

(VII-1)

A pharmaceutical composition, including the antibody (I) or the cell (VI).

(VII-2)

5 A pharmaceutical composition according to the item (VII-1), in which the cell is the chimeric antigen receptor T-cell (VI-4).

(VII-3)

A pharmaceutical composition according to the item (VII-1) or (VII-2), which is for use in treatment of cancer.

10 (VII-4)

A pharmaceutical composition according to the item (VII-3), in which the cancer is blood cancer.

(VII-5)

15 A pharmaceutical composition according to the item (VII-4), in which the blood cancer is a disease causing neoplastic growth of plasma cells.

[0023] (VIII) Treatment or Prevention Method for Disease

A treatment or prevention method (VIII) for a disease encompasses treatment or prevention methods for a disease
20 described in the following items (VIII-1) to (VIII-6).

(VIII-1)

A treatment or prevention method for a disease, including administering a therapeutically effective amount of the antibody (I) or the cell (VI) to a subject.

25 (VIII-2)

A treatment or prevention method according to the item (VIII-1), in which the cell is the chimeric antigen receptor T-cell (VI-4).

(VIII-3)

30 A treatment or prevention method according to the item (VIII-1) or (VIII-2), in which the disease is cancer, and in which the subject is a patient who has developed cancer or an animal having a risk of developing cancer.

(VIII-4) A treatment or prevention method according to the
35 item (VIII-3), in which the cancer is blood cancer.

(VIII-5)

A treatment or prevention method according to the item (VIII-4), in which the blood cancer is a disease causing neoplastic growth of plasma cells.

5 (VIII-6)

A treatment or prevention method for multiple myeloma, targeting active-form human integrin β_7 .

[0024] (IX) Use

10 A use (IX) encompasses uses described in the following items (IX-1) to (IX-5).

(IX-1)

A use of the antibody (I) or the cell (VI), for producing a pharmaceutical composition.

(IX-2)

15 A treatment or prevention method according to the item (IX-1), in which the cell is the chimeric antigen receptor T-cell (VI-4).

(IX-3)

20 A use according to the item (IX-1) or (IX-2), which is for treatment of cancer.

(IX-4)

A use according to the item (IX-3), in which the cancer is blood cancer.

(IX-5)

25 A use according to the item (IX-4), in which the blood cancer is a disease causing neoplastic growth of plasma cells.

[0025] (X) Screening Method

A screening method (X) encompasses screening methods described in the following (X-1) to (X-5).

30 (X-1)

A screening method for an active ingredient of a pharmaceutical composition for treating or preventing cancer, the method including selecting, from a compound library, a candidate substance that specifically binds to human integrin β_7 and binds
35 to a region of the amino acid residue positions 20 to 109 of the

human integrin β_7 .

(X-2)

A screening method according to the item (X-1), further including selecting a substance having cytotoxic activity.

5 (X-3)

A screening method according to the item (X-1) or (X-2), in which the substance to be selected is a monoclonal antibody.

(X-4)

A screening method according to any one of the items (X-1) to (X-3), in which the cancer is blood cancer.

(X-5)

A screening method according to the item (X-4), in which the blood cancer is a disease causing neoplastic growth of plasma cells.

15 [0026] (XI) Diagnosis Method

A diagnosis method (XI) encompasses diagnosis methods described in the following items (XI-1) to (XI-5).

(XI-1)

A diagnosis method for cancer, including bringing a sample collected from a subject into contact with the antibody (I).

(XI-2)

A diagnosis method according to the item (XI-1), in which the sample collected from a subject is blood or bone marrow fluid.

(XI-3)

25 A diagnosis method according to the item (XI-1) or (XI-2), further including judging that the subject has developed, or has a risk of developing, cancer when cells that bind to the antibody (I) are detected.

(XI-4)

30 A diagnosis method according to the item (XI-3), in which the cancer is blood cancer.

(XI-5)

A diagnosis method according to the item (XI-4), in which the cells are plasma cells, and in which the cancer is a disease causing neoplastic growth of plasma cells.

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myeloma progenitor cell fraction, myeloma plasma cell fraction, and CD45⁺ leukocytes of a plurality of myeloma patient-derived bone marrow cells (UPN1 to UPN5).

FIG. 3 is an illustration of a process of identifying an antigen protein recognized by the MMG49 antibody by an expression cloning method in Example 3. There is illustrated a process of concentrating BaF3 cells that bind to the MMG49 antibody, from an initial concentration of 0.1% or less, by FACS sorting.

FIGS. 4 are graphs for showing results obtained in Example 4 by staining ITGB7-deficient U266 cells generated using a Crisp-cas9 system with the MMG49 antibody or an FIB27 antibody (commercially available anti-integrin β_7 antibody), followed by FACS analysis.

FIG. 5 is an image for showing results obtained in Example 4 by subjecting a product immunoprecipitated from a cell lysate derived from MM1s myeloma cells with the MMG49 antibody or an isotype control antibody, to SDS-PAGE, and then performing western blot with a commercially available anti-integrin β_7 antibody (Abcam plc).

FIGS. 6 are graphs for showing results obtained in Example 5 by analyzing the binding of each of the MMG49 antibody, the FIB27 antibody, and an FIB504 antibody to each cell fraction of healthy person peripheral blood cells (in the figures, B-cells, T-cells, monocytes, neutrophils, red blood cells, and platelets are shown in the stated order from the left-hand side) through use of FACS.

FIGS. 7 are graphs for showing results obtained in Example 5 by analyzing, by FACS, the binding of the MMG49 antibody to each of cell fractions of myeloma patient-derived bone marrow cells. On the left-hand side, a method of identifying each cell fraction is illustrated, and on the right-hand side, graphs for showing the binding of MMG49 to each fraction are shown. In FIGS. 7A, a comparison between hematopoietic stem cell and progenitor cell fractions, and myeloma cells is shown, and in FIGS. 7B, a comparison between B/T lymphocyte fractions, and myeloma

progenitor cell and myeloma plasma cell fractions is shown.

FIGS. 8 are graphs for showing results obtained in Example 6 by analyzing the binding of each of the MMG49 antibody and the FIB27 antibody to each of various myeloma cell lines, and T-cells and B-cells derived from peripheral blood through use of FACS. There are also shown results of confirming the expression of ITGA4 (binding of an anti-integrin α_4 antibody) and the expression of ITGAE (binding of an anti-integrin α_E antibody) in the above-mentioned cells by FACS analysis.

FIGS. 9 are graphs for showing results obtained in Example 6 by analyzing, by FACS, the binding of the MMG49 antibody and the FIB27 antibody to U266 cells and ITGA4 (integrin α_4)-deficient U266 cells. There are also shown results obtained by analyzing, by FACS, the expression of ITGA4 (binding of the anti-integrin α_4 antibody) in the above-mentioned cells.

FIGS. 10 are graphs for showing results obtained in Example 7 by allowing integrin $\alpha_4\beta_7$ -forcibly expressing K562 cells and human normal peripheral blood-derived T-cells treated in the presence of $\text{Ca}^{2+}/\text{Mg}^{2+}$ or Mn^{2+} at 37°C for 20 minutes to react with the MMG49 antibody or an isotype antibody, then staining the cells using an anti-mouse IgG antibody as a secondary antibody, and subjecting the stained cells to FACS analysis.

FIG. 11 is a diagram for illustrating the construction of human/mouse chimeric integrin β_7 proteins and the presence or absence of the binding of the MMG49 antibody to 293T cells caused to transiently express the proteins in Example 8.

FIGS. 12 are graphs for showing results obtained in Example 8 by analyzing, by FACS, the binding of the MMG49 antibody to 293T cells caused to transiently express the human/mouse chimeric integrin β_7 proteins.

FIG. 13 is a graph for summarizing the results shown in FIGS. 12. In the graph in FIG. 13, the axis of ordinate represents the percentage of cells bound to the antibody, and the axis of abscissa represents various human/mouse chimeric integrin β_7 proteins.

FIGS. 14 are graphs for showing results obtained by staining MM1s cells and KMS12BM cells with a chimerized MMG49 antibody generated by linking variable regions of the MMG49 antibody to a human IgG4 antibody constant domain.

5 FIG. 15 is a scheme for illustrating a method of generating a CAR construct using variable regions of the MMG49 antibody.

FIG. 16 are graphs for showing results obtained by staining T-cells caused to express the CAR construct using variable regions of the MMG49 antibody with a PE-anti-human F(ab')₂ antibody.

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FIG. 17 are graphs for showing results obtained in Example 11 by quantitatively determining, by ELISA, the amounts of IFN- γ and IL2 produced through coculture of MMG49 antibody-derived CAR-T cells or T-cells having introduced therein GFP (control) with K562 cells expressing no integrin β_7 or K562 cells caused to forcibly express integrin $\alpha_4\beta_7$. *: $p < 0.05$.

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FIGS. 18 are graphs for showing results obtained in Example 11 by quantitatively determining, by ELISA, the amount of IFN- γ produced through coculture of MMG49 antibody-derived CAR-T cells or T-cells having introduced therein GFP (control) with MMG49 antigen-expressing cells or non-expressing cells.

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FIGS. 19 are graphs for showing results obtained in Example 11 by quantitatively determining, by ELISA, the amount of IL2 produced through coculture of MMG49 antibody-derived CAR-T cells or T-cells having introduced therein GFP (control) with MMG49 antigen-expressing cells or non-expressing cells.

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FIGS. 20 are graphs for showing results obtained in Example 11 by measuring, by ⁵¹Cr killing assay, the degree of cell damage caused by MMG49 antibody-derived CAR-T cells or T-cells having introduced therein GFP (control) with respect to K562 cells expressing no integrin β_7 or K562 cells caused to forcibly express integrin $\alpha_4\beta_7$. The y-axis of each of the graphs in FIGS. 20 represents a cell damage percentage (%).

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FIGS. 21 are graphs for showing results obtained in Example 11 by measuring, by ⁵¹Cr killing assay, the degree of cell damage

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caused by MMG49 antibody-derived CAR-T cells or T-cells having introduced therein GFP (control) with respect to MMG49 antigen-expressing cells or non-expressing cells.

FIGS. 22 are a diagram and graphs for illustrating and showing the design of a therapeutic experiment for a myeloma cell line MM1s engrafted in the bone marrow of an NOG mouse and results thereof in Example 11. Bone marrow cells after 1 week from the transfer of MMG49 antibody-derived CAR-T cells or T-cells having introduced therein GFP (control) were collected and analyzed by FACS. MM1s cells can be identified as human CD138⁺ cells. In an MMG49 antibody-derived CAR-T cell-administered group, MM1s cells in the bone marrow have almost completely disappeared.

FIGS. 23 are a diagram, images, and a graph for illustrating and showing the design of a therapeutic experiment for the myeloma cell line MM1s systemically engrafted to an NOG mouse and results thereof in Example 12. The amounts of myeloma cells before and after the transfer of MMG49 antibody-derived CAR-T cells or T-cells having introduced therein GFP (control) were evaluated by fluorescence intensity measurement based on IVIS imaging. In an MMG49 antibody-derived CAR-T cell-administered group, MM1s cells in the bone marrow have almost completely disappeared.

FIG. 24 is a view for illustrating a comparison between the amino acid sequence of integrin β_7 of human origin and the amino acid sequence of integrin β_7 of mouse origin.

FIGS. 25 are a diagram and graphs for illustrating and showing the construction of human/mouse chimeric integrin β_7 proteins and the presence or absence of the binding of the MMG49 antibody to 293T cells caused to transiently express the proteins in Example 13.

FIG. 26 is a graph for showing results of an experiment for investigating an epitope of the MMG49 antibody in Example 14. MFI on the axis of abscissa represents binding strength to the MMG49 antibody, and a higher numerical value indicates a higher avidity.

given as an example corresponding to the conformational epitope,
and a case in which a region of non-consecutive amino acid
residues included in the region of the amino acid residue
positions 20 to 109 serves as the epitope is also encompassed in
5 the conformational epitope.

[0047] Of the above-mentioned epitopes, it is preferred that the
amino acid residue at position 48 be strongly related to the
epitope of the antibody (I) or be included in the epitope of the
antibody (I).

10 [0048] A person skilled in the art can understand about specific
linear epitopes and conformational epitopes with reference to,
for example, JP 2011-527572 A, JP 2009-534401 A, or "Dissecting
antibodies with regards to linear and conformational epitopes."
Forsstrom B, Axnas BB, Rockberg J, Danielsson H, Bohlin A, Uhlen
15 M. PLoS One. 2015 Mar 27; 10(3): e0121673. doi:
10.1371/journal.pone.0121673. eCollection 2015.

[0049] In other words, the foregoing means that the antibody (I)
is an antibody that specifically binds to the region of the amino
acid residue positions 20 to 109 of the human integrin β_7 , and in
20 particular, preferably specifically binds to the region at
positions from 23 to 109, more preferably specifically binds to
the region at positions from 23 to 48 and/or positions from 93 to
109.

[0050] In addition, the property of the antibody (I) of binding to
25 the region of the amino acid residue positions 20 to 109 of the
integrin β_7 , which serves as the epitope, is sometimes referred to
as affinity for the epitope. Accordingly, the term "affinity for
the epitope is increased" has the same meaning as "specific
binding capacity for the epitope is increased."

30 [0051] The term "specific" may be distinguished from the term
"selective".

[0052] As another embodiment of the antibody (I), it is preferred
that the affinity of the antibody (I) for the epitope be
increased in the presence of at least part of the region of the
35 amino acid residue positions 379 to 721 of the human integrin β_7 .

[0053] The "at least part of the region of the amino acid residue positions 379 to 721" means that any one of the region of the amino acid residue positions 379 to 721 and a partial region thereof may be adopted. Specific examples of the "partial region
5 thereof" include: at least part of the region of the amino acid residue positions 417 to 721 of the human integrin β_7 ; at least part of the region of the amino acid residue positions 564 to 721 of the human integrin β_7 ; at least part of the region of the amino acid residue positions 379 to 563 of the human integrin β_7 ; at
10 least part of the region of the amino acid residue positions 417 to 563 of the human integrin β_7 ; and at least part of the region of the amino acid residue positions 379 to 416 of the human integrin β_7 . That is, the affinity of the antibody (I) for the epitope can be increased in the presence of any of those regions.

[0054] The term "in the presence of" means that the region of the amino acid residue positions 20 to 109 of the human integrin β_7 and at least part of the region of the amino acid residue positions 379 to 721 of the human integrin β_7 may be present in the same molecule, or the two regions may be present as separate
20 molecules. It is preferred that the two regions be present in the same molecule. The term "in the presence of" may be read as "by".

[0055] A person skilled in the art can easily confirm that the affinity of the above-mentioned antibody (I) for the epitope is increased, by a commonly used immunoassay method described in,
25 for example, Examples to be described below.

[0056] For example, cells caused to express a human/mouse chimeric integrin β_7 protein (#4960), which is various human/mouse chimeric integrin β_7 proteins described in Example 8, and which includes the region of the amino acid residue positions 1 to 109 of
30 integrin β_7 of human origin and includes the region of the amino acid residue positions 722 to 798 of the integrin β_7 of human origin, are prepared, and a human/mouse chimeric integrin β_7 protein (#4961) in which the region of #4960 of the amino acid residue positions 379 to 721 of the integrin β_7 of human origin is
35 replaced with the region of the amino acid residue positions 379

immunizing an animal suited for antibody production, such as a mouse, a rat, or a rabbit, with a peptide corresponding to the region of the amino acid residue positions 20 to 109 of the human integrin β_7 ; then recovering B-cells and subjecting the recovered
5 B-cells to a hybridoma method; and performing screening through use of the function exhibited by the antibody (I) described above as an indicator, to thereby produce the antibody (I).

[0129] In addition to the foregoing, there may be given a method involving: generating cells expressing chimeric integrin β_7 in
10 which only the region of the amino acid residue positions 20 to 109 of integrin β_7 is of human origin and the rest is of non-human origin, such as mouse origin; immunizing an animal suited for antibody production, such as a mouse, a rat, or a rabbit (preferably a mouse), with the cells; then recovering B-cells and
15 subjecting the recovered B-cells to a hybridoma method; and performing screening through use of the function exhibited by the antibody (I) described above as an indicator, to thereby produce the antibody (I).

[0130] Examples of the function exhibited by the antibody (I) may
20 include: an increase in affinity for the region of the amino acid residue positions 20 to 109 of the human integrin β_7 in the presence of at least part of the region of the amino acid residue positions 380 to 721 of the human integrin β_7 ; and an increase in the affinity through activation of the human integrin β_7 .

25 Accordingly, the antibody (I) may be obtained by a method shown in a screening method (X) to be described below, which utilizes such function.

[0131] An epitope for the antibody (I) is present in the region of the amino acid residue positions 20 to 109 of the human
30 integrin β_7 , and hence the antibody (I) is expected to exhibit cytotoxic activity or the like on cells expressing the integrin β_7 by combining one or two or more of not only the above-mentioned ADCC activity and CDC activity, but also apoptosis-inducing activity, survival signal-blocking activity, and the like on such
35 cells. Accordingly, a composition including the antibody (I) is

useful as a pharmaceutical composition (VII) as described in detail below.

[0132] In particular, the antibody (I) uses the region of the amino acid residue positions 20 to 109 of the human integrin β_7 as an epitope, and the affinity of the antibody (I) for the epitope is increased through activation of the integrin β_7 . Active-form integrin β_7 is expressed in blood cells, such as plasma cells, and hence the antibody (I) is used as an active ingredient of a pharmaceutical composition for cancer thereof (e.g., blood cancer). In particular, the antibody (I) is effectively used as a pharmaceutical composition for a disease causing an abnormality in the above-mentioned cells (e.g., myeloma or multiple myeloma).

[0133] (II) Polynucleotide

A polynucleotide (II) is a polynucleotide having a base sequence encoding the amino acid sequence of the antibody (I). The term "polynucleotide" includes, for example, a single-stranded or double-stranded form in which a ribonucleotide, a deoxyribonucleotide, any one nucleotide thereof, or the like is appropriately modified by a known method.

[0134] A person skilled in the art can appropriately determine the base sequence of the polynucleotide (II), for example, in silico on the basis of the amino acid sequence of the antibody (I). The kinds of codons to be used for determining such base sequence are not limited. The base sequence is preferably determined in consideration of the codon frequency of a host in which the polynucleotide is to be used.

[0135] A specific base sequence of the polynucleotide (II) is not particularly limited. Correspondence between each SEQ ID NO in which an amino acid sequence identified as one embodiment of the antibody (I) is set forth and the SEQ ID NO in which a base sequence encoding such amino acid sequence is set forth is shown in Table 2 below. That is, preferred base sequences of the polynucleotide (II) are base sequences set forth in SEQ ID NOS: 11 to 20.

[0136]

preferably a disease causing neoplastic growth of plasma cells (e.g., myeloma or multiple myeloma).

[0204] The kit (XII) may be appropriately accompanied by a manual. In such manual, the method described in detail in the above-mentioned diagnosis method (XI) may be described to serve as a criterion for the diagnosis of cancer.

Examples

[0205] Now, Examples for describing the present invention in more detail are described. Needless to say, the present invention is not limited to Examples described below.

[0206] Test Method: Flow Cytometry and Sorting

In the following Examples, flow cytometry (FACS) used for selecting cells was performed in the following manner.

[0207] Bone marrow mononuclear cells collected from an ilium of a myeloma patient who had given informed consent were suspended in an ACK solution (150 mM NH_4Cl and 10 mM KHCO_3), and the whole was left at rest at 4°C for 3 minutes to remove red blood cells. The bone marrow mononuclear cells after the removal were washed with PBS supplemented with 2% fetal bovine serum, and then, in order to prevent the binding of a non-specific antibody, blocking was performed at 4°C for 20 minutes in PBS containing 10% human AB serum.

[0208] After that, each antibody (see below) labeled with a fluorescent dye was added thereto to perform staining at 4°C for 30 minutes. After that, the cells were washed with PBS, and then suspended in PBS containing 1 $\mu\text{g/ml}$ propidium iodide (PI), followed by FACS analysis. The analysis of the cells and cell sorting were performed using FACS Aria Cell Sorter (manufactured by Becton Dickinson Immunocytometry Systems).

[0209] For the staining of the cells, the following monoclonal antibodies were appropriately selected and used.

- APC-conjugated anti-human CD34 antibody (manufactured by BD Pharmingen)

- PE-Cy7-conjugated anti-human CD34 antibody (manufactured by BD

Pharmingen)

•APC/Cy7-conjugated anti-human CD19 antibody (manufactured by Biolegend)

5 •FITC-conjugated anti-human CD38 antibody (manufactured by eBioscience)

•APC-conjugated anti-human CD138 antibody (manufactured by Biolegend)

•PE/Cy7-conjugated anti-human CD3 antibody (manufactured by Biolegend)

10 •FITC-conjugated anti-human CD14 antibody (manufactured by BD Pharmingen)

•PE/Cy7-conjugated anti-human CD45 antibody (manufactured by Biolegend)

[0210] [Example 1]

15 Generation of Monoclonal Antibody Library that binds to Myeloma Cell Line and does not bind to Healthy Person Peripheral Blood

In antibody therapy against multiple myeloma, it is important to use an antibody that binds to myeloma cells but does not bind to normal blood cells. In view of this, such antibody
20 was identified by the following method. First, 10,000 clones or more of monoclonal antibodies that bound to various myeloma cell lines were generated using the following technique.

[0211] Balb/c mice were immunized at the footpad twice a week for from 2 weeks to 3 weeks through use of six kinds of human myeloma
25 cell lines (MM.1s cells, RPMI8226 cells, INA6 cells, U266 cells, OPM2 cells, and KMS12BM cells) as antigens. After that, a lymph node below the knee was removed, and a cell suspension was generated and subjected to cell fusion with a SP2/0 mouse myeloma cell line to generate hybridomas. The cell fusion was performed
30 using a method using polyethylene glycol (PEG method). After that, the cells were cultured in hypoxanthine-aminopterin-thymidine medium (HAT medium) to select hybridomas (>10,000 clones).

[0212] Finally, through use of culture supernatants of the hybridomas, a supernatant including antibodies that bound to the
35 myeloma cell line used for the immunization and did not bind to

which the MMG49 antibody was known to bind, using a superscript choice system for cDNA synthesis (Invitrogen), and was inserted into a pMXs retrovirus vector (donated by Professor Toshio Kitamura at the Institute of Medical Science of the University of Tokyo) using a BstXI adaptor (Invitrogen). The thus generated cDNA library was introduced into plat-E cells (donated by Professor Toshio Kitamura), and BaF3 cells were infected with the resultant retrovirus. Thus, BaF3 cells expressing an MM.1s-derived cDNA library were obtained.

[0219] Next, the cells were repeatedly concentrated by staining the cells with the MMG49 antibody and sorting positive cells by FACS (FIG. 3). After the third sorting, most cells were cells that bound to the MMG49 antibody. Then, the retrovirus insert carried by those cells was amplified by PCR, and then sequenced to identify its base sequence. As a result, it was revealed that the insert carried by the cells was ITGB7.

[0220] [Example 4]

Confirmation that Antigen to which MMG49 Antibody binds is ITGB7-expressed Protein by Generation of ITGB7-deficient Myeloma Cells

An ITGB7-deficient U266 myeloma cell line was generated using a Crisp-cas9 system.

[0221] First, a vector was generated by inserting a double-stranded DNA sequence serving as an ITGB7-specific target sequence into a PX330 (addgene) vector. The vector was introduced together with a linear hygromycin-resistance gene expression vector (Clontech) serving as a vector for drug selection into U266 cells through use of Nucleofector (trademark) II (Lonza). After that, for clones that had grown in a medium supplemented with hygromycin, the expression of ITGB7 was stained using an FIB27 antibody (anti-integrin β_7 antibody; Biolegend), followed by analysis by FACS, to thereby identify ITGB7-deficient cells.

[0222] Next, the resultant ITGB7-deficient cells were stained using the MMG49 antibody and analyzed by FACS. As a result, it was found that the MMG49 antibody bound to wild-type U266 cells, whereas the binding of the MMG49 antibody had completely

finally stained using a Pacific blue-conjugated anti-human CD235 antibody (BD Pharmingen) or an FITC-conjugated anti-human CD41 antibody (BD Pharmingen), and thus the presence or absence of the binding of each antibody to CD235⁺ red blood cells and platelets was also similarly investigated by FACS analysis (FIGS. 6). The results of the foregoing show that the FIB27 antibody strongly binds to many lymphoid cells, whereas the binding of the MMG49 antibody to the above-mentioned normal blood cells is extremely weak.

[0228] Further, in order to elucidate whether the binding of the MMG49 antibody to each normal cell fraction except for myeloma cells was absent in bone marrow, bone marrow cells of a myeloma patient were also similarly stained using the MMG49 antibody, and finally stained using an APC-conjugated anti-human CD34 antibody (manufactured by BD Pharmingen), Alexa647-conjugated human CD3 (manufactured by BD Pharmingen), a Cy7APC-conjugated anti-CD19 human antibody (manufactured by BD Pharmingen), a PE-Cy7-conjugated anti-CD38 human antibody (manufactured by BD Pharmingen), or an FITC-conjugated anti-CD14 human antibody (manufactured by BD Pharmingen). Those myeloma patient-derived bone marrow cells after the staining were analyzed using FACS, and thus the binding of the MMG49 antibody in each fraction was measured (FIGS. 7). The results of the foregoing show that the MMG49 antibody strongly binds to myeloma cells, but hardly binds to all normal blood cells including hematopoietic stem cell and progenitor cell fractions.

[0229] [Example 6]

Analysis of Binding of MMG49 to Various Cell Lines

The binding of the MMG49 antibody and the FIB27 antibody in various cell lines (MM1s cells, U266 cells, RPMI8226 cells, and JJN3 cells) was analyzed using FACS. A staining method is the same as in the case of peripheral blood or the like described above in Example 5.

[0230] Integrin β_7 is known to form a heterodimer with integrin α_4 or integrin α_E and be expressed on a cell surface. Therefore, the

expression thereof was also analyzed at the same time using FACS, with an Alexa647-cojugated anti-human CD49d antibody (Biolegend) and an APC-conjugated anti-human CD103 antibody (Biolegend).

CD103 represents the integrin α_E , and CD49d represents the integrin α_4 . In the same manner as in Example 5 described above, the expression levels of the integrin α_E and the integrin α_4 in healthy person-derived peripheral blood were also investigated (FIGS. 8).

[0231] As a result, ITGA4 was expressed in most of the myeloma cell lines, and ITGAE was expressed in none of the cell lines. The FIB27 antibody was bound to all myeloma cell lines, but the binding of the MMG49 antibody did not coincide with the expression level of the FIB27 antibody. Further, the binding of the MMG49 antibody or the FIB27 antibody to ITGA4-deficient U266 cells generated using a Crisp-cas9 system was investigated by FACS, and as a result, it was found that the binding of both the antibodies to U266 cells had disappeared due to ITGA4 deficiency. That is, it was found that both the MMG49 antibody and the FIB27 antibody recognized β_7 integrin expressed as $\alpha_4\beta_7$ integrin.

[0232] [Example 7]

Analysis of Correlation between Activation of Integrin and Binding of MMG49 Antibody

In consideration of the unique binding manner of the MMG49 antibody described above, it was supposed that the MMG49 antibody recognized integrin β_7 that had been changed in structure through activation.

[0233] In view of the foregoing, K562 cells caused to forcibly express $\alpha_4\beta_7$ and human normal peripheral blood CD4T-cells concentrated using a CD4 T-cell enrichment kit (BD pharmingen) were washed with 5 mM EDTA/HBS, and then incubated in 1 mM Ca^{2+} /1 mM Mg^{2+} /HBS (buffer for low activity) or 2 mM Mn^{2+} /HBS (activating buffer) together with the MMG49 antibody or the FIB27 antibody at room temperature for 30 minutes, followed by washing. A PE-conjugated anti-mouse IgG antibody was added as a secondary antibody, and the cells were further incubated at room

	CDR2 (SEQ ID NO: 17)	GCCACATCC
	CDR3 (SEQ ID NO: 18)	CAGCAGTGGAGTAGTGACCCACCGACG
	Variable region (SEQ ID NO: 19)	ATGGATTTTCAAGTGCAGATTTTCAGCTTCCTGCTA ATCAGTGCTTCAGTCATAATGTCCAGAGGACAAATT GTTCTCTCCCAGTCTCCAGCAATCCTGTCTGCATCT CCAGGGGAGAAGGTCACAATGACTTGAGGGCCAGC TCAAGTGTAGGTTACATGCACTGGTTCCAGCAGAAG CCAGGATCCTCCCCCAAACCCTGGATTTATGCCACA TCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTCAGT GGCAGTGAGTCTGGGACCTCTTACTCTCTCACAATC AGCAGAGTGGAGGCTGAAGATGCTGCCACTTATTAC TGCCAGCAGTGGAGTAGTGACCCACCGACGTTCCGT GGAGGCACCAAGCTGGAAATCAAA

[0248] [Example 10]

Generation of Chimeric Antigen Receptor T-cells using Antibody Molecule Variable Regions of MMG49 Antibody

5 Chimeric antigen receptor T-cells (hereinafter referred to as MMG49 antibody-derived chimeric antigen receptor T-cells) were generated using the MMG49 antibody molecule variable region sequences by the following procedure with reference to Non Patent Literatures 2 to 4 and the like.

10 [0249] (1) Cloning of CD28 and CD3z:

RNAs were collected from Jurkat cells through use of Trizol (Invitrogen), and cDNAs were generated using a Superscript III cDNA synthesis kit (Invitrogen). Then, the cDNAs were used as templates to amplify cDNAs of CD28 and CD3z by PCR, each of which
15 was cloned using a TA cloning kit (Invitrogen), and their base sequences were confirmed by sequencing.

[0250] (2) Binding of Four Fragments, i.e., MMG49 antibody-derived VL/VH, and CD28/CD3z:

Through use of an overlapping PCR method, respective gene
20 fragments of the MMG49 antibody-derived VL region and VH region, and the CD28 and CD3z cloned above were bound to each other to generate chimeric cDNA. A procedure therefor and primers used are illustrated in FIG. 15. The base sequences of the primers used are shown in Table 5 below.

[0251]

Table 5

No.	Primer name	Base sequence
23	49 car vk-s5	gaattccaccatggattttcaagtgcagatt
24	49 car vk-as6	gccggaaccgctagtggagccccgtttgatttccagcttgg
25	593 car vk as4	gctgccttctccgctgccaggtttgccggaaccgctagtggagcc
26	49 car vh-s5	aaacctggcagcggagaaggcagccaggttcagctgcagcagtc
27	49 car vh-as6	tgaggagacggtgaccgtgg
28	49 VKVH28as8	atacataacttcaattgcggccgctgaggagacggtgaccgtgg
29	49carinfus1	ctaggcgccggaattccaccatggattttc
30	tcrzcarinfuas4	aatgtcgacctcgagtggctgttagcgag

[0252] The bound chimeric cDNA was cloned using a ZeroBlunt PCR
5 cloning kit (Invitrogen), and then sequenced to confirm its base
sequence. In addition, an amino acid sequence (SEQ ID NO: 21)
confirmed on the basis of the confirmed base sequence and the
base sequence (SEQ ID NO: 22) are shown in the sequence listing.
The amino acid sequence set forth in SEQ ID NO: 21 is one
10 obtained by conversion into an amino acid sequence from the start
codon (atg) immediately following the Kozak sequence (gaattccacc)
shown above in SEQ ID NO: 23, which was excluded.

[0253] (3) Insertion into Expression Vector:

Subsequently, the chimeric cDNA bound in (2) was cleaved
15 with two restriction enzymes EcoRI/SalI, and inserted into an
MSCV-ires-GFP vector.

[0254] The MMG49 antibody-derived chimeric antigen receptor cDNA
retrovirus vector generated by the foregoing was introduced into
293T cells together with a gag/pol and VSV-G envelope expression
20 vector through use of lipofectamine 2000 (Invitrogen), to thereby
generate a retrovirus. After 48 hours from the gene introduction,
a supernatant was recovered and used as a virus solution.

[0255] (4) Introduction into T-cells:

Subsequently, cDNA of an MMG49 antibody-derived chimeric
25 antigen receptor was introduced into human T-cells as described
below.

[0256] First, human peripheral blood mononuclear cells were added
to a 48-well plate coated with an anti-CD3 antibody (eBioscience)

and cultured for 72 hours. X-VIVO15 (Lonza) supplemented with 10% human AB serum and IL-2 (175 IU/L) was used as a culture medium, and the peripheral blood mononuclear cells were stimulated. After that, the virus solution generated above was added to a 48-well plate coated with Retronectin (Takara), and the virus was adsorbed to Retronectin by centrifugation at 1,700×g for 120 minutes. After that, the peripheral blood mononuclear cells (including T-cells) after the stimulation were added, and the gene was introduced thereinto. After that, culture was continued in the above-mentioned medium to amplify MMG49 antibody-derived chimeric antigen receptor T-cells, which were used in the following investigation. The T-cells caused to express a CAR construct using variable regions of the MMG49 antibody were stained using a PE-anti-human F(ab')₂ antibody (Jackson Laboratory), and as a result, the expression of human F(ab')₂ was detected in proportion to the expression of GFP indicating the introduction of the construct (FIGS. 16). That is, it was confirmed that the introduced CAR was expressed on a cell surface. [0257] [Example 11]

20 Analysis of Recognition of ITGB7-expressing Tumor Cells by MMG49 Antibody-derived Chimeric Antigen Receptor T-cells and Cytotoxic Activity thereof

The MMG49 antibody-derived chimeric antigen receptor T-cells generated by the above-mentioned method or control T-cells having introduced therein only GFP were cocultured with K562 cells expressing no integrin β_7 or K562 cells caused to forcibly express integrin $\alpha_4\beta_7$, and the amount of a produced cytokine was quantitatively determined. Specifically, 1×10^5 each of the T-cells and the target cells were added to a 96-well plate. After 24 hours, a supernatant was recovered, and the amount of production of IFN- γ was measured by ELISA. The measurement was performed using a Quantikine kit (R&D). As a result, only in the coculture of the K562 cells caused to forcibly express integrin $\alpha_4\beta_7$ with the MMG49 antibody-derived chimeric antigen receptor T-cells, higher production of IFN- γ and IL2 than in the control (T-

with a γ -counter. A cell damage percentage (%) was determined on the basis of the following expression (1).

$$[0261] \quad (A-B)/(C-D) \times 100 \quad (1)$$

A: Amount of ^{51}Cr released from cells used in experiment

5 B: Spontaneous ^{51}Cr release amount under antibody-free state

C: Maximum ^{51}Cr release amount with addition of 1% Triton X-100

D: Spontaneous ^{51}Cr release amount under antibody-free state.

[0262] As a result, in the K562 cells caused to forcibly express integrin $\alpha_4\beta_7$ to which the MMG49 antibody bound, higher cell
10 damage caused by the MMG49 antibody-derived chimeric antigen receptor T-cells than in the T-cells expressing only GFP serving as a control was observed (FIGS. 20).

[0263] Next, the MMG49 antibody-derived chimeric antigen receptor T-cells or control T-cells having introduced therein only GFP
15 were cocultured with myeloma cell lines to which the MMG49 antibody bound (MM1s cells, RPMI8226 cells, and JJN3 cells) or cells to which the MMG49 antibody did not bind (KMS12BM, Molt4, and Raji cells), and a similar investigation was performed. As a result, only in the K562 cells caused to forcibly express
20 integrin $\alpha_4\beta_7$ to which the MMG49 antibody bound, higher cell damage caused by the MMG49 antibody-derived chimeric antigen receptor T-cells than in the T-cells expressing only GFP serving as a control was observed (FIGS. 21).

[0264] The above-mentioned results show that the MMG49 antibody-
25 derived chimeric antigen receptor T-cells can specifically damage cells expressing an antigen to be recognized by the MMG49 antibody.

[0265] [Example 12]

Analysis of Myeloma Cell-eliminating Ability exhibited by MMG49
30 Antibody-derived Chimeric Antigen Receptor T-cells in vivo

A therapeutic effect on multiple myeloma was investigated in vivo using the MMG49 antibody-derived chimeric antigen receptor T-cells.

[0266] Myeloma cell line MM1s cells (4×10^5 cells) were
35 transplanted into the bone marrow of NOG mice subjected to

radiation exposure at 2.4 Gy. After 5 days, the mice were grouped into an MMG49 antibody-derived chimeric antigen receptor T-cell-administered group and a control T-cell-administered group, and each group was intravenously administered with 5×10^6 cells per mouse. After 7 days from the administration, the bone marrow was analyzed. As a result, marked growth of myeloma cells was clearly observed in all mice of the control T-cell-administered group, whereas the tumor had almost completely disappeared in the MMG49 antibody-derived chimeric antigen receptor T-cell-administered group. The results show that the administration of the MMG49 antibody-derived chimeric antigen receptor T-cells has an ability to eliminate a tumor expressing the MMG49 antigen even in vivo (FIGS. 22).

[0267] Further, a therapeutic effect on multiple myeloma was investigated in vivo using a myeloma-systemically seeded model. [0268] Myeloma cell line MM1s cells (5×10^6 cells) having introduced therein luciferase gene were intravenously transplanted into NOG mice subjected to radiation exposure at 2.4 Gy. After 5 days from the transplantation, the degree of engraftment of the tumor cells was measured using an IVIS imaging system (PerkinElmer). After that, the mice were grouped into an MMG49 antibody-derived chimeric antigen receptor T-cell-administered group and a control T-cell-administered group, and each group was intravenously administered with 3×10^6 cells per mouse 5 days and 7 days after the transplantation. After 7 days from the second T-cell administration, a tumor volume was measured again using the IVIS imaging system. As a result, marked growth of myeloma cells was clearly observed in all mice of the control T-cell-administered group, whereas the tumor had almost completely disappeared in the MMG49 antibody-derived chimeric antigen receptor T-cell-administered group (FIGS. 23). The results show that the administration of the MMG49 antibody-derived chimeric antigen receptor T-cells has an ability to eliminate a tumor expressing the MMG49 antigen even in vivo.

[0269] [Example 13]

For the epitope of the MMG49 antibody, an experiment for investigating, in more detail, the results of the investigation in Example 8 was performed. Vectors for expressing three kinds of human/mouse chimeric integrin β_7 protein as illustrated in FIGS.

5 25 were generated, and each of the expression vectors was introduced into 293T cells by a lipofection method. After 48 hours, the presence or absence of the binding of the MMG49 antibody was analyzed by FACS.

[0270] As a result, it was revealed that the MMG49 antibody
10 strongly bound to a chimeric integrin β_7 protein (ch5.1 in FIGS. 25) in which regions of the amino acid residue positions 1 to 32 and positions 91 to 798 of integrin β_7 protein were of mouse origin, and the rest (region of the amino acid residue positions 33 to 90) had a sequence of human origin in almost the same
15 manner as in the case of the integrin β_7 protein entirely of human origin (#4927 in FIG. 11) (FIGS. 25).

[0271] Thus, it was strongly suggested that the epitope of the MMG49 antibody was included in the amino acid residues at positions 33 to 90 of the human integrin β_7 protein.

20 [0272] [Example 14]

Vectors expressing the human integrin β_7 , the mouse integrin β_7 , and various variants obtained by mutating only one or two amino acids of the human integrin β_7 into an amino acid sequence of mouse origin (R35E/N36D, H38D, M41L/L42Q, and A48V)
25 were introduced into 293T cells by a lipofection method, and an experiment was performed in the same manner as in Example 8 thereafter. As a result, as shown in FIGS. 26, it was revealed that only the A48V variant had a remarkably reduced avidity for the MMG49 antibody as compared to the human integrin β_7 , and had a
30 numerical value close to that of the mouse integrin β_7 . The results revealed that the amino acid residue at position 48 of the human integrin β_7 was strongly related to the epitope of the MMG49 antibody, or included in the epitope of the MMG49 antibody.

[0273] Base sequences and amino acid sequences described
35 herein are shown below.