VH chain of SEQ ID NO: 2, a VL chain of SEQ ID NO: 3, a VH chain of SEQ ID NO: 8, and a VL chain of SEQ ID NO: 9, which were prepared at a concentration of 1 µg/mL with Acetate 5.5 (GE Healthcare) were immobilized using an Amine coupling kit (GE Healthcare) so that the

amount of an immobilized antibody was 200 RU. Subsequently, surface plasmon resonance was measured using a peptide having cysteine added to the N-terminus of the amino acid sequence (RRMMRTKMR) contained in SEQ ID NO: 1 prepared at a concentration of 20, 10, 5, 2.5, and 1.25 nM with an HBS-EP buffer, and the dissociation constant was acquired with Biacore T200 Evaluation Software.

As another method, measurements were performed using the molecular interaction analysis system BLItz (ForteBio), which applies biolayer interferometry and surface plasmon resonance technologies. Specifically, a protein having an Fc region and a His-tag added to the N-terminus of SEQ ID NO: 1 was immobilized at a concentration of 0.1 mg/mL using the Anti-Penta-HIS or ProteinA biosensor (ForteBio). Subsequently, the antibodies 1 to 114 made were prepared at a concentration of 500, 100, and 50 nM and reacted to acquire the dissociation constant.

[0096]

As a result, as shown in Figure 5, Table 14, and Table 16-1 to 16-2, the bispecific antibodies of the present invention strongly bind to the mutant CALR protein.

[0097]

[Table 14]

Name	K _D value (M)	Name	K _D value (M)
Antibody 1	6.55E-11	Antibody 19	7.28E-10
Antibody 3	1.55E-8	Antibody 20	1.24E-8
Antibody 4	1.25E-9	Antibody 22	<1E-12
Antibody 6	1.34E-10	Antibody 23	6.79E-9
Antibody 9	6.55E-9	Antibody 24	5.31E-10
Antibody 11	N.D.	Antibody 25	N.D.
Antibody 13	<1E-12	Antibody 26	1.23E-8
Antibody 14	<1E-12	Antibody 27	1.36E-8
Antibody 15	6.68E-9	Antibody 28 (SEQ ID NO: 110 and 111)	1.48E-3
Antibody 16	2.83E-9	Antibody 29 (SEQ ID NO: 112 and 113)	5.70E-6
Antibody 17	<1E-12	Antibody 30 (SEQ ID NO: 114 and 115)	-
Antibody 18	<1E-12	B3 chimeric antibody (SEQ ID NO: 48 and 60)	<1E-12

:- No antibody expression

N.D.: Not Determined

[0098]

[Example 5] Evaluation of in vitro cytotoxic activity

Material and test method

The cytotoxic activity of the bispecific antibody was measured in a cytolysis assay based on fluorescent dye release. It was verified whether the antibodies of the present invention induce cytolysis of target cells by immune effector cells in vitro. The immune effector cells used were T-LAK cells, but very similar assays can

be used for NK cells, macrophages, monocytes, dendritic cells, PBMCs, or the like.

UT-7/TPO CALR Ins 5 cells expressing the mutant CALR were labeled using about 10 μ M Calcein-AM (PromoKine) at 37°C for 30 minutes and washed with HBSS three times. After counting the appropriate number of labeled target cells, the cells were placed in 96-well round-bottom culture plates and incubated at 37°C for 2 hours in the presence or absence of various concentrations of bispecific antibodies and control proteins, or in the absence of antibodies added. T-LAK cells were induced by culturing PBMCs isolated from healthy human donors for two days in plates coated with an anti-CD3 antibody (Takara Bio Inc.) from 4 to 24 hours, and further culturing in an RPMI-1640 medium containing IL-2 and 10% FBS from 10 to 21 days. The immune effector cells and labeled target cells were added at a ratio of 10:1. The ratio of other immune effector cells to labeled target cells is also appropriate. Plates containing the labeled target cells, bispecific antibodies, and immune effector cells were cultured at 5% CO₂ from 1 to 6 hours at 37°C. After the reaction for 1 to 6 hours, the cells were centrifuged (125 rpm $\times$ 5 mins) to collect the supernatants. Subsequently, the supernatants were placed in 96-well flat-bottom culture plates, and the released dye in the incubation medium was measured for absorbance at 485/526 nm with a fluorescence reader (SynergyH1M, Biotech Japan Corporation). For normalization, the

maximum lysis rate (=100%) of labeled target cells was obtained by the addition of a 10 × lysis solution. The minimum lysis rate (=0%) was calculated from wells incubated with the labeled target cells without any constructs or antibodies. The cell cytolysis rate was calculated using the following formula:
$$\frac{[(\text{lysis rate of tumor cells in the presence of the bispecific antibody}) - (\text{lysis rate of tumor cells in the absence of the bispecific antibody})]}{[(\text{maximum lysis rate}) - (\text{minimum lysis rate})]}$$
. S-shaped dose-response curves were calculated by Prism Software (GraphPad Software Inc., San Diego), and EC50 values (50% effective concentration) were calculated.

[0099]

As shown in Table 15, the bispecific antibody of the present invention showed cytotoxic activity against UT-7/TPO CALR Ins 5 cells expressing mutant CALR.

[0100]

[Table 15]

Name	EC50(pM)	Name	EC50(pM)
Antibody 1	20	Antibody 19	3714
Antibody 3	19	Antibody 20	1463
Antibody 4	23	Antibody 22	101
Antibody 6	85	Antibody 23	370
Antibody 9	1528	Antibody 24	138
Antibody 11	1262	Antibody 25	56
Antibody 13	4473	Antibody 26	39
Antibody 14	989	Antibody 27	36
Antibody 15	1290	Antibody 28 (SEQ ID NO: 110 and 111)	5433
Antibody 16	334	Antibody 29 (SEQ ID NO: 112 and 113)	634
Antibody 17	46	Antibody 30 (SEQ ID NO: 114 and 115)	-
Antibody 18	81	B3 chimeric antibody (SEQ ID NO: 48 and 60)	>10000

-: No antibody expression

[0101]

As shown in Tables 16-1 to 16-2, the humanized antibodies (Antibody 56 to Antibody 114) were strongly bound to the mutant CALR proteins and showed cytotoxic activity against UT-7/TPO CALR Ins 5 cells expressing the mutant CALR.

[0102]

[Table 16-1]

Name	Binding affinity KD (M)	Binding affinity EC50 (pM)
Antibody 56	<1E-12	45
Antibody 57	<1E-12	398
Antibody 58	<1E-12	1531
Antibody 59	<1E-12	469
Antibody 60	<1E-12	77
Antibody 61	<1E-12	9763
Antibody 62	<1E-12	91
Antibody 63	2.62E-10	197
Antibody 64	<1E-12	829
Antibody 65	<1E-12	397
Antibody 66	<1E-12	115
Antibody 67	<1E-12	5899
Antibody 68	<1E-12	27
Antibody 69	<1E-12	162
Antibody 70	<1E-12	507
Antibody 71	5.54E-10	448
Antibody 72	<1E-12	93
Antibody 73	<1E-12	N.D.
Antibody 74	<1E-12	31
Antibody 75	<1E-12	77
Antibody 76	<1E-12	300
Antibody 77	<1E-12	129
Antibody 78	<1E-12	63
Antibody 79	<1E-12	N.D.
Antibody 80	<1E-12	28
Antibody 81	<1E-12	N.D.
Antibody 82	<1E-12	22
Antibody 83	<1E-12	110
Antibody 84	<1E-12	277
Antibody 85	<1E-12	389

[0103]

[Table 16-2]

Name	Binding affinity KD (M)	Binding affinity EC50 (pM)
Antibody 86	<1E-12	58
Antibody 87	<1E-12	N.D.
Antibody 88	<1E-12	28
Antibody 89	<1E-12	77
Antibody 90	<1E-12	269
Antibody 91	<1E-12	230
Antibody 92	<1E-12	47
Antibody 93	<1E-12	N.D.
Antibody 94	<1E-12	32
Antibody 95	<1E-12	N.D.
Antibody 96	<1E-12	N.D.
Antibody 97	<1E-12	N.D.
Antibody 98	<1E-12	N.D.
Antibody 99	<1E-12	N.D.
Antibody 100	<1E-12	1081
Antibody 101	<1E-12	1664
Antibody 102	<1E-12	850
Antibody 103	<1E-12	399
Antibody 104	<1E-12	621
Antibody 105	1.54E-9	26
Antibody 106	4.99E-9	2565
Antibody 107	1.21E-8	3869
Antibody 109	9.91E-9	3796
Antibody 110	5.33E-9	2541
Antibody 111	1.28E-8	6108
Antibody 112	<1E-12	N.D.
Antibody 113	3.24E-11	N.D.
Antibody 114	6.06E-9	790

N.D.: Not Determined

Claims

[Claim 1]

A bispecific antibody comprising a first domain which specifically binds to a mutant calreticulin protein and a second domain which specifically binds to a CD3 antigen.

[Claim 2]

The bispecific antibody according to claim 1, wherein the first domain competes with an antibody which binds to an epitope of a mutant calreticulin protein of SEQ ID NO: 1.

[Claim 3]

The bispecific antibody according to claim 1 or 2, wherein the mutant calreticulin protein has an antigen-recognition site in a polypeptide chain consisting of the amino acid sequence of SEQ ID NO: 1 or a polypeptide chain consisting of an amino acid sequence of SEQ ID NO: 1 but having deletion, substitution, or addition of one or several amino acids.

[Claim 4]

The bispecific antibody according to any one of claims 1 to 3, wherein the first domain does not bind to a wild-type calreticulin protein but has high affinity to the mutant calreticulin protein.

[Claim 5]

The bispecific antibody according to any one of claims 1 to 4, wherein the first domain has a high affinity to a mutant calreticulin protein after cleavage.

[Claim 6]

The bispecific antibody according to any one of claims 1 to 5, wherein binding of the first domain with the mutant calreticulin protein has a K_D of 10^{-7} M or less.

[Claim 7]

The bispecific antibody according to any one of claims 1 to 6, wherein a CDR of the first domain has (a) an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 10, SEQ ID NO: 11 or 126, and SEQ ID NO: 12 (VHCDR) and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 13, SEQ ID NO: 14 or 127, and SEQ ID NO: 15 (VLCDR); (b) an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 16 to 18 (VHCDR) and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 19 to 21 (VLCDR); or (c) an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 22 to 24 (VHCDR) and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 25 to 27 (VLCDR).

[Claim 8]

The bispecific antibody according to any one of claims 1 to 7, wherein the first domain has (d) a VH

region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 2, 90, 91, 92, 93, 94, 133, 134, 135, and 136, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 3, 95, 96, 97, 98, 99, 137, 138, 139, 140, and 141; (e) a VH region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 4, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 5; or (f) a VH region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 6, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequence of SEQ ID NO: 7.

[Claim 9]

The bispecific antibody according to any one of claims 1 to 8, wherein a CDR of the second domain has an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 28, SEQ ID NO: 29 or 128, and SEQ ID NO: 30, 129 or 130 (VHCDR), and an amino acid sequence having 80% or more homology to the amino acid sequences of SEQ ID NO: 31 or 131, SEQ ID NO: 32 or 132, and SEQ ID NO: 33 (VLCDR).

[Claim 10]

The bispecific antibody according to any one of claims 1 to 9, wherein the second domain has a VH region having an amino acid sequence having 80% or more homology

to the amino acid sequences of SEQ ID NO: 8, SEQ ID NO: 204, SEQ ID NO: 205, and 206, and a VL region having an amino acid sequence having 80% or more homology to the amino acid sequences of in SEQ ID NO: 9, SEQ ID NO: 207, and 208.

[Claim 11]

The bispecific antibody according to any one of claims 1 to 10, wherein a dimerization module is a hetero-H chain having one or more knob-into-hole structures, wherein an amino acid(s) at one or more positions selected from the group consisting of 349, 354, 366, 368, and 407 in a constant region of the H chain are replaced by Y349C, T366S, L368A, Y407V (hole or cavity), S354C, or T366W (knob or protrusion), the bispecific antibody comprising an Fc region of SEQ ID NO: 209 or 210.

[Claim 12]

The bispecific antibody according to any one of claims 1 to 11, comprising a mutated Fc region of SEQ ID NO: 211 with substitution of an amino acid(s) at one or more positions selected from the group consisting of 234 and 235 in a H-chain Fc region to each introduce L234A or L235A mutation.

[Claim 13]

The bispecific antibody according to any one of claims 1 to 12, which is a human antibody, a humanized antibody, a chimeric antibody of an animal-derived antibody and a human antibody, or a synthetic antibody.

[Claim 14]

A pharmaceutical composition comprising the bispecific antibody according to any one of claims 1 to 13 or a functional fragment thereof.

[Claim 15]

The pharmaceutical composition according to claim 14, which is a pharmaceutical composition for treatment or diagnosis of a myeloproliferative neoplasm.

[Claim 16]

A method for detecting a mutant calreticulin protein, comprising detecting the following polypeptide (A) or (B) in a biological sample using the bispecific antibody according to any one of claims 1 to 13 or a functional fragment thereof:

(A) a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1; or

(B) a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1 but having deletion, substitution, or addition of one or several amino acids.

[Claim 17]

The bispecific antibody according to any one of claims 1 to 13 or a functional fragment thereof, for use in detection of a mutant calreticulin protein.

[Claim 18]

A diagnostic method for a myeloproliferative neoplasm, comprising detecting the following polypeptide (A) or (B) in a biological sample using the bispecific

antibody according to any one of claims 1 to 13 or a functional fragment thereof:

(A) a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1; or

(B) a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1 but having deletion, substitution, or addition of one or several amino acids.

[Claim 19]

The bispecific antibody according to any one of claims 1 to 13 or a functional fragment thereof, for use in treatment or diagnosis of a myeloproliferative neoplasm.

[Claim 20]

Use of the bispecific antibody according to any one of claims 1 to 13 or a functional fragment thereof, for producing an agent for treatment or diagnosis of a myeloproliferative neoplasm.

[Claim 21]

A method for prevention or treatment of tumor, comprising administering the bispecific antibody according to any one of claims 1 to 13 or a functional fragment thereof.

[Claim 22]

The method according to claim 21, wherein the tumor is a hematologic tumor, and preferably a myeloproliferative neoplasm, myeloid leukemia, thrombocytosis, polycythemia, primary myelofibrosis, or secondary myelofibrosis.