

Description

Title of Invention: ANTI-CD147 ANTIBODY

Technical Field

[0001]

The present invention relates to an anti-CD147 antibody exhibiting potent antitumor efficacy, a method for producing the anti-CD147 antibody, and an antitumor agent comprising the anti-CD147 antibody.

Background Art

[0002]

Advances in methods and remedies for treating cancers have revealed that cancers previously considered to be incurable are treatable and completely curable. A CTLA4 antibody and a PD1 antibody, which have been developed as antibody drugs exhibiting particularly excellent drug stability and specificity, exhibit high objective response rates against, and occasionally cure, melanoma and some solid cancers through activation of immune cells including T cells, bringing good news to cancer patients. Treatments of most intractable solid cancers with these remedies have been attempted. However, most pancreatic cancers and liver cancers, etc., do not show sensitivity to these drugs, and exhibit high recurrence rates even when treated by surgical extraction

or treated based on the use of conventional anticancer agents. Hence, treatments and remedies by which cancers can be completely cured are earnestly sought.

[0003]

CD147 is a single-pass transmembrane protein having 2 to 3 immunoglobulin-like domains, and is known to activate downstream signal-related molecules, such as FAK, MEK, Erk, JAK/STAT, AKT and MAPK family molecules through interactions between multiple CD147 proteins, or through interactions with extracellular or cell membrane surface molecules, such as CD44, Integrin family molecules, CD98, VEGFR, CypA/B, and MCT1/3/4 which are involved in growth, invasion, and inflammation, thereby accelerating the production of proteases such as MMPs, and cancer growth, metastasis, and invasion. Moreover, it has been reported that a high CD147 expression level leads to a short survival period and a poor prognosis. Hence, CD147 is considered as one of the target molecules for cancer treatment.

[0004]

Antibodies targeting CD147, ABX-CBL and Licartin have, in fact, been administered clinically to humans. ABX-CBL inhibits binding between CD147 and cyclophilin A, and not only suppresses T cell activity, but also exhibits cytotoxic activity against normal cells including CD147-positive T cells in a complement-dependent manner in blood. Clinical trials performed for

GVHD as a target disease have revealed insufficient efficacy and have resulted in observations of severe muscle pains, and thus this antibody has not been approved as a drug (Patent Literature 1, Non Patent Literature 1).

[0005]

Licartin is a biopharmaceutical prepared by adding a radioactive isotope, iodine I 131, to the Fab'2 portion of a HAb18 antibody, and has been approved as a drug in China, the relevant type of cancer being liver cancer (Non Patent Literature 2, Non Patent Literature 3). Licartin lacks the Fc portion of the antibody for activating immune cells and complements, and has not been reported to have immune-mediated toxicity and has not been reported clinically to cure liver cancer completely.

[0006]

As to other antibodies targeting CD147, an anti-CD147 monoclonal antibody blocking biological activity related to CD147, such as vascularization or VEGF-producing matrix metalloprotease production (Patent Literature 2), an anti-CD147 monoclonal antibody inhibiting T cell activation (Non Patent Literature 4), and an antibody specifically binding to the CD147 molecule characterized by having ADCC activity and CDC activity (Patent Literature 3) are known. However, a CD147 antibody lacking effector functions and exhibiting antitumor efficacy is unknown. Moreover, the association

between activation of the cell signal transduction system through CD147 and antitumor efficacy is unknown.

Citation List

Patent Literature

[0007]

Patent Literature 1: WO1999/045031

Patent Literature 2: WO2010/036460

Patent Literature 3: WO2017/061602

Non Patent Literature

[0008]

Non Patent Literature 1: Deeg H, et al., J. Blood., 2052-2058, 2001

Non Patent Literature 2: Chen Z, et al., Int. J. Radiation Oncology Biol. Phys., 435-444, 2006

Non Patent Literature 3: Xu J, et al., Hepatology, 269-276, 2007

Non Patent Literature 4: Koch C, et al., International Immunology, 777-786, 1999

Summary of Invention

Problem to be solved by the Invention.

[0009]

An object of the present invention is to provide a novel anti-CD147 antibody having novel pharmacologic effects, excellent safety, and high antitumor efficacy, a pharmaceutical product comprising the antibody, and a

method for treating tumors using the antibody or the pharmaceutical product and the like.

Solution

[0010]

As a result of intensive studies to achieve the above object, the present inventors have discovered for the first time that the activation of signal-related molecules through CD147 is associated with antitumor efficacy. The present inventors have successfully obtained a CD147-specific antibody activating CD147 and exhibiting high antitumor efficacy. The antibody of the present invention has the feature of exhibiting high antitumor efficacy in a manner independent of effector functions. Antibodies exhibiting antitumor efficacy in an effector function-dependent manner have been reported. However, the antibody of the present invention has the feature of not acting on T cells and PBMC, and exhibiting high antitumor efficacy in a manner independent of effector functions, and thus is an excellent antibody expected to be safe as a pharmaceutical product. The antibody of the present invention exhibits efficacy in liver cancer cells which is remarkably more strong than that of sorafenib that is used as one of the standard care drugs for liver cancer. The antibody of the present invention exhibits efficacy in pancreatic cancer cells which is remarkably more strong than that of gemcitabine

that is used as one of the standard of care drugs for pancreatic cancer. The antibody of the present invention exhibits efficacy in chronic myeloid leukemia cells which is remarkably more strong than that of imatinib that is used as one of the standard of care drugs for chronic myeloid leukemia. The present inventors have identified that the CD147 antibody of the present invention activates the p38MAPK and SMAD signal transduction system in cancer cells. The present inventors have identified that the CD147 antibody of the present invention exhibits excellent antitumor efficacy in SMAD4-positive cells.

[0011]

The invention of the present application encompasses the following aspects.

[1]

An antibody against human CD147 or an antigen-binding fragment thereof, which competes with at least one antibody selected from the group consisting of the following (A) to (F) for binding to human CD147 and which activates signal transduction through CD147:

(A) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 71 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 69;

(B) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented

by SEQ ID NO: 51 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 49;

(C) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 61 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 59,

(D) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 81 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 79;

(E) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 10 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 8; and

(F) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 20 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 18.

[2]

An antibody against human CD147 or an antigen-binding fragment thereof, which binds to an epitope to which at least one antibody selected from the group

consisting of the following (A) to (F) binds, and which activates signal transduction through CD147:

(A) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 71 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 69;

(B) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 51 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 49;

(C) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 61 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 59;

(D) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 81 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 79;

(E) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 10 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 8; and

(F) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 20 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 18.

[3]

The antibody against human CD147 or the antigen-binding fragment thereof according to [1] or [2], which has decreased ADCC activity or lacks ADCC activity.

[4]

The antibody against human CD147 or the antigen-binding fragment thereof according to any one of [1] to [3], which has decreased CDC activity or lacks CDC activity.

[5]

The antibody against human CD147 or the antigen-binding fragment thereof according to any one of [1] to [4], which has decreased ADCP activity or lacks ADCP activity.

[0012]

[6]

The antibody or the antigen-binding fragment thereof according to any one of [1] to [5], which binds to an epitope comprising residues of arginine (Arg) at position 106 to glycine (Gly) at position 165 in SEQ ID NO: 3.

[7]

The antibody or the antigen-binding fragment thereof according to any one of [1] to [6], which binds to an epitope comprising residues of arginine (Arg) at position 106, lysine (Lys) at position 108, alanine (Ala) at position 109, valine (Val) at position 110, lysine (Lys) at position 127, serine (Ser) at position 128, glutamic acid (Glu) at position 129, serine (Ser) at position 130, valine (Val) at position 131, proline (Pro) at position 132, proline (Pro) at position 133, valine (Val) at position 134, glutamine (Gln) at position 164 and glycine (Gly) at position 165 in the amino acid sequence set forth in SEQ ID NO: 3.

[8]

The antibody or the antigen-binding fragment thereof according to any one of [1] to [7], wherein:

in the heavy chain sequence, which comprises a variable region having CDRH1, CDRH2 and CDRH3, the CDRH1 consists of an amino acid sequence represented by SEQ ID NO: 75, the CDRH2 consists of an amino acid sequence represented by SEQ ID NO: 76, and the CDRH3 consists of an amino acid sequence represented by SEQ ID NO: 77; and

in the light chain sequence, which comprises a variable region having CDRL1, CDRL2 and CDRL3, the CDRL1 consists of an amino acid sequence represented by SEQ ID NO: 72, the CDRL2 consists of an amino acid sequence represented by SEQ ID NO: 73, and the CDRL3 consists of an amino acid sequence represented by SEQ ID NO: 74.

[9]

The antibody or the antigen-binding fragment thereof according to any one of [1] to [5], which binds to an epitope comprising an amino acid sequence set forth in SEQ ID NO: 143 or an amino acid sequence in which one or several amino acids are deleted, substituted or added in the sequence of SEQ ID NO: 143.

[10]

The antibody or the antigen-binding fragment thereof according to any one of [1] to [5] or (9), wherein:

in the heavy chain sequence, which comprises a variable region having CDRH1, CDRH2 and CDRH3, the CDRH1 consists of an amino acid sequence represented by SEQ ID NO: 55, the CDRH2 consists of an amino acid sequence represented by SEQ ID NO: 56, and the CDRH3 consists of an amino acid sequence represented by SEQ ID NO: 57; and

in the light chain sequence, which comprises a variable region having CDRL1, CDRL2 and CDRL3, the CDRL1 consists of an amino acid sequence represented by SEQ ID NO: 52, the CDRL2 consists of an amino acid sequence represented by SEQ ID NO: 53, and the CDRL3 consists of an amino acid sequence represented by SEQ ID NO: 54.

[0013]

[11]

The antibody or the antigen-binding fragment thereof according to any one of [1] to [5] or (9), wherein:

in the heavy chain sequence, which comprises a variable region having CDRH1, CDRH2 and CDRH3, the CDRH1 consists of an amino acid sequence represented by SEQ ID NO: 65, the CDRH2 consists of an amino acid sequence represented by SEQ ID NO: 66, and the CDRH3 consists of an amino acid sequence represented by SEQ ID NO: 67; and

in the light chain sequence, which comprises a variable region having CDRL1, CDRL2 and CDRL3, the CDRL1 consists of an amino acid sequence represented by SEQ ID NO: 62, the CDRL2 consists of an amino acid sequence represented by SEQ ID NO: 63, and the CDRL3 consists of an amino acid sequence represented by SEQ ID NO: 64.

[12]

The antibody or the antigen-binding fragment thereof according to any one of [1] to [5] or (9), wherein:

in the heavy chain sequence, which comprises a variable region having CDRH1, CDRH2 and CDRH3, the CDRH1 consists of an amino acid sequence represented by SEQ ID NO: 85, the CDRH2 consists of an amino acid sequence represented by SEQ ID NO: 86, and the CDRH3 consists of an amino acid sequence represented by SEQ ID NO: 87; and

in the light chain sequence, which comprises a variable region having CDRL1, CDRL2 and CDRL3, the CDRL1 consists of an amino acid sequence represented by SEQ ID NO: 82, the CDRL2 consists of an amino acid sequence represented by SEQ ID NO: 83, and the CDRL3 consists of an amino acid sequence represented by SEQ ID NO: 84.

[13]

The antigen-binding fragment of the antibody according to any one of [1] to [12], which is selected from the group consisting of Fab, F(ab')₂, Fab' and Fv.

[14]

The antibody according to any one of [1] to [12], which is scFv.

[15]

The antibody or the antigen-binding fragment thereof according to any one of [1] to [12], which is a chimeric antibody.

[0014]

[16]

The antibody or the antigen-binding fragment thereof according to any one of [1] to [12], which is humanized.

[17]

The antibody according to any one of [1] to [16], wherein the heavy chain comprises a human immunoglobulin G1 heavy chain constant region, a human immunoglobulin G2 heavy chain constant region or a human immunoglobulin G4 heavy chain constant region, and the light chain comprises a human immunoglobulin κ light chain constant region.

[18]

The antibody according to [17], wherein the heavy chain comprises the human immunoglobulin G4 heavy chain constant region.

[19]

The antibody according to [18], wherein within the human immunoglobulin G4 heavy chain constant region, the serine (Ser) at position 228 as indicated by the EU index is substituted with a proline (Pro).

[20]

The antibody according to [18], wherein within the human immunoglobulin G4 heavy chain constant region, the phenylalanine (Phe) at position 234 as indicated by the EU index is substituted with an alanine (Ala), and the leucine (Leu) at position 235 as indicated by the EU index is substituted with an alanine (Ala).

[0015]

[21]

The antibody according to [18], wherein within the human immunoglobulin G4 heavy chain constant region, the serine (Ser) at position 228 as indicated by the EU index is substituted with a proline (Pro), the phenylalanine (Phe) at position 234 as indicated by the EU index is substituted with an alanine (Ala), and the leucine (Leu) at position 235 as indicated by the EU index is substituted with an alanine (Ala).

[22]

The antibody according to [17], wherein the heavy chain comprises the human immunoglobulin G2 heavy chain constant region.

[23]

An antibody against human CD147 or an antigen-binding fragment thereof, which comprises the following (c) and (d), and activates signal transduction through CD147:

(c) a heavy chain variable region selected from the group consisting of the following (c1) to (c4):

(c1) a heavy chain variable region consisting of amino acid residues at positions 20 to 136 in an amino acid sequence represented by SEQ ID NO: 135;

(c2) a heavy chain variable region consisting of amino acid residues at positions 20 to 136 in an amino acid sequence represented by SEQ ID NO: 147;

(c3) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (c1) or (c2); and

(c4) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence according to any one of (c1) to (c3); and

(d) a light chain variable region selected from the group consisting of the following (d1) to (d5):

(d1) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 137;

(d2) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 149;

(d3) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 151;

(d4) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence according to any one of (d1) to (d3); and

(d5) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence according to any one of (d1) to (d4).

[24]

The antibody or the antigen-binding fragment thereof according to [23], which comprises:

the heavy chain variable region consisting of amino acid residues at positions 20 to 136 in the amino acid sequence represented by SEQ ID NO: 135; and

the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 137.

[0016]

[25]

The antibody or the antigen-binding fragment thereof according to [23], which comprises:

the heavy chain consisting of amino acid residues at positions 20 to 463 in the amino acid sequence represented by SEQ ID NO: 135; and

the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 137.

[26]

The antibody or the antigen-binding fragment thereof according to [23], which comprises:

the heavy chain variable region consisting of amino acid residues at positions 20 to 136 in the amino acid sequence represented by SEQ ID NO: 147; and

the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 149.

[27]

The antibody or the antigen-binding fragment thereof according to [23], which comprises:

the heavy chain consisting of amino acid residues at positions 20 to 463 in the amino acid sequence represented by SEQ ID NO: 147; and

the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 149.

[28]

The antibody or the antigen-binding fragment thereof according to [23], which comprises:

the heavy chain variable region consisting of amino acid residues at positions 20 to 136 in the amino acid sequence represented by SEQ ID NO: 147; and

the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 151.

[29]

The antibody or the antigen-binding fragment thereof according to [23], which comprises:

the heavy chain consisting of amino acid residues at positions 20 to 463 in the amino acid sequence represented by SEQ ID NO: 147; and

the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 151.

[30]

An antibody against human CD147 or an antigen-binding fragment thereof, which comprises the following (a) and (b), and activates signal transduction through CD147:

(a) a heavy chain variable region selected from the group consisting of the following (a1) to (a4):

(a1) a heavy chain variable region consisting of amino acid residues at positions 20 to 140 in an amino acid sequence represented by SEQ ID NO: 123;

(a2) a heavy chain variable region consisting of amino acid residues at positions 20 to 140 in an amino acid sequence represented by SEQ ID NO: 125;

(a3) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (a1) or (a2); and

(a4) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence according to any one of (a1) to (a3); and

(b) a light chain variable region selected from the group consisting of the following (b1) to (b3):

(b1) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 127;

(b2) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (b1); and

(b3) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence of (b1) or (b2).

[0017]

[31]

The antibody or the antigen-binding fragment thereof according to [30], which comprises:

the heavy chain variable region consisting of amino acid residues at positions 20 to 140 in the amino acid sequence represented by SEQ ID NO: 123 or the heavy chain variable region consisting of amino acid residues at positions 20 to 140 in the amino acid sequence represented by SEQ ID NO: 125; and

the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 127.

[32]

The antibody or the antigen-binding fragment thereof according to [30], which comprises:

the heavy chain consisting of amino acid residues at positions 20 to 466 in the amino acid sequence represented by SEQ ID NO: 123 or the heavy chain consisting of amino acid residues at positions 20 to 467 in the amino acid sequence represented by SEQ ID NO: 125; and

the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 127.

[33]

An antibody against human CD147 or an antigen-binding fragment thereof, which comprises the following

(e) and (f), and activates signal transduction through CD147:

(e) a heavy chain variable region selected from the group consisting of the following (e1) to (e4):

(e1) a heavy chain variable region consisting of amino acid residues at positions 20 to 137 in an amino acid sequence represented by SEQ ID NO: 129;

(e2) a heavy chain variable region consisting of amino acid residues at positions 20 to 137 in an amino acid sequence represented by SEQ ID NO: 131;

(e3) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (e1) or (e2); and

(e4) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence according to any one of (e1) to (e3); and

(f) a light chain variable region selected from the group consisting of the following (f1) to (f3):

(f1) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 133;

(f2) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework

region outside each CDR sequence in the sequence of (f1);
and

(f3) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence of (f1) or (f2).

[34]

The antibody or the antigen-binding fragment thereof according to [33], which comprises:

the heavy chain variable region consisting of amino acid residues at positions 20 to 137 in the amino acid sequence represented by SEQ ID NO: 129 or the heavy chain variable region consisting of amino acid residues at positions 20 to 137 in the amino acid sequence represented by SEQ ID NO: 131; and

the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 133.

[35]

The antibody or the antigen-binding fragment thereof according to [33], which comprises:

the heavy chain consisting of amino acid residues at positions 20 to 463 in the amino acid sequence represented by SEQ ID NO: 129 or the heavy chain consisting of amino acid residues at positions 20 to 464 in the amino acid sequence represented by SEQ ID NO: 131; and

the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 133.

[0018]

[36]

An antibody against human CD147 or an antigen-binding fragment thereof, which comprises the following (g) and (h), and activates signal transduction through CD147:

(g) a heavy chain variable region selected from the group consisting of the following (g1) to (g3):

(g1) a heavy chain variable region consisting of amino acid residues at positions 20 to 138 in an amino acid sequence represented by SEQ ID NO: 139;

(g2) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (g1); and

(g3) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence of (g1) or (g2); and

(h) a light chain variable region selected from the group consisting of the following (h1) to (h3):

(h1) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 141;

(h2) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (h1); and

(h3) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence of (h1) or (h2).

[37]

The antibody or the antigen-binding fragment thereof according to [36], which comprises:

the heavy chain variable region consisting of amino acid residues at positions 20 to 138 in the amino acid sequence represented by SEQ ID NO: 139; and

the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 141.

[38]

The antibody or the antigen-binding fragment thereof according to [36], which comprises:

the heavy chain consisting of amino acid residues at positions 20 to 464 in the amino acid sequence represented by SEQ ID NO: 139; and

the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 141.

[39]

The antibody against human CD147 or the antigen-binding fragment thereof according to any one of [23] to [38], which has decreased ADCC activity or lacks ADCC activity.

[40]

The antibody against human CD147 or the antigen-binding fragment thereof according to any one of [23] to [39], which has decreased CDC activity or lacks CDC activity.

[0019]

[41]

The antibody against human CD147 or the antigen-binding fragment thereof according to any one of [23] to [40], which has decreased ADCP activity or lacks ADCP activity.

[42]

A pharmaceutical composition comprising at least one of the antibodies or antigen-binding fragments thereof according to any one of [1] to [41].

[43]

The pharmaceutical composition according to [42], which is an antitumor agent.

[44]

The pharmaceutical composition according to [43], wherein the tumor is a tumor expressing CD147.

[45]

The pharmaceutical composition according to [43] or [44], wherein the tumor is pancreatic cancer, liver cancer, gastric cancer, colon cancer, renal cancer, breast cancer, uterine cancer, ovarian cancer, lung cancer, lymphoma, thyroid cancer, skin cancer, head and neck cancer, sarcoma, prostate cancer, bladder cancer, brain tumor, gastrointestinal stromal tumor (GIST), leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), malignant lymphoma, B-cell lymphoma, non-Hodgkin's lymphoma or diffuse large B-cell lymphoma (DLBCL).

[0020]

[46]

The pharmaceutical composition according to any one of [43] to [45], wherein the tumor is pancreatic cancer, liver cancer, gastric cancer, colon cancer, renal cancer, leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), malignant lymphoma, B-cell lymphoma, non-Hodgkin's lymphoma or diffuse large B-cell lymphoma (DLBCL).

[47]

The pharmaceutical composition according to any one of [43] to [46], wherein the tumor is a SMAD4-positive tumor or a tumor having a decreased expression level of or lacking the expression of KLF5.

[48]

The pharmaceutical composition according to any one of [42] to [47], further comprising another antitumor agent.

[49]

A method for treating a tumor, comprising administering the antibody or the antigen-binding fragment thereof according to any one of [1] to [41] or the pharmaceutical composition according to any one of [42] to [48] to a patient.

[50]

The method according to [49], wherein the tumor is a tumor expressing CD147.

[0021]

[51]

The method according to any one of [49] or [50], wherein the tumor is pancreatic cancer, liver cancer, gastric cancer, colon cancer, renal cancer, breast cancer, uterine cancer, ovarian cancer, lung cancer, lymphoma, thyroid cancer, skin cancer, head and neck cancer, sarcoma, prostate cancer, bladder cancer, brain tumor, gastrointestinal stromal tumor (GIST), leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), malignant lymphoma, B-cell lymphoma, non-Hodgkin's lymphoma or diffuse large B-cell lymphoma (DLBCL).

[52]

The method according to any one of [49] to [51], wherein the tumor is pancreatic cancer, liver cancer, gastric cancer, colon cancer, renal cancer, leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), malignant lymphoma, B-cell lymphoma, non-Hodgkin's lymphoma or diffuse large B-cell lymphoma (DLBCL).

[53]

The method according to any one of [49] to [52], wherein the tumor is a SMAD4-positive tumor or a tumor having a decreased expression level of or lacking the expression of KLF5.

[54]

The method according to any one of [49] to [53], wherein the antibody or the antigen-binding fragment thereof, or the pharmaceutical composition is administered in combination with another antitumor agent.

[55]

A polynucleotide which encodes the antibody or the antigen-binding fragment thereof according to any one of [1] to [41].

[0022]

[56]

The polynucleotide according to [55], which comprises a polynucleotide selected from the group consisting of the following (j1) to (j3):

(j1) a polynucleotide encoding CDRH1 consisting of an amino acid sequence represented by SEQ ID NO: 75, CDRH2 consisting of an amino acid sequence represented by SEQ ID NO: 76 and CDRH3 consisting of an amino acid sequence represented by SEQ ID NO: 77, and a polynucleotide encoding CDRL1 consisting of an amino acid sequence represented by SEQ ID NO: 72, CDRL2 consisting of an amino acid sequence represented by SEQ ID NO: 73 and CDRL3 consisting of an amino acid sequence represented by SEQ ID NO: 74;

(j2) a polynucleotide having at least 95% sequence identity with the nucleotide sequence according to (j1); and

(j3) a polynucleotide in which one to several nucleotides are substituted, deleted or added in the polynucleotide according to (j1) or (j2).

[57]

The polynucleotide according to [55], which comprises a polynucleotide selected from the group consisting of the following (i1) to (i3):

(i1) a polynucleotide encoding CDRH1 consisting of an amino acid sequence represented by SEQ ID NO: 55, CDRH2 consisting of an amino acid sequence represented by SEQ ID NO: 56 and CDRH3 consisting of an amino acid

sequence represented by SEQ ID NO: 57, and a polynucleotide encoding CDRL1 consisting of an amino acid sequence represented by SEQ ID NO: 52, CDRL2 consisting of an amino acid sequence represented by SEQ ID NO: 53 and CDRL3 consisting of an amino acid sequence represented by SEQ ID NO: 54;

(i2) a polynucleotide having at least 95% sequence identity with the nucleotide sequence according to (i1); and

(i3) a polynucleotide in which one to several nucleotides are substituted, deleted or added in the polynucleotide according to (i1) or (i2).

[58]

The polynucleotide according to [55], which comprises a polynucleotide selected from the group consisting of the following (k1) to (k3):

(k1) a polynucleotide encoding CDRH1 consisting of an amino acid sequence represented by SEQ ID NO: 65, CDRH2 consisting of an amino acid sequence represented by SEQ ID NO: 66 and CDRH3 consisting of an amino acid sequence represented by SEQ ID NO: 67, and a polynucleotide encoding CDRL1 consisting of an amino acid sequence represented by SEQ ID NO: 62, CDRL2 consisting of an amino acid sequence represented by SEQ ID NO: 63 and CDRL3 consisting of an amino acid sequence represented by SEQ ID NO: 64;

(k2) a polynucleotide having at least 95% sequence identity with the nucleotide sequence according to (k1); and

(k3) a polynucleotide in which one to several nucleotides are substituted, deleted or added in the polynucleotide according to (k1) or (k2).

[59]

The polynucleotide according to [55], which comprises a polynucleotide selected from the group consisting of the following (m1) to (m3):

(m1) a polynucleotide encoding CDRH1 consisting of an amino acid sequence represented by SEQ ID NO: 85, CDRH2 consisting of an amino acid sequence represented by SEQ ID NO: 86 and CDRH3 consisting of an amino acid sequence represented by SEQ ID NO: 87, and a polynucleotide encoding CDRL1 consisting of an amino acid sequence represented by SEQ ID NO: 82, CDRL2 consisting of an amino acid sequence represented by SEQ ID NO: 83 and CDRL3 consisting of an amino acid sequence represented by SEQ ID NO: 84;

(m2) a polynucleotide having at least 95% sequence identity with the nucleotide sequence according to (m1); and

(m3) a polynucleotide in which one to several nucleotides are substituted, deleted or added in the polynucleotide according to (m1) or (m2).

[60]

An expression vector comprising the polynucleotide according to any one of [55] to [59].

[0023]

[61]

A host cell transformed with the expression vector according to [60].

[62]

A method for producing the antibody or the antigen-binding fragment thereof according to any one of [1] to [41], comprising a step of culturing the host cell according to [61] and collecting a target antibody or an antigen-binding fragment thereof from a culture supernatant.

[63]

The antibody or the antigen-binding fragment thereof according to any one of [1] to [41], wherein the activation of signal transduction through CD147 is an activation of p38 and/or an activation of SMAD4.

[64]

The antibody or the antigen-binding fragment thereof according to [63], wherein the activation of p38MAPK and/or the activation of SMAD4 is an increase in p38MAPK expression level, the phosphorylation of p38MAPK, the phosphorylation of HSP27, an increase in CXCL8 expression level, an increase in rhoB expression level, a decrease in KLF5 mRNA or a decrease in KLF5 protein expression level.

[65]

A method for treating a tumor, comprising administering the antibody or the antigen-binding fragment thereof according to [63] or [64].

[0024]

[66]

A method for predicting responsiveness to cancer treatment, comprising:

detecting the expression of SMAD4 or the expression of KLF5 in a biological sample derived from a cancer patient ; and

determining a patient's responsiveness to cancer treatment with the antibody or the antigen-binding fragment thereof according to any one of [1] to [41] or the pharmaceutical composition according to any one of [42] to [48] to be positive, when SMAD4 expression is detected in the patient, or a decrease in KLF5 expression level or a lack of KLF5 expression is detected in the patient.

[67]

A method for selecting subjects for cancer treatment, comprising:

detecting the expression of SMAD4 or the expression of KLF5 in a biological sample derived from a cancer patient; and

selecting a patient as a subject of cancer treatment with the antibody or the antigen-binding fragment thereof

according to any one of [1] to [41] or the pharmaceutical composition according to any one of [42] to [48], when SMAD4 expression is detected in the patient or a decrease in KLF5 expression level or a lack of KLF5 expression is detected in the patient.

[68]

A method for treating cancer, comprising:

using a biological sample derived from a cancer patient to detect the presence or the absence of the expression of SMAD4 or to detect the expression of KLF5 in the biological sample; and

administering the antibody or the antigen-binding fragment thereof according to any one of [1] to [41] or the pharmaceutical composition according to any one of [42] to [48] to a patient in whom SMAD4 expression is detected or to a patient in whom a decrease in KLF5 expression level or a lack of KLF5 expression is detected.

[69]

A kit for determining responsiveness to cancer treatment with the antibody or the antigen-binding fragment thereof according to any one of [1] to [41] or the pharmaceutical composition according to any one of [42] to [48], the kit comprising at least one means for detecting the expression of SMAD4 or the expression of KLF5 in a biological sample derived from a cancer patient.

[70]

An antibody-drug complex comprising the antibody or the antigen-binding fragment thereof according to any one of [1] to [41] conjugated to another drug.

[0025]

[71]

A bispecific antibody comprising: the antigen-binding fragment of the antibody according to any one of [1] to [41]; and an antigen-binding fragment which binds to an antigen other than CD147.

Advantageous Effects of Invention

[0026]

The antibody of the present invention is an antibody that specifically recognizes CD147, and is characterized by activating signal-related molecules through CD147, and having high antitumor efficacy. CD147 is expressed not only in tumor cells, but also in blood cells. However, the antibody of the present invention does not act on T cells or PBMC and is independent of effector functions, and thus has an advantage of posing fewer safety concerns in development thereof as an antitumor agent. The antibody of the present invention exhibits extremely high antitumor efficacy. The antibody of the present invention exhibits efficacy in liver cancer cells which is remarkably more strong than that of sorafenib that is used as one of the standard of care drugs for liver cancer. The antibody of the present invention exhibits

efficacy in pancreatic cancer cells which is remarkably more strong than that of gemcitabine that is used as one of the standard of care drugs for pancreatic cancer. The antibody of the present invention exhibits efficacy in chronic myeloid leukemia cells which is remarkably more strong than that of imatinib that is used as one of the standard of care drugs for chronic myeloid leukemia.

[Brief Description of Drawings]

[0027]

[Figure 1] Figures 1(a) to (c) show changes in tumor volume in a PANC-1 xenograft model.

[Figure 2-1] Figure 2-1 shows the binding activity to human CD147 or monkey CD147.

[Figure 2-2] Figure 2-2 shows the binding activity to human CD147 or monkey CD147.

[Figure 2-3] Figure 2-3 shows the binding activity to human CD147 or monkey CD147.

[Figure 3] Figure 3 shows a comparison of the amino acid sequences between human CD147 and cynomolgus monkey CD147 and the positions of mu1 to mu9 in human CD147 and cynomolgus monkey CD147.

[Figure 4-1] Figure 4-1 shows the nucleotide sequence and the amino acid sequence of the LN22R8 light chain variable region and the amino acid sequences of CDRL1, CDRL2, and CDRL3 of the LN22R8 light chain.

[Figure 4-2] Figure 4-2 shows the nucleotide sequence and the amino acid sequence of the LN22R8 heavy chain variable region and the amino acid sequences of CDRH1, CDRH2, and CDRH3 of the LN22R8 heavy chain.

[Figure 5-1] Figure 5-1 shows the nucleotide sequence and the amino acid sequence of the 2P10F2 light chain variable region and the amino acid sequences of CDRL1, CDRL2, and CDRL3 of the 2P10F2 light chain.

[Figure 5-2] Figure 5-2 shows the nucleotide sequence and the amino acid sequence of 2P10F2 heavy chain variable region and the amino acid sequences of CDRH1, CDRH2, and CDRH3 of the 2P10F2 heavy chain.

[Figure 6] Figure 6 shows the ADCC activities of human chimeric antibodies.

[Figure 7] Figure 7 shows the CDC activities of human chimeric antibodies.

[Figure 8] Figure 8 shows the ADCP activities of human chimeric antibodies.

[Figure 9-1] Figures 9-1(a) to (d) show the antitumor efficacy of antibodies in a MIA PaCa-2 subcutaneous implantation model using NOD-scid mice.

[Figure 9-2] Figures 9-2(e) to (g) show the antitumor efficacy of antibodies in MIA PaCa-2 subcutaneous implantation model using NOD-scid mice.

[Figure 10] Figure 10 shows the antitumor efficacy of LN22R8chIgG4P in a MIA PaCa-2 subcutaneous implantation model using NOG mice.

[0028]

[Figure 11] Figures 11(a) to (d) show the antitumor efficacy of r#84, r#101, r#110, and r#131 in a MIA PaCa-2 subcutaneous implantation model using NOD-scid mice.

[Figure 12] Figure 12 shows a competition assay of 2P10F2 with r#84, r#101, r#110, and r#131.

[Figure 13-1] Figures 13-1(a) to (d) show the antitumor efficacy of chimeric antibodies in a MIA PaCa-2 subcutaneous implantation model.

[Figure 13-2] Figures 13-2(e) to (h) show the antitumor efficacy of chimeric antibodies in a MIA PaCa-2 subcutaneous implantation model.

[Figure 13-3] Figures 13-3 (i) to (l) show the antitumor efficacy of chimeric antibodies in a MIA PaCa-2 subcutaneous implantation model.

[Figure 13-4] Figure 13-4 (m) and (n) show the antitumor efficacy of chimeric antibodies in a MIA PaCa-2 subcutaneous implantation model.

[Figure 14] Figure 14(a) shows the design of the variable region of humanized antibody heavy chain #84H1h, and Figure 14(b) shows the design of the variable region of humanized antibody light chain #84L2h.

[Figure 15] Figure 15(a) shows the design of the variable region of humanized antibody heavy chain #101H1h, and Figure 15(b) shows the design of the variable region of humanized antibody light chain #101L2h.

[Figure 16] Figure 16(a) shows the design of the variable region of humanized antibody heavy chain #110H1h, Figure 16(b) shows the design of the variable region of humanized antibody heavy chain #110H13h, Figure 16(c) shows the design of the variable region of humanized antibody light chain #110L4h, Figure 16(d) shows the design of the variable region of humanized antibody light chain #110L2h, and Figure 16(e) shows the design of the variable region of humanized antibody light chain #110L12h.

[Figure 17] Figure 17(a) shows the design of the variable region of humanized antibody heavy chain #131H2h, and Figure 17(b) shows the design of the variable region of humanized antibody light chain #131L2h.

[Figure 18] Figures 18(a) to (d) show the antitumor efficacy of humanized antibodies in a MIA PaCa-2 subcutaneous implantation model.

[Figure 19] Figure 19 shows p38MAPK phosphorylation by an anti-human CD147 human chimeric antibody.

[Figure 20] Figures 20 (a) and (b) show HSP27 phosphorylation by an anti-human CD147 human chimeric antibody.

[0029]

[Figure 21] Figures 21(a) to (c) show p38MAPK phosphorylation by an anti-human CD147 human chimeric antibody in tumors.

[Figure 22] Figures 22(a) to (c) show expression of cxcl8 and rhoB by anti-human CD147 human chimeric antibodies.

[Figure 23] Figures 23 (a) to (c) show expression of cxcl8 and rhoB by an anti-human CD147 rat antibody and its human chimeric antibody.

[Figure 24] Figure 24 shows the results of evaluating the antitumor efficacy of anti-CD147 antibodies and gemcitabine using SMAD4-negative pancreatic cancer cells BxPC-3.

[Figure 25] Figures 25(a) to (c) show the results of evaluating the antitumor efficacy of anti-CD147 human chimeric antibodies using BxPC-3 stably expressing SMAD4.

[Figure 26] Figures 26(a) and (b) show p38MAPK phosphorylation by an anti-CD147 human chimeric antibody in a subcutaneous implantation model with pancreatic cancer cells BxPC-3 expressing SMAD4.

[Figure 27] Figure 27 shows the antitumor efficacy of an anti-CD147 human chimeric antibody in a gemcitabine-resistant pancreatic cancer tumor model.

[Figure 28] Figure 28(a) shows the confirmation of CD147 expression in Hep G2 cells using a flow cytometer.

Figure 28(b) shows p38MAPK activation by humanized CD147 antibodies in liver cancer cells.

[Figure 29] Figures 29(a) to (d) show comparisons of the antitumor efficacy of CD147 antibodies with sorafenib in liver cancer.

[Figure 30] Figure 30 shows CD147-APC binding in CD3- and CD4-positive cells and CD3- and CD8-positive cells.

[0030]

[Figure 31] Figure 31 shows evaluation of the influence of anti-human CD147 antibodies on the growth of human peripheral blood mononuclear cells.

[Figure 32] Figure 32 shows evaluation of the influence of anti-human CD147 antibodies on cytokine production by human peripheral blood lymphocytes.

[Figure 33-1] Figure 33-1 shows the nucleotide sequence and the amino acid sequence of the rat_CD147_#84 light chain variable region, and the amino acid sequences of CDRL1, CDRL2, and CDRL3 of the rat_CD147_#84 light chain.

[Figure 33-2] Figure 33-2 shows the nucleotide sequence and the amino acid sequence of the rat_CD147_#84 heavy chain variable region and the amino acid sequences of CDRH1, CDRH2, and CDRH3 of the rat_CD147_#84 heavy chain.

[Figure 34-1] Figure 34-1 shows the nucleotide sequence and the amino acid sequence of the rat_CD147_#101 light chain variable region, and the amino acid sequences of CDRL1, CDRL2, and CDRL3 of the rat_CD147_#101 light chain.

[Figure 34-2] Figure 34-2 shows the nucleotide sequence and the amino acid sequence of the rat_CD147_#101 heavy chain variable region and the amino acid sequences of CDRH1, CDRH2, and CDRH3 of the rat_CD147_#101 heavy chain.

[Figure 35-1] Figure 35-1 shows the nucleotide sequence and the amino acid sequence of the rat_CD147_#110 light

chain variable region, and the amino acid sequences of CDRL1, CDRL2, and CDRL3 of the rat_CD147_#110 light chain.

[Figure 35-2] Figure 35-2 shows the nucleotide sequence and the amino acid sequence of the rat_CD147_#110 heavy chain variable region and the amino acid sequences of CDRH1, CDRH2, and CDRH3 of the rat_CD147_#110 heavy chain.

[Figure 36-1] Figure 36-1 shows the nucleotide sequence and the amino acid sequence of the rat_CD147_#131 light chain variable region, and the amino acid sequences of CDRL1, CDRL2, and CDRL3 of the rat_CD147_#131 light chain.

[Figure 36-2] Figure 36-2 shows the nucleotide sequence and the amino acid sequence of the rat_CD147_#131 heavy chain variable region and the amino acid sequences of CDRH1, CDRH2, and CDRH3 of the rat_CD147_#131 heavy chain.

[Figure 37-1] Figure 37-1 shows the amino acid sequence and the nucleotide sequence of #84H1hIgG2.

[Figure 37-2] Figure 37-2 shows the amino acid sequence and the nucleotide sequence of #84H1hIgG4P.

[Figure 37-3] Figure 37-3 shows the amino acid sequence and the nucleotide sequence of #84L2h.

[Figure 38-1] Figure 38-1 shows the amino acid sequence and the nucleotide sequence of #101H1hIgG2.

[Figure 38-2] Figure 38-2 shows the amino acid sequence and the nucleotide sequence of #101H1hIgG4P.

[Figure 38-3] Figure 38-3 shows the amino acid sequence and the nucleotide sequence of #101L2h.

[Figure 39-1] Figure 39-1 shows the amino acid sequence and the nucleotide sequence of #110H1hIgG4P.

[Figure 39-2] Figure 39-2 shows the amino acid sequence and the nucleotide sequence of #110H13hIgG4P.

[Figure 39-3] Figure 39-3 shows the amino acid sequence and the nucleotide sequence of #110L4h.

[Figure 39-4] Figure 39-4 shows the amino acid sequence and the nucleotide sequence of #110L2h.

[Figure 39-5] Figure 39-5 shows the amino acid sequence and the nucleotide sequence of #110L12h.

[Figure 40-1] Figure 40-1 shows the amino acid sequence and the nucleotide sequence of #131H2hIgG2.

[Figure 40-2] Figure 40-2 shows the amino acid sequence and the nucleotide sequence of #131L2h.

[0031]

[Figure 41] Figure 41 shows a ribbon diagram showing two complexes contained in the asymmetric unit. CD147 is shown in black, and the heavy chain (H CHAIN) and the light chain (L CHAIN) of the antibody are shown in gray.

[Figure 42] Figure 42 shows the interaction surface between CD147 and the antibody. The amino acids of CD147 in the vicinity of the antibody are represented by stick models and are labeled with characters. The other parts of CD147 are represented by black ribbon models.

Meanwhile, the amino acids of the antibody in the vicinity of CD147 are represented by thin line models,

and the other parts of the antibody are represented by gray ribbon models.

[Figure 43] Figure 43 shows the antitumor efficacy of chimeric and humanized CD147 antibodies in a gastric cancer model.

[Figure 44] Figure 44 shows the antitumor efficacy of a humanized CD147 antibody in a chronic myeloid leukemia (CML) model.

[Figure 45] Figure 45 shows the antitumor efficacy of humanized CD147 antibodies in a colon cancer model.

[Figure 46] Figure 46 shows the antitumor efficacy of humanized CD147 antibodies in a renal cancer model.

[Figure 47] Figure 47 shows the antitumor efficacy of a humanized CD147 antibody in an acute myeloid leukemia (AML) model.

[Figure 48] Figure 48 shows the antitumor efficacy of humanized CD147 antibodies in a pancreatic cancer model.

[Figure 49] Figure 49 shows the results of competitive ELISA.

[Figure 50] Figure 50 (a) shows the antitumor efficacy of a humanized CD147 antibody using MIA PaCa-2 cells, and Figure 50 (b) shows the antitumor efficacy of the humanized CD147 antibody using MIA PaCa-2 cells expressing KLF5 for comparison.

Description of Embodiments

[0032]

Preferred embodiments of the present invention are as described below with reference to the drawings. The embodiments described as follows illustrate examples of representative embodiments of the invention, and the scope of the invention is not to be narrowly interpreted by the embodiments.

[0033]

(Definition) The terms "cancer(s)" and "tumor(s)" as used herein are used as synonyms, and each term is used to refer to, unless otherwise specifically limited, solid cancer, non-solid cancer, or both of them.

[0034]

Examples of the term "gene(s)" as used herein include not only DNA, but also mRNA thereof, and cDNA and cRNA thereof.

[0035]

The term "polynucleotide(s)" or "nucleotide(s)" as used herein is used synonymously with "nucleic acid" and examples thereof include DNA, RNA, probes, oligonucleotides, and primers.

[0036]

The terms "polypeptide(s)" and "protein(s)" are used herein indistinguishably.

[0037]

Examples of the term "cells" as used herein include cells within an individual animal and cultured cells.

[0038]

The term "CD147" as used herein is used synonymously with "CD147 protein".

[0039]

The term "functional fragment of an antibody" also called "antigen-binding fragment of an antibody" as used herein refers to a partial fragment of an antibody having binding activity to an antigen. Examples thereof include Fab, F(ab')₂, Fv, scFv, a diabody, a linear antibody and a multi-specific antibody formed of antibody fragments. Examples of an antigen-binding fragment of an antibody also include Fab', which is a monovalent fragment of an antibody variable region, and is prepared by treating F(ab')₂ under reducing conditions. However, examples thereof are not limited to these molecules as long as they are capable of binding to antigens. Examples of these antigen-binding fragments include not only fragments prepared by treating the full-length antibody protein molecules with appropriate enzymes, but also proteins produced by appropriate host cells using antibody genes modified by genetic engineering techniques.

[0040]

The term "effector activity" as used herein refers to either one or more of antibody-dependent cellular cytotoxicity (hereinafter, referred to as "ADCC") activity, complement-dependent cytotoxicity (hereinafter, referred to as "CDC") activity, and antibody-dependent

cellular phagocytosis (hereinafter, referred to as "ADCP") activity.

[0041]

The term "effector functions" used herein refers to exhibition of one or more "effector activity".

[0042]

Antibody-dependent cellular cytotoxicity (ADCC) activity can be measured by a ^{51}Cr release assay method, which measures cell death caused when immune cells having effector activity, an antibody and ^{51}Cr -labeled target cells are brought into contact. The ADCC activity of the anti-human CD147 antibody of the present invention is measured as follows. Regarding the ADCC activity of the anti-human CD147 antibody, human peripheral blood mononuclear cells (PBMC) are used as effector cells, and a CD147-positive human cancer cell line (for example, pancreatic cancer cell line MIA PaCa-2) is used as ADCC target cells. Cancer cells labeled with a radioisotope, ^{51}Cr , and the antibody to be evaluated are treated at a concentration of 0.5 or 5 $\mu\text{g/ml}$ at 4°C for 30 minutes, PBMC separated from human peripheral blood is added at 20 times the proportion of cancer cells, and then cells are cultured for 4 hours at 37°C in the presence of 5% CO_2 . A Total release value is obtained by measuring ^{51}Cr released in the supernatant using TopCount NXT v2.53. The measured value of ^{51}Cr released from ^{51}Cr -labeled cancer cells treated with Triton-100 is designated as a "Maximum

release value" and the measured value of ^{51}Cr released from ^{51}Cr -labeled cancer cells treated with antibody in the absence of PBMC is designated as a "Spontaneous release value". With these values, %Specific release is calculated from the following formula. As a negative control sample, human IgG (hIgG, ChromPure Human IgG, Jackson ImmunoResearch Laboratories, Cat. 009-000-003) is used. The measurement is performed three times, and then the average value and the standard deviation are calculated.

[0043]

$$\% \text{specific release} = (\text{Total release} - \text{Spontaneous release}) / \text{Maximum release}$$

Complement-dependent cytotoxicity (CDC) activity can be evaluated by measuring cell death caused when complement contained in blood, an antibody and target cells are brought into contact. The CDC activity of the anti-human CD147 antibody of the present invention is measured as follows. The complement-dependent cytotoxicity activity (CDC activity) of the anti-human CD147 antibody is evaluated using human pancreatic cell line MIA PaCa-2 as target cells. Commercially available rabbit complement (Low Tox-M Rabbit Complement, CEDARLANE LABORATORIES LIMITED, Cat. CL3051) is used as the complement. Human IgG (hIgG, ChromPureHuman IgG, Jackson ImmunoResearch Laboratories, Cat. 009-000-003) is used as a CDC-negative control antibody. Target cells are

treated with the antibody to be evaluated and the negative control antibody at concentrations of 0, 0.1, 1 or 10 $\mu\text{g/ml}$ for 1 hour at 4°C, the rabbit complement is added in such a manner that the final concentration is 7.5%, the resultant mixture is heated at 37°C in the presence of 5% CO₂ for 3 hours, and then intracellular ATP contained in living cells is measured using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Cat. G7572). Luminescent signals obtained using the CellTiter-Glo Luminescent Cell Viability Assay are quantitatively determined using an EnVision 2104 Multilabel Reader (Perkin Elmer). The measurement is performed three times and the average value and the standard deviation are calculated. Luminescent signals obtained from untreated cells are designated as 100%, and decreased luminescent signals which are dependent on the antibody and the complement are designated as CDC activity.

[0044]

Antibody-dependent cellular phagocytosis (ADCP) activity can be evaluated by measuring phagocytosis caused when immune cells capable of phagocytosis, an antibody and target cells are brought into contact by a double fluorescent labeling method.

[0045]

The ADCP activity of the anti-human CD147 antibody of the present invention is measured as follows. It has

been reported that a human IgG antibody exhibits cytotoxic activity against cancer cells through induction of antibody-dependent cellular phagocytosis (ADCP) by monocytes and macrophages via interaction with mouse Fcγ receptor (Overdijk et al., Journal of Immunology, 1-9, 2012). The ADCP activity of the human chimeric antibody of the present invention is evaluated using RAW264.7 (ATCC, TIB-71) as effector cells and human pancreatic cell line PANC-1 or MIA PaCa-2 as ADCP target cells. After 1 hour of treatment of ADCP target cells labeled using a PKH67 Green Fluorescent Cell Linker Mini Kit for General Cell Membrane Labeling (SIGMA, Cat. MINI67-1KIT) with an antibody to be evaluated having a concentration of 20 µg/ml at 4°C, RAW264.7 cells labeled using a PKH26 Red Fluorescent Cell Linker Lit for General Cell Membrane Labeling (SIGMA, Cat. PKH26GL-1KT) are added in an amount 5 times that of the ADCP target cells, followed by 3 hours of heating at 37°C in the presence of 5% CO₂. With the use of a flow cytometer (BD (Becton, Dickinson and Company), CantoII), the proportion of PKH26-positive cells, which are transferred to a PKH67 signal positive status due to phagocytosis, is measured. As a negative control sample, the same measurement is performed for a sample treated with human IgG (hIgG, ChromPure Human IgG, Jackson ImmunoResearch Laboratories, Cat. 009-000-003). The measurement is performed three times and the average value and the standard deviation are calculated.

[0046]

The expression "substantially lacking effector activity" or "having decreased effector activity or lacking effector activity" as used herein means that the antibody does not exhibit at least one of ADCC activity, CDC activity or ADCP activity, or that the levels of these activities are so low that the functions are not sufficiently exhibited. The expression "substantially lacking effector activity" or "having decreased effector activity or lacking effector activity" means, for example, that the activity of an antibody to be evaluated is at the same level as that of a negative control as determined by one of the above methods for evaluating activity.

[0047]

The expression "having decreased ADCC activity or lacking ADCC activity" as used herein means that an antibody to be evaluated does not exhibit ADCC activity, or that the ADCC activity is at a low level such that it is not exhibited sufficiently. The expression "having decreased ADCC activity or lacking ADCC activity" means, for example, that the activity of an antibody to be evaluated is at the same level as that of a negative control as determined by the above method for evaluating activity.

[0048]

The expression "having decreased CDC activity or lacking CDC activity" means that an antibody to be evaluated does not exhibit CDC activity, or, that the CDC activity is at a low level such that it is not exhibited sufficiently. The expression "having decreased CDC activity or lacking CDC activity" means, for example, that the activity of an antibody to be evaluated is at the same level as that of a negative control as determined by the above method for evaluating activity.

[0049]

The expression "having decreased ADCP activity or lacking ADCP activity" means that an antibody to be evaluated does not exhibit ADCP activity, or, that the ADCP activity is at a low level such that it is not exhibited sufficiently. The expression "having decreased ADCP activity or lacking ADCP activity" means, for example, that the activity of an antibody to be evaluated is at the same level as that of a negative control as determined by the above method for evaluating activity.

[0050]

The expression "having decreased ADCC activity or lacking ADCC activity", "having decreased CDC activity or lacking CDC activity" or "having decreased ADCP activity or lacking ADCP activity" means, for example, that the activity of each antibody to be evaluated is at the same level as that of a negative control as determined by the above methods for evaluating activity.

[0051]

The expression "activates signal transduction through CD147", "activation of signal-related molecules through CD147", "activation of CD147" or "activates CD147" as used herein refers to the activation of one or more cellular signal transduction systems through CD147 and means that at least one of the signal-related molecules located downstream of CD147 is activated. The activation of signal transduction through CD147 means that the expression of a gene located downstream of the CD147 signal is accelerated or decreased, the expression of the protein is accelerated or decreased, or the phosphorylation of the protein is accelerated or decreased. Examples of the signal-related molecules which are located downstream of CD147 include, FAK, MEK, Erk, JAK/STAT, AKT or MAP kinase (MAPK), or activation of signal molecules located further downstream thereof. Examples of MAPK include ERK1/2, JNK or p38MAPK, and more preferably p38MAPK. Examples of signal molecules located further downstream of MAPK include HSP27, cxcl8 or SMAD (for example, SMAD2, SMAD3 and/or SMAD4). Examples of the "activation of CD147" include increased p38MAPK mRNA expression level, increased p38MAPK protein expression level, phosphorylation of p38MAPK, phosphorylation of HSP27 (for example, phosphorylation of Ser82 of HSP27 or phosphorylation of Ser15 of HSP27), increased cxcl8 mRNA expression level, increased cxcl8 protein expression

level, increased rhoB mRNA expression level or increased rhoB protein expression level through SMAD signal activation, or, decreased KLF5 mRNA or decreased KLF5 protein expression level.

[0052]

The term "epitope" used herein refers to a partial peptide of, or a partial three-dimensional structure of, CD147, to which a specific anti-CD147 antibody binds. The above epitope that is a partial peptide of CD147 can be determined by a method well known by persons skilled in the art, such as an immunoassay method. First, various partial structures of antigens are prepared. Upon preparation of the partial structures, known oligonucleotide synthesis techniques can be employed. For example, a series of polypeptides are prepared by gene recombination techniques known by persons skilled in the art in such a manner that lengths of polypeptides from the C-terminus or the N-terminus of CD147 are sequentially shortened, the reactivity of the antibody to these polypeptides is examined, recognition sites are roughly determined, peptides that are even shorter than the polypeptides are synthesized, and then the reactivity to these peptides is examined, so that an epitope can be determined. Further, when the epitope of an antibody, which binds to a membrane protein consisting of plurality of extracellular domains, is a three-dimensional structure consisting of a plurality of domains, the amino

acid sequence of a specific extracellular domain can be modified to modify the three-dimensional structure, so that the specific domain to which the antibody binds can be determined. An epitope that is a partial three-dimensional structure of an antigen, to which a specific antibody binds, can also be determined by specifying the amino acid residues of an antigen adjacent to the above antibody by X-ray structure analysis.

[0053]

If a second antibody binds to a partial peptide or a partial three-dimensional structure, to which a first antibody binds, it can be determined that the first antibody and the second antibody share a common epitope. Through confirmation of a second antibody cross-competing with a first antibody in terms of its binding to the antigen (specifically, the second antibody inhibits the binding of the first antibody to the antigen), it can be determined that the first antibody and the second antibody bind to the same epitope, even if the specific sequence or structure of the epitope has not determined. When a first antibody and a second antibody bind to the same epitope, and the first antibody has a special effect such as antitumor efficacy, the second antibody can be expected to have a similar activity.

[0054]

Each of the heavy chains and the light chains of an antibody molecule is known to have 3 complementarity

determining regions (CDRs). The complementarity determining regions are also referred to as hypervariable regions (hypervariable domains), are located in the heavy chain and the light chain variable regions of an antibody, are sites where the variability of the primary structure is particularly high, and are located separately in the primary structure of the polypeptide chains of the heavy chains and the light chains. Regarding the term "complementarity determining regions of an antibody" as used herein, the heavy chain complementarity determining regions are denoted as CDRH1, CDRH2, and CDRH3 from the amino terminal side of the heavy chain amino acid sequence, and the light chain complementarity determining regions are denoted as CDRL1, CDRL2, and CDRL3 from the amino terminal side of the light chain amino acid sequence. These sites are adjacent to each other on the three-dimensional structure, and determine the specificity to an antigen to which the antibody binds.

[0055]

The term "several" as used herein in the expressions "1 to several" and "1 or several" refers to 2 to 10, preferably, 10 or less, more preferably, 5 or 6 or less, further more preferably, 2 or 3.

[0056]

(CD147)

CD147 is a single-pass transmembrane protein having 2 to 3 immunoglobulin-like domains, and is known to

activate downstream signal-related molecules, such as FAK, MEK, Erk, JAK/STAT, AKT and MAPK family molecules through interactions between multiple CD147 proteins, or through interactions with extracellular or cell membrane surface molecules involved in growth, invasion, and inflammation, such as CD44, Integrin family molecules, CD98, VEGFR, CypA/B, and MCT1/3/4, thereby accelerating the production of proteases such as MMP, and cancer growth, metastasis, and invasion.

[0057]

Three variants of human CD147 are known. Variant 1 is expressed in a retina-specific manner and is a single-pass transmembrane protein having 3 immunoglobulin-like domains (these domains may be referred to as D0, D1 and D2, respectively herein.). Variant 2 is expressed in T cells or various normal cells, and, is a single-pass transmembrane protein having 2 immunoglobulin-like domains (D1, D2), the increased expression of which in various cancer tissues has been reported. Variant 3 is a single-pass transmembrane protein having 1 immunoglobulin-like domain.

[0058]

The amino acid sequence and the nucleotide sequence of variant 1 of human CD147 are available with reference to GenBank Accession Nos. NP_001719.2 and NM_001728.3, and, the amino acid sequence is disclosed herein as SEQ ID NO: 1, and the nucleotide sequence is disclosed herein

as SEQ ID NO: 2. The three immunoglobulin-like domains of variant 1 range from amino acid number 22 to 138 (D0), amino acid number 140 to 218 (D1), and amino acid number 223 to 323 (D2), respectively, in SEQ ID NO: 1, (Redzic, J., J. Mol. Biol., 2011, 68-82) (Grass et al., Biosol. Rep, 2016, 1-16). The transmembrane region of variant 1 ranges from amino acid number 324 to 344 in SEQ ID NO: 1.
[0059]

The amino acid sequence and the nucleotide sequence of variant 2 of human CD147 are available with reference to GenBank Accession Nos. NP_940991.1 and NM_198589.2, and the amino acid sequence is disclosed herein as SEQ ID NO: 3, and the nucleotide sequence is disclosed herein as SEQ ID NO: 4. The two immunoglobulin-like domains (D1, D2) of variant 2 range from amino acid number 24 to 102 (D1) and amino acid number 107 to 207 (D2), respectively, in SEQ ID NO: 3. The transmembrane region of variant 2 ranges from amino acid number 208 to 228 in SEQ ID NO: 3 (Grass et al., Biosol. Rep, 2016, 1-16).
[0060]

The amino acid sequence and the nucleotide sequence of variant 3 of human CD147 are available with reference to GenBank Accession Nos. NP_940992.1 and NM_198590.2. The human CD147 gene is also available from a commercial source.
[0061]

The amino acid sequence and the nucleotide sequence of cynomolgus monkey CD147 (also referred to as monkey CD147 herein) are available with reference to GenBank Accession Nos. XP_005587354.1 and XM_005587297.1. The monkey CD147 gene is also available from a commercial source. The amino acid sequence and the nucleotide sequence of mouse CD147 are available with reference to GenBank Accession Nos. NP_001070652.1 and NM_001077184. 1. The mouse CD147 gene is also available from a commercial source.

[0062]

CD147 to be used in the present invention can be obtained by synthesizing CD147 *in vitro*, or by using genetically engineered host cells to produce CD147. Specifically, CD147 cDNA is incorporated into a vector capable of expressing it, and then CD147 is synthesized in a solution containing an enzyme, a substrate and energy substances required for transcription and translation, or host cells of other prokaryotes or eukaryotes are transformed to express CD147, so that the protein can be obtained.

[0063]

The cDNA of CD147 can be obtained by a polymerase chain reaction (hereinafter, referred to as "PCR"), namely, a PCR method, using a cDNA library expressing the cDNA of CD147 as a template, and primers for specific

amplification of the cDNA of CD147 (Saiki, R. K., et al., Science, (1988)239, 487-49), for example.

[0064]

Examples of the cDNA of CD147 include a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to a nucleotide sequence encoding human, monkey or mouse CD147, and, encoding a protein having biological activity equivalent to that of CD147. Further, examples of the cDNA of CD147 include a polynucleotide that is a splicing variant transcribed from the human, monkey or mouse CD147 gene locus or a polynucleotide hybridizing under stringent conditions thereto, and, encodes a protein having biological activity equivalent to that of CD147.

[0065]

Examples of CD147 further include a protein: consisting of the amino acid sequence of human, monkey or mouse CD147, or an amino acid sequence in which one or several amino acids are substituted, deleted, or added in the amino acid sequence of human, monkey or mouse CD147 from which a signal sequence is removed; and having biological activity equivalent to that of CD147. Further, examples of CD147 include a protein: consisting of an amino acid sequence encoded by a splicing variant transcribed from the human, monkey or mouse CD147 gene locus, or the amino acid sequence in which one or several

amino acids are substituted, deleted, or added; and having biological activity equivalent to that of CD147.

[0066]

(Production of anti-CD147 antibody)

The antibody against CD147 of the present invention can be obtained as follows. Specifically, a non-human animal is immunized with an antigen of interest, a lymph, a lymphatic tissue, a hemocyte sample or bone marrow-derived cells are collected from the animal after establishment of immunity, and then according to a known method (for example, Kohler and Milstein, Nature(1975)256, p.495-497, Kennet, R. ed., Monoclonal Antibodies, p.365-367, Plenum Press, N. Y. (1980)), antibody-producing cells, which produce an antibody against CD147, and myeloma cells are fused to establish hybridomas, and thus a monoclonal antibody can be obtained. Specific examples of such a method are described in WO2009/48072 (disclosed on April 16, 2009) and WO2010/117011 (disclosed on October 14, 2010). Examples of the thus obtained monoclonal antibody can include LN22R8, 2P10F2, rat_CD147_#84, rat_CD147_#101, rat_CD147_#110 and rat_CD147_#131. However, methods for obtaining a monoclonal antibody fall under the category of an already established field, and examples thereof are not limited to the above listed specific examples.

[0067]

Examples of the antibody of the present invention include, in addition to the above monoclonal antibody against CD147, a gene recombinant antibody, which is artificially modified in order to lower heterologous antigenicity against humans, for example, a chimeric antibody, a humanized antibody, and a human antibody. These antibodies can be produced using known methods.

[0068]

Examples of a chimeric antibody can include antibodies in which the antibody variable regions and constant regions are heterologous to each other, for example, a chimeric antibody, in which a mouse or rat-derived antibody variable region is joined to a human-derived constant region (see Proc. Natl. Acad. Sci. USA., 81, 6851-6855, (1984)). An example of an LN22R8-derived chimeric antibody is an antibody consisting of a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 471 of SEQ ID NO: 33, a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 467 of SEQ ID NO: 35 or a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 468 of SEQ ID NO: 37, and a light chain having an amino acid sequence consisting of amino acid residues at positions 21 to 234 of SEQ ID NO: 31 in the sequence listing. An example of a 2P10F2-derived chimeric antibody is an antibody consisting of a heavy chain having an amino acid

sequence consisting of amino acid residues at positions 20 to 466 of SEQ ID NO: 43, a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 462 of SEQ ID NO: 45 or a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 463 of SEQ ID NO: 47, and a light chain having an amino acid sequence consisting of amino acid residues at positions 21 to 234 of SEQ ID NO: 41 in the sequence listing.

[0069]

An example of a rat_CD147_#84-derived chimeric antibody is an antibody consisting of a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 470 of SEQ ID NO: 92, a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 466 of SEQ ID NO: 94, and a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 467 of SEQ ID NO: 96, a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 470 of SEQ ID NO: 98 or a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 467 of SEQ ID NO: 100, and, a light chain having an amino acid sequence consisting of amino acid residues at positions 21 to 234 of SEQ ID NO: 90 in the sequence listing.

[0070]

An example of a rat_CD147_#101-derived chimeric antibody is an antibody consisting of a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 463 of SEQ ID NO: 104, a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 464 of SEQ ID NO: 106 or a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 464 of SEQ ID NO: 108, and, a light chain having an amino acid sequence consisting of amino acid residues at positions 21 to 234 of SEQ ID NO: 102 in the sequence listing.

[0071]

An example of a rat_CD147_#110-derived chimeric antibody is an antibody consisting of a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 462 of SEQ ID NO: 112, a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 463 of SEQ ID NO: 114 or a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 463 of SEQ ID NO: 116, and, a light chain having an amino acid sequence consisting of amino acid residues at positions 21 to 234 of SEQ ID NO: 110 in the sequence listing.

[0072]

An example of a rat_CD147_#131-derived chimeric antibody is an antibody consisting of a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 464 of SEQ ID NO: 120 or a heavy chain having an amino acid sequence consisting of amino acid residues at positions 20 to 465 of SEQ ID NO: 122, and, a light chain having an amino acid sequence consisting of amino acid residues at positions 21 to 234 of SEQ ID NO: 118 in the sequence listing.

[0073]

Examples of a humanized antibody can include, an antibody in which only CDRs are incorporated into a human-derived antibody (see Nature(1986)321, p.522-525), an antibody in which amino acid residues of a partial framework in addition to a CDR sequence are also incorporated into a human antibody by a CDR grafting method (International Publication WO90/07861).

[0074]

Examples of the antibody of the present invention include any rat_CD147_#84 antibody-derived humanized antibody, as long as it retains all the 6 types of CDR sequence of rat_CD147_#84, has binding activity to CD147, and, is an antibody activating CD147. Note that the heavy chain variable region of the rat_CD147_#84 antibody comprises CDRH1 consisting of the amino acid sequence represented by SEQ ID NO: 55, CDRH2 consisting of the amino acid sequence represented by SEQ ID NO: 56, and

CDRH3 consisting of the amino acid sequence represented by SEQ ID NO: 57. The light chain variable region of the rat_CD147_#84 antibody comprises CDRL1 consisting of the amino acid sequence represented by SEQ ID NO: 52, CDRL2 consisting of the amino acid sequence represented by SEQ ID NO: 53, and CDRL3 consisting of the amino acid sequence represented by SEQ ID NO: 54. The amino acid sequence and the nucleotide sequence of the light chain variable region or the heavy chain variable region of the rat_CD147_#84 antibody, and the amino acid sequences of the CDRs are also described in FIG. 33-1 and FIG. 33-2. [0075]

Examples of the antibody of the present invention include a rat_CD147_#101 antibody-derived humanized antibody, as long as it retains all the 6 types of CDR sequence of rat_CD147_#101, has binding activity to CD147, and, is an antibody activating CD147. Note that the heavy chain variable region of the rat_CD147_#101 antibody comprises CDRH1 consisting of the amino acid sequence represented by SEQ ID NO: 65, CDRH2 consisting of the amino acid sequence represented by SEQ ID NO: 66, and CDRH3 consisting of the amino acid sequence represented by SEQ ID NO: 67. The light chain variable region of the rat_CD147_#101 antibody comprises CDRL1 consisting of the amino acid sequence represented by SEQ ID NO: 62, CDRL2 consisting of the amino acid sequence represented by SEQ ID NO: 63, and CDRL3 consisting of the

amino acid sequence represented by SEQ ID NO: 64. The amino acid sequence and the nucleotide sequence of the light chain variable region or the heavy chain variable region of the rat_CD147_#101 antibody, and the amino acid sequences of CDRs are also described in FIG. 34-1 and FIG. 34-2.

[0076]

Examples of the antibody of the present invention include a rat_CD147_#110 antibody-derived humanized antibody, as long as it retains all the 6 types of CDR sequence of rat_CD147_#110, has binding activity to CD147, and, is an antibody activating CD147. Note that the heavy chain variable region of the rat_CD147_#110 antibody comprises CDRH1 consisting of the amino acid sequence represented by SEQ ID NO: 75, CDRH2 consisting of the amino acid sequence represented by SEQ ID NO: 76, and CDRH3 consisting of the amino acid sequence represented by SEQ ID NO: 77. The light chain variable region of the rat_CD147_#110 antibody comprises CDRL1 consisting of the amino acid sequence represented by SEQ ID NO: 72, CDRL2 consisting of the amino acid sequence represented by SEQ ID NO: 73, and CDRL3 consisting of the amino acid sequence represented by SEQ ID NO: 74. The amino acid sequence and the nucleotide sequence of the light chain variable region or the heavy chain variable region of the rat_CD147_#110 antibody and the amino acid

sequences of the CDRs are also described in FIG. 35-1 and FIG. 35-2.

[0077]

Examples of the antibody of the present invention include a rat_CD147_#131 antibody-derived humanized antibody, as long as it retains all the 6 types of CDR sequence of rat_CD147_#131, has binding activity to CD147, and, is an antibody activating CD147. Note that the heavy chain variable region of the rat_CD147_#131 antibody comprises CDRH1 consisting of the amino acid sequence represented by SEQ ID NO: 85, CDRH2 consisting of the amino acid sequence represented by SEQ ID NO: 86, and CDRH3 consisting of the amino acid sequence represented by SEQ ID NO: 87. The light chain variable region of the rat_CD147_#131 antibody comprises CDRL1 consisting of the amino acid sequence represented by SEQ ID NO: 82, CDRL2 consisting of the amino acid sequence represented by SEQ ID NO: 83, and CDRL3 consisting of the amino acid sequence represented by SEQ ID NO: 84. The amino acid sequence and the nucleotide sequence of the light chain variable region or the heavy chain variable region of the rat_CD147_#131 antibody and the amino acid sequences of CDRs are also described in FIG. 36-1 and FIG. 36-2.

[0078]

Examples of the antibody of the present invention further include a CDR-modified humanized antibody in

which 1 to 3 amino acid residues in each CDR are substituted with other amino acid residues, as long as it has binding activity to CD147, and, is an antibody activating CD147.

Examples of a rat_CD147_#84 antibody-derived humanized antibody include a humanized anti-CD147 antibody or an antigen-binding fragment of the antibody, having the following (a) and (b):

(a) a heavy chain variable region selected from the group consisting of the following (a1) to (a4):

(a1) a heavy chain variable region consisting of amino acid residues at positions 20 to 140 in an amino acid sequence represented by SEQ ID NO: 123;

(a2) a heavy chain variable region consisting of amino acid residues at positions 20 to 140 in an amino acid sequence represented by SEQ ID NO: 125;

(a3) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (a1) or (a2); and

(a4) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence according to any one of (a1) to (a3); and
(b) a light chain variable region selected from the group consisting of the following (b1) to (b3):

(b1) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 127;

(b2) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (b1); and

(b3) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence of (b1) or (b2).

[0079]

Preferred examples of a rat_CD147_#84 antibody-derived humanized antibody include an antibody comprising the heavy chain variable region consisting of amino acid residues at positions 20 to 140 in the amino acid sequence represented by SEQ ID NO: 125 and the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 127, or, an antibody comprising the heavy chain variable region consisting of amino acid residues at positions 20 to 140 in the amino acid sequence represented by SEQ ID NO: 123 and the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 127.

[0080]

Preferred examples of a rat_CD147_#84 antibody-derived humanized antibody include an antibody comprising a heavy chain consisting of amino acid residues at positions 20 to 467 in the amino acid sequence represented by SEQ ID NO: 125 and a light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 127, or, an antibody comprising a heavy chain consisting of amino acid residues at positions 20 to 466 in the amino acid sequence represented by SEQ ID NO: 123 and a light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 127.

[0081]

Examples of a rat_CD147_#101 antibody-derived humanized antibody include the humanized anti-CD147 antibody or an antigen-binding fragment of the antibody, having the following (e) and (f):

(e) a heavy chain variable region selected from the group consisting of the following (e1) to (e4):

(e1) a heavy chain variable region consisting of amino acid residues at positions 20 to 137 in an amino acid sequence represented by SEQ ID NO: 129;

(e2) a heavy chain variable region consisting of amino acid residues at positions 20 to 137 in an amino acid sequence represented by SEQ ID NO: 131;

(e3) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (e1) or (e2); and

(e4) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence according to any one of (e1) to (e3); and

(f) a light chain variable region selected from the group consisting of the following (f1) to (f3):

(f1) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 133;

(f2) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (f1); and

(f3) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence of (f1) or (f2).

[0082]

Preferred examples of a rat_CD147_#101 antibody-derived humanized antibody include an antibody comprising the heavy chain variable region consisting of amino acid residues at positions 20 to 137 in the amino acid sequence represented by SEQ ID NO: 129 and the light

chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 133, or, an antibody comprising the heavy chain variable region consisting of amino acid residues at positions 20 to 137 in the amino acid sequence represented by SEQ ID NO: 131 and the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 133.

[0083]

Preferred examples of a rat_CD147_#101 antibody-derived humanized antibody include an antibody comprising the heavy chain consisting of amino acid residues at positions 20 to 463 in the amino acid sequence represented by SEQ ID NO: 129 and the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 133, or, an antibody comprising the heavy chain consisting of amino acid residues at positions 20 to 464 in the amino acid sequence represented by SEQ ID NO: 131 and the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 133.

[0084]

Examples of a rat_CD147_#110 antibody-derived humanized antibody include the humanized anti-CD147

antibody or an antigen-binding fragment of the antibody, having the following (c) and (d):

(c) a heavy chain variable region selected from the group consisting of the following (c1) to (c4):

(c1) a heavy chain variable region consisting of amino acid residues at positions 20 to 136 in an amino acid sequence represented by SEQ ID NO: 135;

(c2) a heavy chain variable region consisting of amino acid residues at positions 20 to 136 in an amino acid sequence represented by SEQ ID NO: 147;

(c3) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (c1) or (c2); and

(c4) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence according to any one of (c1) to (c3),

(d) a light chain variable region selected from the group consisting of the following (d1) to (d5):

(d1) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 137;

(d2) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 149;

(d3) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 151;

(d4) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence according to any one of (d1) to (d3); and

(d5) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence according to any one of (d1) to (d4).

[0085]

Preferred examples of a rat_CD147_#110 antibody-derived humanized antibody include: an antibody comprising the heavy chain variable region consisting of amino acid residues at positions 20 to 136 in the amino acid sequence represented by SEQ ID NO: 135 and the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 137; an antibody comprising the heavy chain variable region consisting of amino acid residues at positions 20 to 136 in the amino acid sequence represented by SEQ ID NO: 147 and the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 149; or, an antibody comprising the heavy chain variable region consisting of amino acid

residues at positions 20 to 136 in the amino acid sequence represented by SEQ ID NO: 147 and the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 151.

[0086]

Preferred examples of a rat_CD147_#110 antibody-derived humanized antibody include: an antibody comprising the heavy chain consisting of amino acid residues at positions 20 to 463 in the amino acid sequence represented by SEQ ID NO: 135 and the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 137; an antibody comprising the heavy chain consisting of amino acid residues at positions 20 to 463 in the amino acid sequence represented by SEQ ID NO: 147, and, the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 149; or, an antibody comprising the heavy chain consisting of amino acid residues at positions 20 to 463 in the amino acid sequence represented by SEQ ID NO: 147, and, the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 151.

[0087]

Examples of a rat_CD147_#131 antibody-derived humanized antibody include the humanized anti-CD147

antibody or an antigen-binding fragment of the antibody, having the following (g) and (h):

(g) a heavy chain variable region selected from the group consisting of the following (g1) to (g3):

(g1) a heavy chain variable region consisting of amino acid residues at positions 20 to 138 in an amino acid sequence represented by SEQ ID NO: 139;

(g2) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (g1); and

(g3) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence of (g1) or (g2), and,

(h) a light chain variable region selected from the group consisting of the following (h1) to (h3):

(h1) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 141;

(h2) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (h1); and

(h3) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the

sequence of a framework region outside each CDR sequence in the sequence of (h1) or (h2).

[0088]

Preferred examples of a rat_CD147_#131 antibody-derived humanized antibody include: an antibody comprising the heavy chain variable region consisting of amino acid residues at positions 20 to 138 in the amino acid sequence represented by SEQ ID NO: 139 and the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 141.

[0089]

Preferred examples of a rat_CD147_#131 antibody-derived humanized antibody include an antibody comprising the heavy chain consisting of amino acid residues at positions 20 to 464 in the amino acid sequence represented by SEQ ID NO: 139 and the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 141.

[0090]

The above rat_CD147_#84 antibody-derived humanized antibody, rat_CD147_#101 antibody-derived humanized antibody, rat_CD147_#110 antibody-derived humanized antibody or rat_CD147_#131 antibody-derived humanized antibody preferably activates p38MAPK signal transduction and/or SMAD4 signal transduction through CD147.

[0091]

Examples of the antibody of the present invention can further include human antibodies. The term "anti-CD147 human antibody" refers to a human antibody having only the gene sequence of a human chromosome-derived antibody. Such an anti-CD147 human antibody can be obtained by, for example, a method using mice producing a human antibody having a human chromosome fragment comprising the genes of the heavy chains and the light chains of a human antibody (see Tomizuka, K. et al., Nature Genetics (1997)16, p.133-143; Kuroiwa, Y. et al., Nucl. Acids Res. (1998) 26, p.3447-3448; Yoshida, H. et al., Animal Cell Technology: Basic and Applied Aspects vol. 10, p.69-73 (Kitagawa, Y., Matsuda, T. and Iijima, S. eds.), Kluwer Academic Publishers, 1999; Tomizuka, K. et al., Proc. Natl. Acad. Sci. USA (2000) 97, p.722-727 etc.).

[0092]

Specifically, such human antibody-producing mice can be obtained by: producing gene-recombinant animals through disruption of the gene loci of the endogenous immunoglobulin heavy chain and light chain, and then introducing the gene loci of the human immunoglobulin heavy chain and light chain via a vector such as a human artificial chromosome (HAC) vector or a mouse artificial chromosome (MAC) vector in place of the disrupted loci; and recombinant animals are then created by crossing these animals.

[0093]

Eukaryotic cells are transformed by gene recombination techniques with cDNA encoding each of such a human antibody heavy chain and light chain, preferably a vector comprising the cDNA, and then the transformed cells producing the gene-recombinant human monoclonal antibody are cultured, so that the antibody can be obtained from the culture supernatant. Here, as host cells, eukaryotic cells, preferably CHO cells, mammalian cells such as lymphocytes and myeloma cells can be used.

[0094]

Methods for obtaining a phage display-derived human antibody selected from a human antibody library (see Wormstone, I. M. et al., *Investigative Ophthalmology & Visual Science*. (2002)43(7), p.2301-2308; Carmen, S. et al., *Briefings in Functional Genomics and Proteomics* (2002), 1(2), p.189-203; Siriwardena, D. et al., *Ophthalmology* (2002)109(3), p.427-431, etc.) are also known.

[0095]

For example, a phage display method can be employed whereby a human antibody variable region is expressed on the phage surface as a single-chain antibody (scFv), and then phage binding to the antigen is selected (*Nature Biotechnology* (2005), 23, (9), p.1105-1116). The gene of the phage selected based on its binding to an antigen is analyzed, so that the DNA sequence encoding the human

antibody variable region binding to the antigen can be determined. Once the DNA sequence of scFv binding to the antigen is revealed, an expression vector having the sequence is prepared, and then introduced into an appropriate host for expression, and thus a human antibody can be obtained (WO92/01047, WO92/20791, WO93/06213, WO93/11236, WO93/19172, WO95/01438, WO95/15388, Annu. Rev. Immunol. (1994)12, p.433-455, Nature Biotechnology (2005) 23(9), p.1105-1116).

[0096]

Examples of the antibody of the present invention also include an antibody having the same epitope as that of an antibody provided by the present invention. Examples thereof include an antibody having the same epitope as that of at least one of LN22R8, 2P10F2, rat_CD147_#84, rat_CD147_#101, rat_CD147_#110 and rat_CD147_#131.

[0097]

LN22R8 and 2P10F2 of the present invention recognized an epitope denoted as mu3 in FIG. 3 (human CD147v1: DALPGQKTEFKVDSDDQ (SEQ ID NO: 143), monkey CD147: DTLPGQKTDFEVDSDDL (SEQ ID NO: 144)). Examples of the antibody of the present invention also include an antibody that recognizes or binds to a sequence comprising an amino acid sequence in which 1 or several, preferably 1 to 3, more preferably 1 or 2 amino acids are deleted, substituted, or added in the sequence of SEQ ID

NO: 143 or SEQ ID NO: 144, or SEQ ID NO: 143 or SEQ ID NO: 144. The antibody preferably activates signal transduction through CD147.

[0098]

Examples of the antibody of the present invention include an antibody that recognizes the same epitope as that of rat_CD147_#110, preferably, humanized #110H1L4. The antibody preferably activates signal transduction through CD147. The results of the analysis of the epitope of humanized #110H1L4 are described in Example 17.

[0099]

A suitable antibody can be selected through evaluation of the binding activity to an antigen as known by persons skilled in the art. The dissociation constant of an antibody and the antigen (CD147) can be measured using Biacore T200 (GE Healthcare Bioscience) with surface plasmon resonance (SPR) as the detection principle. For example, an antibody at an appropriate concentration and an analyte are reacted to an antigen immobilized as a ligand, the binding and dissociation thereof are measured, and thus the association rate constant k_{a1} , the dissociation rate constant k_{d1} and the dissociation constant (KD; $KD=k_{d1}/k_{a1}$) can be obtained.

[0100]

A device to be used for the evaluation of binding activity to CD147 is not limited to Biacore T200. Binding activity to CD147 can also be evaluated using an

instrument with surface plasmon resonance (SPR) as the detection principle, KinExA (Sapidyne Instruments) with kinetic exclusion assay as the detection principle, BLItz system (Pall) with bio-layer interferometry as the detection principle, or ELISA (Enzyme-Linked ImmunoSorbent Assay), for example.

[0101]

An example of another indicator for comparison of the properties of antibodies can be the stability of antibodies. Differential scanning calorimetry (DSC) is a method capable of quickly and accurately measuring the thermal unfolding midpoint (T_m), serving as a good indicator of the relative structural stability of proteins. T_m values are measured using DSC, the T_m values are compared, and then differences in thermal stability can be compared. The storage stability of an antibody is known to be correlated with the thermal stability of the antibody to some extent (Lori Burton, et al., Pharmaceutical Development and Technology (2007)12, p.265-273). Hence, a suitable antibody can be selected with thermal stability as an indicator. Examples of other indicators for selection of an antibody can include a high yield in appropriate host cells, and low aggregability in an aqueous solution. For example, since an antibody exhibiting the highest yield does not always exhibit the highest thermal stability, an optimum antibody for administration to humans should be selected

through a comprehensive determination based on the above indicators.

[0102]

A method for obtaining a single chain immunoglobulin, which involves linking the full-length sequences of an antibody heavy chain and light chain with an appropriate linker, is also known (Lee, H-S, et al., Molecular Immunology (1999)36, p.61-71; Shirmann, T. et al., mAbs (2010), 2, (1) p.1-4). Such a single chain immunoglobulin can retain the structure and activity analogous to those of an antibody that is originally a tetramer through dimerization. The antibody of the present invention may also be an antibody having a single heavy chain variable region, but lacking a light chain sequence. Such an antibody is referred to as a single domain antibody (sdAb) or a nanobody, and has been actually observed among camels or lamas and reported as retaining antigen binding ability (Muyldemans S. et al., Protein Eng. (1994)7(9), 1129-35, Hamers-Casterman C. et al., Nature (1993) 363 (6428) 446-8). The above antibody can also be construed as a type of an antigen-binding fragment of the antibody in accordance with the present invention.

[0103]

Antibody-dependent cellular cytotoxic activity can be enhanced by regulating the glycosylation of the sugar chain binding to the antibody of the present invention.

Examples of known techniques for regulating the glycosylation of antibodies include, but are not limited to, WO99/54342, WO2000/61739, and WO2002/31140.

[0104]

When an antibody is prepared by isolating an antibody gene and then introducing the isolated gene into an appropriate host, such an appropriate host and an expression vector can be used in combination.

[0105]

Specific examples of an antibody gene can include combinations of genes encoding antibody heavy chain sequences and genes encoding light chain sequences described herein. Upon transformation of host cells, a heavy chain sequence gene and a light chain sequence gene can be inserted into the same expression vector, or can be inserted into different expression vectors. When eukaryotic cells are used as host cells, animal cells, plant cells, and eukaryotic microorganisms can be used. Examples of animal cells can include mammalian cells, for example, monkey cells; that is, COS cells (Gluzman, Y. Cell(1981)23, p.175-182, ATCC CRL-1650), dihydrofolate reductase deficient cell lines of mouse fibroblasts NIH3T3 (ATCC, CRL-1658) or Chinese hamster ovary cells (CHO cells, ATCC CCL-61) (Urlaub, G. and Chasin, L. A. Proc. Natl. Acad. Sci. USA. (1980) 77, p.4126-4220). When prokaryotic cells are used, examples thereof can include *Escherichia coli* and *Bacillus subtilis*. An

antibody gene of interest is introduced into these cells by transformation, and then the thus transformed cells are cultured *in vitro*, thereby obtaining the antibody. The above culture methods may vary in yield depending on the sequence of an antibody. Hence, an antibody that would be easy to produce as a drug can be selected by using the yield as an indicator by which to select between antibodies having equivalent binding activity.

[0106]

An isotype of the antibody of the present invention is not limited and examples thereof can include IgG (IgG1, IgG2, IgG3, IgG4), IgM, IgA (IgA1, IgA2), IgD or IgE. Such an isotype is preferably IgG or IgM, and another example thereof is preferably IgG.

[0107]

Human IgG1 exhibits very strong effector functions including CDC activity through mediation of complement fixation and antibody-dependent cellular cytotoxic activity out of the existing 5 types of human IgG subclass (Bruggemann et al., J. Exp. Med., 1351-1361, 1987). When a molecule that is highly expressed in cancer is targeted by a therapeutic antibody, human IgG1 is used as it is an IgG isotype that exhibits therapeutic effects by promoting the induction of cancer-cell death due to cytotoxicity mediated by effector functions (e.g., trastuzumab and rituximab). However, it has been reported that a human IgG1 antibody targeting HLA-DR with

its original CDC activity, led to cynomolgus monkeys dying after administration thereof. The use of such an antibody as an antibody drug which targets a molecule that is also expressed in normal organs causes a concern that the effector functions could induce a severe adverse reaction (Tawara, T., J. Immunology, 2008, 2294-2298). When IgG1 is used as an isotype of the antibody of the present invention, the IgG1 antibody may have a mutation, and specifically, effector functions can be adjusted by substitution of some of the amino acid residues of the constant regions (see WO88/007089, WO94/28027, WO94/29351). Examples of a variant of IgG1 with attenuated effector functions include IgG1 LALA (IgG1-L234A, L235A), and IgG1 LAGA (IgG1-L235A, G237A).

[0108]

Human IgG2 has very weak effector functions including CDC activity through complement fixation and antibody-dependent cellular cytotoxic activity among the existing 5 types of human IgG subclass (Bruggemann et al., J. Exp. Med., 1351-1361, 1987). When a molecule that is expressed in normal organs is targeted by a therapeutic antibody, IgG2 is used as one of the IgG formats to avoid toxicity due to cytotoxicity mediated by effector functions (Denosumab, Evolocumab, Brodalumab).

[0109]

Human IgG4 has very weak effector functions including CDC activity through complement fixation and

antibody-dependent cellular cytotoxic activity among the existing 5 types of human IgG subclass (Bruggemann et al., J. Exp. Med., 1351-1361, 1987). When a molecule that is expressed in normal organs is targeted by a therapeutic antibody, IgG4 is used as one of the IgG formats to avoid toxicity due to cytotoxicity mediated by effector functions (Opdivo). When IgG4 is used as an isotype of the antibody of the present invention, splitting, a distinct feature of IgG4, is suppressed and the half-life can be extended by substitution of some of amino acid residues of the constant regions (see Molecular Immunology, 30, 1 105-108 (1993)).

[0110]

When IgG4 is used as an isotype of the antibody of the present invention, the IgG4 antibody may have a mutation. Examples of a mutation of an IgG4 variant include (Proc Natl Acad Sci USA. 1969, 63(1), 78-85; Kabat et al., Sequences of proteins of immunological interest, 1991 Fifth edition) a substitution (F234A) of phenylalanine at position 234 according to the EU Index with alanine and substitution (L235A) of leucine at position 235 according to the EU Index with alanine (Parekh et al., mAbs, 310-318, 2012). Such a mutation of an antibody is referred to as FALA mutation. IgG4P FALA further attenuates effector functions through substitution of two amino acid residues required for interaction with FcγRs (for example, FcγRI, FcγRII or

FcγRIII) existing in the CH2 domain with alanine. Since S-S bond formation between antibody heavy chains is unstable in IgG4, a mutation for accelerating S-S bond formation between antibody heavy chains is introduced into IgG4 in order to increase the stability. Examples of such a mutation include a substitution (S228P) of serine at position 228 according to the EU Index with proline (ANGAL et al., Molecular Immunology, 105-108, 1993). Such mutation of an antibody is referred to as Pro mutation. Into the constant region of the antibody of the present invention, both FALA mutation and Pro mutation described above may be introduced simultaneously (Vafa et al., Methods, 65, 114-126, 2014). An IgG4 heavy chain having the FALA mutation is referred to as "IgG4FALA"-type heavy chain, an IgG4 heavy chain having the Pro mutation is referred to as "IgG4P"-type heavy chain, and an IgG4 heavy chain having both FALA mutation and Pro mutation is referred to as "IgG4PFALA"-type heavy chain.

[0111]

An antibody heavy chain constant region consists of CH1, hinge, CH2 and CH3 regions, wherein CH1 is defined as ranging from EU Index No. 118 to 215, the hinge is defined as ranging from EU Index No. 216 to 230, CH2 is defined as ranging from EU Index No. 231 to 340, and CH3 is defined as ranging from EU Index No. 341 to 446. According to the EU Index, proline resulting from

substitution of serine at position 228, alanine resulting from substitution of phenylalanine at position 234, and alanine resulting from substitution of leucine at position 235 correspond to: proline at position 248, alanine at position 254 and alanine at position 255, respectively, in SEQ ID NO: 100 representing the amino acid sequence of human chimeric rat_CD147_#84 heavy chain IgG4PFALA; proline at position 245, alanine at position 251, and alanine at position 252, respectively, in SEQ ID NO: 108 representing the amino acid sequence of human chimeric rat_CD147_#101 heavy chain IgG4PFALA; and proline at position 244, alanine at position 250, and alanine at position 251, respectively, in SEQ ID NO: 108 representing the amino acid sequence of human chimeric rat_CD147_#110 heavy chain IgG4PFALA.

[0112]

Preferred examples of an isotype of the antibody of the present invention include IgG1, IgG2, IgG4, IgG4P or IgG4PFALA, particularly preferred examples thereof include IgG2, IgG4P or IgG4PFALA, and even more preferred examples thereof include IgG2 or IgG4P.

[0113]

The antibody of the present invention may also be an antigen-binding fragment of an antibody, which has an antigen-binding site of the antibody or a modified product thereof. An antibody is treated with protease such as papain or pepsin, or, an antibody gene is

modified by genetic engineering techniques and then the resultant is expressed in appropriate cultured cells, so that a fragment of the antibody can be obtained. Of these antibody fragments, fragments retaining all or part of functions of the full-length antibody molecule can be referred to as an antigen-binding fragment of the antibody. An example of the functions of an antibody is activation of antigen-related signal transduction.

[0114]

CD147 is also expressed in blood cells including erythrocytes and normal organs essential for survival (Spring, et al., Eur. J. Immunol., 1997, 891-897), and thus antitumor efficacy exerted using effector functions accompanying an antibody has a high risk for adverse reaction. It has been actually reported that erythrocytes are sensitive to effector functions (ADCC, CDC, ADCP) resulting from antibody binding. (Flegel, W., Transfusion, 2015, S47-S58), and the thus increased antibody *in vivo* against erythrocytes is known to cause autoimmune hemolytic anemia (Gibson, J., Aust. N. Z. J. Med., 1988. 625-637). The antibody of the present invention as a therapeutic antibody targeting CD147 that is also expressed in normal cells is characterized in that any one of or all of ADCC activity, ADCP activity and CDC activity, which cause serious adverse reaction, are detected at low levels or none of these activities are detected.

[0115]

The present inventors have discovered for the first time that the anti-human CD147 antibody exhibits antitumor efficacy independent of the antibody's effector functions by activating cell signal transduction through CD147. In the present invention, the antibody retains the functions of binding activity to CD147 and/or activating CD147. The antibody of the present invention activates preferably downstream signal-related molecules mediating CD147, for example, FAK, MEK, Erk, JAK/STAT, AKT or MAP kinase (MAPK) or activates signal-related signal located further downstream thereof. The antibody of the present invention activates more preferably, molecules located downstream of MAPK or MAPK. An example of MAPK is, preferably, p38MAPK. Examples of signal molecules located further downstream of MAPK include HSP27, cxcl8 and SMAD (for example, SMAD2, SMAD3 or SMAD4, preferably, SMAD4). Examples of the "activation of CD147" include increased p38MAPK mRNA expression level, increased p38MAPK protein expression level, phosphorylation of p38MAPK, phosphorylation of HSP27 (for example, phosphorylation of Ser82 of HSP27 or phosphorylation of Ser15 of HSP27), increased cxcl8 mRNA expression level, increased cxcl8 protein expression level, increased rhoB mRNA expression level or increased rhoB protein expression level through SMAD signal activation. Examples of the "activation of CD147"

include preferably, increased p38MAPK protein expression level, phosphorylation of p38MAPK, phosphorylation of HSP27 (for example, phosphorylation of Ser82 of HSP27 or phosphorylation of Ser15 of HSP27), increased cxcl8 mRNA expression level, and increased rhoB mRNA expression level through SMAD signal activation.

SMAD2 or SMAD3 is known to be phosphorylated by the TGF β receptor when TGF β binds to the TGF β receptor (TGFBR1/2), so as to form a heterotrimer with SMAD4. After nuclear import thereof, the heterotrimer is known to bind to a transcriptional regulatory domain having a SMAD DNA binding sequence on the chromosome (Smad binding element: SBE), and then positively or negatively controls the mRNA expression of downstream genes (Miyazono, The Official Journal of the Japan Geriatrics Society, 1999, 162-166). Therefore, it is considered that the activation of SMAD4 requires the presence of SMAD2 or SMAD3. SMAD2, SMAD3 and SMAD4 negatively control KLF5 expression level in a TGF β -dependent manner (David et al., Cell, 2016, 164(5), 1015-1030). In SMAD4-deficient pancreatic cancer cells, signals for suppressing the expression of the KLF5 gene through SMAD2, SMAD3 and SMAD4 are cancelled, and thus KLF5 protein is expressed. It is known that when SMAD4 is lost and KLF5 is expressed, TGF β -dependent cell death signaling (SOX4-dependent) is suppressed (above-mentioned David et al, Cell).

[0116]

The present inventors have discovered that the anti-human CD147 antibody of the present invention phosphorylates p38MAPK (FIG. 21), phosphorylates HSP27 (FIG. 20), and increases the expression of cxcl8 (FIG. 22(b) and FIG. 23(b)). Therefore, activation of CD147 by the antibody of the present invention can be confirmed by confirming a change in gene expression, protein expression or phosphorylation of at least one of these molecules before and after the administration of the antibody.

[0117]

The present inventors have discovered that: the anti-human CD147 antibody of the present invention exhibits efficacy on a pancreatic cancer model expressing SMAD4 protein (FIG. 25); the anti-human CD147 antibody of the present invention exhibited partial (up to about 30%) antitumor efficacy in a pancreatic cancer model such as BxPC-3 having a gene mutation in SMAD4 and expressing no SMAD4 (FIG. 24); and downstream molecules of SMAD2, SMAD3 and SMAD4 (rhoB, FIG. 22(c) and FIG. 23(c)) are induced in tumors after administration of the anti-human CD147 antibody of the present invention. Therefore, before and after administration of the antibody of the present invention, the presence or the absence of a change in rhoB gene expression or rhoB protein expression is confirmed, so that if the antibody of the present invention mediates the activation of CD147, this can be

confirmed. The genome sequence, gene expression or protein expression of SMAD4 in patient samples is measured using a method known by persons skilled in the art, patients in which SMAD4 is expressed are selected as subject patients to which the antibody of the present invention is to be administered, and thus the antibody of the present invention can be administered to them.

[0118]

The level of KLF5 expression is known to be high in a SMAD4-negative model such as BxPC-3 (David et al., Cell, 2016, 164(5), 1015-1030). Sensitivity to the anti-human CD147 antibody of the present invention was decreased from 91% to 20% in the KLF5-expressing MIA PaCa-2 model (Example 26). Accordingly, the present inventors consider that KLF5 expression suppresses SMAD2-, SMAD3- and SMAD4-dependent cell death signaling induced by the CD147 antibody. Since KLF5 expression levels are low in liver cancer, ALL, lymphoma, gastrointestinal stromal tumor (GIST), skin cancer, sarcoma, AML or renal cancer, the anti-human CD147 antibody of the present invention is expected to be effective for many patients with these cancers. The gene expression or the protein expression of KLF5 in patient samples is measured using a method known by persons skilled in the art, patients exhibiting decreased KLF5 expression levels or lacking KLF5 expression are selected as subject patients to which the antibody of the present invention is to be administered,

and thus the antibody of the present invention can be administered to them. The degree of a decrease in KLF5 expression in this case can be determined by a method known by persons skilled in the art and performing appropriate clinical trials. For example, patients for which effects can be obtained and patients for which no effect can be obtained are compared for KLF5 expression level, thereby determining an appropriate threshold.

[0119]

Examples of antibody fragments can include Fab, F(ab')₂, Fv, or single-chain Fv (scFv) in which heavy chain Fv and light chain Fv are connected with an appropriate linker, a diabody (diabodies), a linear antibody, and a multi-specific antibody formed of antibody fragments. Examples of antibody fragments include Fab', which is a monovalent fragment of an antibody variable region, and prepared by treating F(ab')₂ under reducing conditions.

[0120]

Moreover, the antibody of the present invention may also be a multi-specific antibody having specificity to at least two different types of antigens. Such a molecule generally binds to two types of antigens (i.e., bispecific antibody), and examples of the "multispecific antibody" in the present invention include an antibody having specificity to three or more (for example, 3 types) antigens.

[0121]

The multispecific antibody of the present invention may be a full-length antibody, or fragments of such an antibody (for example, $F(ab')_2$ bispecific antibody). A bispecific antibody can be prepared by binding a heavy chain and a light chain (HL pair) of two types of antibodies, or, fusing hybridomas producing different monoclonal antibodies to each other, so as to prepare bispecific antibody-producing fused cells (Millstein et al., Nature (1983) 305, P. 537-539).

[0122]

The antibody of the present invention may be a single chain antibody (also described as scFv). A single chain antibody is obtained by connecting an antibody heavy chain variable region and a light chain variable region with a polypeptide linker (Pluckthun, The Pharmacology of Monoclonal Antibodies, 113 (Ed., Rosenberg and Moore, Springer Verlag, New York, p.269-315 (1994), Nature Biotechnology (2005), 23, p.1126-1136). BiscFv fragment prepared by connecting two scFvs with a polypeptide linker can also be used as a bispecific antibody.

[0123]

Methods for preparing a single chain antibody are known in the art (for example, see U.S. Patent No. 4,946,778, U.S. Patent No. 5,260,203, U.S. Patent No. 5,091,513, and U.S. Patent No. 5,455,030.). In the scFv,

a heavy chain variable region and a light chain variable region are connected via a linker that does not form a conjugate, preferably a polypeptide linker (Huston, J. S. et al., Proc. Natl. Acad. Sci. USA. (1988), 85, p.5879-5883). A heavy chain variable region and a light chain variable region in scFv may be derived from the same antibody or different antibodies. As a polypeptide linker for connecting variable regions, for example, an arbitrary single chain peptide consisting of 12 to 19 residues is used.

[0124]

DNA encoding scFv can be obtained by amplifying by the PCR method using as a template, one selected out of: DNA encoding the above antibody heavy chain or heavy chain variable region, and DNA encoding the light chain or light chain variable region, DNA encoding the full-length sequence of each region or a DNA portion encoding a desired amino acid sequence thereof, and a primer pair specifying each end thereof, and then amplifying using DNA encoding a polypeptide linker portion, and a primer pair specifying end so that the ends are connected to a heavy chain and a light chain, respectively, in combination.

[0125]

Once DNA encoding scFv is prepared, an expression vector containing the DNA and a host transformed with the expression vector can be obtained according to a standard

method, and, scFv can be obtained according to a standard method using the host. These antibody fragments can be produced by a host after obtainment of the gene thereof and expression of the gene in the same manner as described above.

[0126]

The antibody of the present invention may be polymerized to have enhanced affinity for an antigen. An antibody to be polymerized may be a single type of antibody or a plurality of antibodies recognizing a plurality of epitopes of the same antigen. Examples of a method for polymerization of an antibody can include, binding of an IgG CH3 domain with two scFvs, binding with streptavidin, and introduction of a helix-turn-helix motif.

[0127]

The antibody of the present invention may also be a polyclonal antibody that is a mixture of multiple types of anti-CD147 antibody differing in amino acid sequence. An example of a polyclonal antibody can be a mixture of multiple types of antibody differing in CDR. As such a polyclonal antibody, a mixture of cells producing different antibodies is cultured and an antibody purified from the culture can be used (see WO2004/061104).

[0128]

The antibody of the present invention may be an antibody having 80% to 99% identity (or homology) with

the above antibody heavy chain and/or light chain. Through combination of sequences exhibiting high homology with the above heavy chain amino acid sequence and light chain amino acid sequence, an antibody having antigen binding ability, activation of CD147, preferably, activation of MAPK, and activation of downstream signal molecules of MAPK equivalent to those of each antibody above can be selected. Such homology is generally 80% or more homology, preferably 90% or more homology, more preferably 95% or more homology, and most preferably 99% or more homology. Through combination of the heavy chain and/or the light chain amino acid sequence with an amino acid sequence in which 1 to several amino acid residues are substituted, deleted and/or added, an antibody having various effects equivalent to those of each antibody above can be selected. The number of amino acid residues to be substituted, deleted and/or added is generally 10 or less amino acid residues, preferably 5 to 6 or less amino acid residues, more preferably 2 to 3 or less amino acid residues, and most preferably 1 amino acid residue. In addition, it is known that the carboxyl terminal lysine residue of an antibody heavy chain produced by cultured mammalian cells is deleted (Tsubaki et al., Int. J. Biol. Macromol, 139-147, 2013). However, deletion and modification of these heavy chain sequences do not affect the antibody's binding ability to an antigen and effector functions (e.g., activation of complement and antibody-

dependent cellular cytotoxicity). Therefore, the present invention includes an antibody subjected to a modification, and examples thereof can include an antibody in which 1 or 2 amino acids are deleted at the heavy chain carboxyl terminus, and an antibody subjected to amidation (for example, a heavy chain in which a proline residue at the carboxyl terminal site is amidated). Note that, as long as the binding ability to an antigen and the function of activating signal-related molecules located downstream of CD147 are maintained, a heavy chain carboxyl terminal-deficient antibody according to the present invention is not limited to the above types. The two heavy chains which compose the antibody of the present invention may be any one type of heavy chain selected from the group consisting of full-length heavy chains and the above-deficient heavy chains, or a combination of two types thereof. The ratio of carboxyl terminal-deficient heavy chains can be affected by the type and culture conditions of the cultured mammalian cells producing the antibody according to the present invention, and an example thereof can be a case in which each of the two heavy chains, as major components of the antibody of the present invention, has a deletion of one amino acid residue at the carboxyl terminus.

[0129]

Homology between two types of amino acid sequence can be determined using default parameters of Blast algorithm version 2.2.2 (Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25: 3389-3402). The Blast algorithm can also be used by accessing the Internet, www.ncbi.nlm.nih.gov/blast. Note that the two types of percentage of Identity (or Identities) and Positivity (or Positivities) are calculated by the Blast algorithm. The former percentage is a value derived from when amino acid residues match when comparing two types of amino acid sequence in which homology is expected to be found, and the latter is a numerical value found when amino acid residues analogous to each other in chemical structure are also taken into consideration. The value of identity when amino acid residues match is designated as the value of homology in the application.

[0130]

As modified products of an antibody, antibodies bound to various molecules such as polyethylene glycol (PEG) can also be used.

[0131]

The antibody of the present invention may also be an immunoconjugate formed between such an antibody and another drug. Examples of such an antibody can include

the antibody bound to a radioactive substance or a compound having pharmacologic effects (Nature Biotechnology(2005)23, p.1137-1146).

[0132]

The thus obtained antibody can be purified to a homogenous state. For separation and purification of an antibody, separation and purification methods used for general proteins may be employed. For example, an antibody can be separated and purified by adequately selecting or combining column chromatography, filter filtration, ultrafiltration, salting-out, dialysis, polyacrylamide gel electrophoresis for preparation, isoelectric focusing (Strategies for Protein Purification and Characterization: A Laboratory Course Manual, Daniel R. Marshak et al. eds., Cold Spring Harbor Laboratory Press (1996); Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)), but separation and purification methods therefor are not limited to these examples.

[0133]

Examples of chromatography can include affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel filtration chromatography, reverse phase chromatography, and adsorption chromatography.

[0134]

These chromatographies can be performed using liquid chromatography such as HPLC and FPLC.

[0135]

Examples of a column to be used for affinity chromatography can include a protein A column and a protein G column.

[0136]

Examples of a column using the protein A column can include Hyper D, POROS, Sepharose F. F. (GE Healthcare).

[0137]

An antibody can also be purified using a carrier to which an antigen is immobilized and the binding activity to the antigen.

[0138]

(Drug comprising anti-CD147 antibody)

The anti-CD147 antibody of the present invention can be obtained from among anti-CD147 antibodies obtained by a method described in the above section "Production of anti-CD147 antibody". The thus obtained antibody can be used as an agent for treating and/or preventing tumor and/or cancer. The anti-CD147 antibody of the present invention has excellent antitumor efficacy, and is useful as a remedy for treating tumor or cancer. The anti-CD147 antibody of the present invention exhibited excellent antitumor efficacy on gemcitabine-resistant cancer cells and cancer cells having low sensitivity to sorafenib. The anti-CD147 antibody of the present invention exhibited significantly stronger efficacy on chronic myeloid leukemia cells, than those of imatinib.

[0139]

Examples of tumors that can be treated using the anti-CD147 antibody of the present invention or a drug comprising the antibody are not particularly limited, as long as they are tumors expressing CD147, and include preferably, pancreatic cancer, liver cancer, gastric cancer, colon cancer, renal cancer, breast cancer, uterine cancer, ovarian cancer, lung cancer, thyroid cancer, skin cancer, head and neck cancer, sarcoma, prostate cancer, bladder cancer, brain tumor, gastrointestinal stromal tumor(GIST), leukemia (for example, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), or chronic lymphocytic leukemia (CLL) or acute lymphocytic leukemia(ALL)), lymphoma or malignant lymphoma (for example, B-cell lymphoma, non-Hodgkin's lymphoma or diffuse large B-cell lymphoma (DLBCL)), and more preferably, pancreatic cancer, liver cancer, gastric cancer, colon cancer, renal cancer, leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), malignant lymphoma, B-cell lymphoma, non-Hodgkin's lymphoma or diffuse large B-cell lymphoma (DLBCL).

[0140]

Examples of tumors that can be treated using the antibody of the present invention or a drug comprising the antibody include SMAD-positive CD147-expressing

tumors. Examples of SMAD-positive CD147-expressing tumors include SMAD-positive CD147-expressing liver cancer and pancreatic cancer. The anti-CD147 antibody of the present invention or a drug comprising the antibody is administered to, preferably, patients confirmed for the expression of CD147 and/or SMAD. Examples of SMAD include, preferably, SMAD2, SMAD3 and/or SMAD4, and more preferably SMAD4. Preferably, together with confirmation of the expression of SMAD4, the expression of at least one of SMAD2 and SMAD3 is confirmed.

[0141]

Alternatively, examples of a tumor that can be treated with the antibody of the present invention or a drug comprising the antibody include a tumor exhibiting decreased expression of KLF5 or lacking the expression of KLF5. Examples of a tumor exhibiting decreased expression of KLF5 or lacking the expression of KLF5 include liver cancer, ALL, lymphoma, gastrointestinal stromal tumor (GIST), skin cancer, sarcoma, AML and renal cancer. The anti-CD147 antibody of the present invention or a drug comprising the antibody is preferably administered to a patient confirmed to exhibit decreased expression of KLF5 or lack the expression of KLF5.

[0142]

The anti-CD147 antibody of the present invention can also be administered with 2, 3 or more other therapeutic agents depending on the purpose of treatment. These

other therapeutic agents can be encapsulated in the same preparation and administered simultaneously. Other therapeutic agents and the anti-CD147 antibody may also be encapsulated in the same preparation and thus can be administered simultaneously. The anti-CD147 antibody and other therapeutic agents may be separately encapsulated in preparations and can then be administered simultaneously. Further, other drugs and the anti-CD147 antibody can also be administered separately, specifically the drugs can be administered before or after the administration of the anti-CD147 antibody. Specifically, after administration of other therapeutic agents, a therapeutic agent comprising the anti-CD147 antibody or an antigen-binding fragment of the antibody as an active component is administered, or, after administration of a therapeutic agent comprising the anti-CD147 antibody or an antigen-binding fragment of the antibody as an active component, other therapeutic agents may be administered.

[0143]

The present invention also provides a pharmaceutical composition comprising a therapeutically and/or prophylactically effective amount of the anti-CD147 antibody, and a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant.

[0144]

The present invention also provides a pharmaceutical composition comprising a therapeutically and/or prophylactically effective amount of the anti-CD147 antibody, a therapeutically and/or prophylactically effective amount of at least one antitumor therapeutic agent, and a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant.

[0145]

Substances to be used for a preparation that are acceptable in the pharmaceutical composition of the present invention are preferably nontoxic to a subject to which the pharmaceutical composition is administered in terms of preferably dosage and concentration for administration.

[0146]

The pharmaceutical composition of the present invention can contain substances for a preparation in order to vary or maintain the pH, osmotic pressure, viscosity, transparency, color, isotonicity, sterility, stability, solubility, sustained release rate, absorptivity, permeability, and the like. Examples of substances for a preparation can include the following, but are not limited to: amino acids such as glycine, alanine, glutamine, asparagine, arginine or lysine, antimicrobial agents, antioxidants such as ascorbic acid, sodium sulfate or sodium hydrogen sulfite, buffering agents such as a phosphate, citrate, or borate buffer,

sodium hydrogen carbonate, and tris-hydrochloric acid (Tris-Hcl) solution, fillers such as mannitol and glycine, chelating agents such as ethylenediaminetetraacetic acid (EDTA), complexing agents such as caffeine, polyvinyl pyrrollysine, β -cyclodextrin, and hydroxypropyl- β -cyclodextrin, extending agents such as glucose, mannose or dextrin, carbohydrates such as monosaccharide and disaccharide, colorants, flavors, diluents, emulsifiers, hydrophilic polymers such as polyvinyl pyrrollysine, low-molecular-weight polypeptides, salt-forming counter ions, benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, antiseptics such as sorbic acid or hydrogen peroxide, glycerin, solvents such as propylene and glycol or polyethylene glycol, sugar alcohols such as mannitol or sorbitol, suspensions, sorbitan esters, polysorbates such as polysorbate 20 and polysorbate 80, triton, tromethamine, surfactants such as lecithin or cholesterol, stabilization enhancers such as sucrose and sorbitol, sodium chloride, potassium chloride, elasticity enhancers such as mannitol and sorbitol, transporting agents, excipients, and/or pharmaceutical adjuvants. The amount of such a substance for a preparation is preferably added in an amount 0.01 to 100 times, and particularly 0.1 to 10 times the weight of the anti-CD147 antibody. The composition of a suitable pharmaceutical composition in a preparation can be

adequately determined by persons skilled in the art according to its applicable disease, applicable route of administration, and the like.

[0147]

An excipient and a carrier in a pharmaceutical composition may be liquid or solid. An appropriate excipient and an appropriate carrier may be water or saline for injection, an artificial cerebrospinal fluid, or other substances that are generally used for parenteral administration. Neutral saline or saline containing serum albumin can also be used as a carrier. A pharmaceutical composition can contain a Tris buffer of pH7.0-8.5, an acetate buffer of pH4.0-5.5, or a citrate buffer of pH3.0-6.2. These buffers may contain sorbitol and other compounds. Examples of the pharmaceutical composition of the present invention can include a pharmaceutical composition comprising the anti-CD147 antibody and a pharmaceutical composition comprising the anti-CD147 antibody and at least one antitumor therapeutic agent. The pharmaceutical composition of the present invention is prepared as a drug having the selected composition and purity as required, a freeze-dried product or a liquid. A pharmaceutical composition comprising the anti-CD147 antibody and a pharmaceutical composition comprising the anti-CD147 antibody and at least one anticancer therapeutic agent can also be

formulated as a freeze-dried product in which an appropriate excipient such as sucrose is used.

[0148]

The pharmaceutical composition of the present invention can also be prepared for parenteral administration, or for oral administration for gastrointestinal absorption. The composition and the concentration of a preparation can be determined depending on the method of administration. When the antibody of the present invention is administered to a human, about 0.1 to 100 mg/kg of the antibody may be administered once or several times during 1 to 180 days. However, the dosage and the frequency of administration should be generally determined in consideration of the gender, body weight, and age of a patient, symptoms, severity, adverse reaction, and the like, and thus doses and methods therefor are not limited to the above examples.

[0149]

Examples of the form of the pharmaceutical composition of the present invention can include injection preparations including preparations for infusion, suppositories, nasal agents, sublingual agents, and transdermal absorbents. Examples of the route of administration include oral administration or parenteral administration. Examples of parenteral administration include intravenous, intraarterial, intramuscular, rectal,

transmucosal or intramucosal, and intradermal administrations.

[0150]

The antibody of the present invention or an antigen-binding fragment of the antibody, a drug complex comprising them, a bispecific antibody comprising them or a pharmaceutical composition comprising them can be provided in combination with a biomarker for selecting patients to which they are administered. Such an antibody or a pharmaceutical composition may also be provided as a kit in combination with a means for detecting a biomarker, or, such an antibody or a pharmaceutical composition may be provided separately with a biomarker. Through the use of a biomarker, the antibody or the pharmaceutical composition of the present invention can be administered to a patient group for whom the antibody of the present invention is expected to be highly effective.

[0151]

The present invention relates to: a method for predicting responsiveness to cancer treatment, comprising using cancer patient-derived biological samples, measuring the expression of SMAD4 or the expression of KLF5 contained in the biological samples, and determining patients in whom SMAD4 is detected or patients in whom decreased expression of KLF5 or the lack of the expression of KLF5 is detected as having responsiveness

to cancer treatment with the antibody of the present invention or a functional fragment of the antibody, or, the pharmaceutical composition of the present invention; a method for selecting subjects of cancer treatment, comprising using cancer patient-derived biological samples, detecting the expression of SMAD4 or the expression of KLF5 in the biological samples, and selecting patients in whom SMAD4 is detected or patients in whom decreased expression of KLF5 or the lack of the expression of KLF5 is detected as subjects of cancer treatment with the antibody of the present invention or a functional fragment of the antibody, or, the pharmaceutical composition of the present invention; a method for treating cancer, comprising using cancer patient-derived biological samples, detecting the expression of SMAD4 or the expression of KLF5 in the biological samples, and administering the antibody of the present invention or a functional fragment of the antibody, or, the pharmaceutical composition of the present invention to patients in whom SMAD4 is detected or patients in whom decreased expression of KLF5 or the lack of the expression of KLF5 is detected; or, a kit for determining responsiveness to cancer treatment with the antibody of the present invention or a functional fragment of the antibody, or, the pharmaceutical composition of the present invention, comprising at least a means for detecting the expression of SMAD4 or the

expression of KLF5 in the cancer patient-derived biological samples.

[0152]

The term "biological sample(s)" as used herein refers to a tissue, a liquid, and cells isolated from an individual and a mixture thereof, and examples thereof can include, but are not limited to, a tumor biopsy, spinal fluids, pleural fluids, intraperitoneal fluids, lymph, skin sections, blood, urine, feces, sputum, respiratory organs, intestinal tract, genitourinary organs, saliva, breast milk, digestive organs, and cells collected therefrom. Examples of the "biological sample(s)" include preferably a sample containing cancer cells, more preferably a tissue or cells obtained by excision or biopsy, or cells derived from a pleural fluid or an intraperitoneal fluid. Further preferred biological samples are samples containing cancer cells or cancerous tissue.

[0153]

The "expression of SMAD4" can be detected or measured using the genome sequence, the gene expression or the protein expression of SMAD4 according to a method known by persons skilled in the art. Examples of such a method include RNA sequencing, microarray, genome sequencing, and immunoassay.

[0154]

The "expression of KLF5" can be detected or measured using the genome sequence, the gene expression or the protein expression of KLF5 according to a method known by persons skilled in the art. Examples of such a method include IHC, RNA sequencing, microarray, genome sequencing, and immunoassay. The term "decreased expression of KLF5" means that when compared with a control (for example, the expression level in a healthy subject or the expression level in a non-cancerous tissue of the same patient), the expression level is found to be lower than that of the control. Alternatively, the degree of the decreased expression of KLF5 at which the responsiveness to cancer treatment resulting from the use of the antibody or the pharmaceutical composition of the present invention can be confirmed, can be determined by performing a method known by persons skilled in the art and appropriate clinical trials. For example, patients for which an effect can be obtained and patients for which no effect can be obtained are compared for KLF5 expression levels, thereby setting an appropriate threshold. Therefore, the term "decreased expression of KLF5" refers to an expression level lower than the thus determined threshold, for example.

Examples

[0155]

Hereinafter, the present invention will be described specifically by way of examples, but the present invention is not limited to the following examples. Moreover, these are not to be interpreted in a limited manner in any sense. In the following examples, procedures relating to genetic engineering were performed according to the methods described in "Molecular Cloning" (Authors: Sambrook, J., Fritsch, E. F., and Maniatis, T., published by Cold Spring Harbor Laboratory Press in 1989), other methods described in experimental manuals and used by those skilled in the art, or instructions of commercially available products in the case of using commercially available reagents or kits, unless otherwise stated. Further, reagents, solvents, and starting materials which are not specifically mentioned in this description can be easily obtained from commercial supply sources. In these examples, ATCC, Cat. CRL-1420 was used as a human pancreatic line MIA PaCa-2, and ATCC, Cat. CRL-1469 was used as a human pancreatic cancer cell line PANC-1.

[0156]

(Example 1) Production of mouse and rat antibodies by cell immunization

1)-1 Production of CD147 expression vector

A clone IOH3378 (Invitrogen, Thermo Fisher Scientific K.K.) of a commercially available human CD147 gene (BSG variant2/CD147v2) was cloned into an expression

vector for mammalian cells pcDNA-DEST40 (Invitrogen, Thermo Fisher Scientific K.K.) using Gateway LR Clonase to produce a human CD147v2 expression vector (pcDNA-DEST40-CD147v2).

[0157]

An expression vector for mammalian cells pCMV6-XL5-hBSGv1 (OriGene Technologies, Inc., Cat. SC303059) of a commercially available human CD147 gene (BSG variant1/CD147v1) was purchased and used as a human CD147v1 expression vector.

[0158]

As a cynomolgus monkey CD147 expression vector, pCMV3-cynoBSG (Sino Biological Inc., Cat. CG90636-UT) was purchased.

[0159]

As a mouse CD147v2 expression vector, pCMV3-mBSGv2 (Sino Biological Inc., Cat. MG50332-UT) was purchased.

[0160]

1)-2 Production of mouse hybridomas

4 to 6 week-old BALB/cAnNCr1Cr1j mice (CHARLES RIVER LABORATORIES JAPAN, INC.) were used. 5×10^6 LNCaP cells (ATCC, CRL-1740) separated using Versene (available from Thermo Fisher SCIENTIFIC K.K.) on day 0, 7, 15, and 24 were suspended in PBS, and the suspension was administered subcutaneously in the back. The same 5×10^6 cells were intravenously administered on day 31, and the spleen was collected on the same day and used for

producing hybridomas. The spleen cells were fused with mouse myeloma P3X63Ag8U.1 cells (ATCC, CRL-1597) using PEG4000 (Immuno-Biological Laboratories Co., Ltd.) to produce hybridomas. For isolating and culturing hybridomas, ClonaCell-HY MediumD (STEMCELL Technologies Inc.) and ClonaCell-HY MediumE (STEMCELL Technologies Inc.) were used.

[0161]

1)-3 Production of rat hybridomas

7 week-old WKY/Izm rats (Japan SLC, Inc.) were used. 13 days after immunization with 1×10^7 cells of a human pancreatic cancer cell line PANC-1 in the buttocks, iliac lymph node cells were collected and used for generation of hybridomas. Rat spleen cells were fused with mouse myeloma SP2/0-Ag14 cells (ATCC, CRL-1581) using a LF301 cell fusion device (BEX CO., LTD.) to produce hybridomas. For isolating and culturing hybridomas, ClonaCell-HY MediumD (STEMCELL Technologies Inc.) and ClonaCell-HY MediumE (STEMCELL Technologies Inc.) were used.

[0162]

1)-4 Identification of antigen by ELISA

A human CD147-Fc fusion protein (Sino Biological Inc., Cat. 10186-H02H) and a mouse CD147-Fc fusion protein (Sino Biological Inc., Cat.50332-M03H) were used. The human CD147-Fc fusion protein and the mouse CD147-Fc fusion protein were prepared by adding a PBS buffer solution and dissolving on ice to 1 µg/ml. 100 µl of the

protein solution was added to a 96-well plate (Nunc, Thermo Fisher Scientific K.K., Cat. 442404), followed by storage overnight at 4°C, and the wells were coated with a CD147-Fc fusion protein. The protein solution was removed, and the wells were blocked with a PBS buffer solution containing 1% BSA (Research Organics, Inc., Cat. 1334A) at 4°C for two hours. The wells were washed three times with a PBS buffer solution containing 0.05% Tween20 (ATTO CORPORATION, Cat. WSE-7235). Thereafter, the hybridoma culture supernatants prepared in Examples 1)-2 and 1)-3 were diluted 20 times with a PBS buffer solution and were each added to wells, followed by incubation at room temperature for one hour. The wells were washed with a PBS buffer solution containing 0.05% Tween20 (ATTO CORPORATION, Cat. WSE-7235) three times. Thereafter, 100 µl of anti-rat-Fab2-igG-HRP (Jackson ImmunoResearch Inc., Cat. 112-036-072) diluted 50000 times with a PBS buffer solution containing 1% BSA was added thereto, followed by shaking at room temperature for 30 minutes. The wells were washed 5 times with a PBS buffer solution containing 0.05% Tween20 (ATTO CORPORATION, Cat. WSE-7235). Thereafter, 100 µl of HRP enzyme coloring reagent (eBioscience, Thermo Fisher SCIENTIFIC K.K., Super AquaBlue ELISA substrate, Cat. 00-4203) was added thereto, followed by heating at room temperature for 10 to 20 minutes, and the absorbance at 405 nm was measured with a plate reader (Envision, PerkinElmer, Inc). An average of

the measured values for absorbance of two to three wells was calculated, and antibodies having an absorbance twice or more the measured value of the control well without primary antibodies were determined to have a binding activity (+), and those having an absorbance less than twice were determined to have no binding activity (-). Table 1 summarizes the results. Color development specific to wells coated with the human CD147-Fc fusion protein was observed in the culture supernatants of LN22R8, 2P1A6, 2P3A9, 2P3G8, 2P8C12, 2P10F2, 2P2D7, 2P2D10, and 2P1B7. Color development specific to wells coated with the human and mouse CD147-Fc fusion proteins was observed in LN24R7, 2P5F5, 2P6A2, and 2P3G8.

[0163]

[Table 1]

	BSA	hCD 147	mCD 147
LN 22R 8	-	+	-
LN 24R 7	-	+	+
2P 1A 6	-	+	-
2P 3A 7	-	-	-
2P 3A 9	-	+	-
2P 3E 12	-	-	-
2P 3G 5	-	-	-
2P 5E 2	-	-	-
2P 5F 5	-	+	+
2P 6A 2	-	+	+
2P 6C 9	-	-	-
2P 6H 2	-	-	-
2P 7B 8	+	+	-
2P 7D 6	-	-	-
2P 8B 12	-	-	-
2P 8C 12	-	+	-
2P 9A 12	+	+	+
2P 9B 10	-	-	-
2P 10E 6	-	-	-
2P 10F 2	-	+	-
2P 10H 2	+	+	+
2P 2D 7	-	+	-
2P 2D 10	-	+	-
2P 2E 6	-	-	-
2P 2H 8	-	-	-
2P 1B 7	-	+	-
2P 3A 9	-	-	-
2P 3G 8	-	+	+

[0164]

1)-5 Preparation of monoclonal antibodies and
determination of antibody isotypes

For hybridomas in which production of anti-human CD147 antibodies was observed in Example 1)-4 and which could be stably cultured, isotypes of the antibodies contained in culture supernatants were determined using a commercially available isotyping kit, and Table 2 shows

the results. Using a CL-1000 flask (Becton, Dickinson and Company), these hybridomas were cultured to prepare hybridoma culture supernatants containing monoclonal antibodies.

[0165]

[Table 2]

CD147 antibodies obtained from cell immunization

Antibody	Isotype
LN22R8	Mouse IgG3
2P1A6	Rat IgG2a/ κ
2P1B7	Rat IgG2a/ κ
2P2D6	Rat IgG2a/ κ
2P3G8	Rat IgG2b/ κ
2P2D7	Rat IgG2a/ κ
2P2D10	Rat IgG2b/ κ
2P8C12	Rat IgG2a/ κ
2P10F2	Rat IgG2b/ κ

[0166]

1)-6 Purification of monoclonal antibodies

Each antibody was purified from the culture supernatant prepared in Example 1)-5. The anti-human CD147 mouse monoclonal antibody was purified by a one-step process of rProtein A affinity chromatography (at 4 to 6°C). A buffer replacement step was performed at 4 to 6°C after the rProtein A affinity chromatographic purification. First, the culture supernatant was applied to a column equilibrated with PBS and filled with MabSelectSuRe (available from GE Healthcare Bioscience). After the culture solution was fully put into the column,

the column was washed with PBS in an amount twice or more the column volume. Next, elution with a 2M arginine hydrochloride solution (pH 4.0) was performed to collect a fraction containing the antibody. The fraction was subjected to liquid replacement with HBSor (25 mM Histidine/5% Sorbitol/pH 6.0) by dialysis (Thermo Scientific, Slide-A-Lyzer Dialysis Cassette). After concentration using a Centrifugal UF Filter Device VIVASPIN20 (fraction molecular weight UF10K, Sartorius AG, at 4°C), the IgG concentration was adjusted to 4.9 mg/ml. Finally, filtration was performed using a Minisart-Plus filter (Sartorius AG) to give a purified sample.

[0167]

The anti-human CD147 rat monoclonal antibody was purified by a one-step process of Protein G affinity chromatography (at 4 to 6°C). A buffer replacement step was performed at 4 to 6°C after the Protein G affinity chromatographic purification. First, the hybridoma culture supernatant was applied to a column equilibrated with PBS and filled with ProteinG (GE Healthcare Bioscience). After the culture supernatant solution was fully put into the column, the column was washed with PBS in an amount twice or more the column volume. Next, elution with a 0.1M glycine/aqueous hydrochloric acid solution (pH 2.7) was performed to collect a fraction containing the antibody. After 1M Tris-HCl (pH 9.0) was added to the collected fraction to adjust the pH to 7.0

to 7.5, buffer replacement with HBSor (25mM Histidine/5% Sorbitol/pH 6.0) was performed together with concentration using a Centrifugal UF Filter Device VIVASPIN20 (fraction molecular weight UF30K, Sartorius AG, at 4 to 6°C) to adjust the antibody concentration to 1 mg/mL or more. Finally, filtration was performed using a Minisart-Plus filter (Sartorius AG) to give a purified sample.

[0168]

1)-7 Antibody screening by measurement of in-vivo antitumor efficacy

1 × 10⁷ cells of human pancreatic line PANC-1 were suspended in PBS, and the suspension was inoculated subcutaneously into the axilla of NOD-scid mice (CHARLES RIVER LABORATORIES JAPAN, INC., NOD. CB17-Prkdc<scid>/J). Grouping was performed based on tumor volume, and the mouse anti-CD147 antibody (LN22R8) or the rat anti-CD147 antibody (2P1A6, 2P1B7, 2P3G8, 2P2D10, 2P8C12, or 2P10F2) was administered intraperitoneally to cancer-bearing mice at 10 mg/kg on the 27th, 34th, and 41st day after the inoculation (n = 6). The rat anti-CD147 antibody (2P2D6) was administered intraperitoneally to cancer-bearing mice at 10 mg/kg on the 27th and 34th day after the inoculation (n = 6). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO

CORPORATION) to calculate the tumor volume by the following formula.

[0169]

Tumor volume (mm³) = 1/2 × minor axis (mm) × minor axis (mm) × major axis (mm)

Figures 1(a) to (c) show the results. The graphs also show the averages and the standard errors of changes in tumor volume. Figure 1(a) shows the results of the 2P2D6 antibody, the 2P3G8 antibody, and the 2P2D10 antibody. The tumor growth inhibition rate on the 48th day after the inoculation, which was the final measurement date, was 10%, 45%, and 40%, respectively, in the 10 mg/kg administration group. Figure 1(b) shows the results of the LN22R8 antibody, the 2P1A6 antibody, and the 2P1B7 antibody. The tumor growth inhibition rate on the 48th day after the inoculation, which was the final measurement date, was 50%, 26%, and 24%, respectively, in the 10 mg/kg administration group. Figure 1(c) shows the results of the 2P8C12 antibody and the 2P10F2 antibody. The tumor growth inhibition rate on the 48th day after the inoculation, which was the final measurement date, was -2% and 62%, respectively, in the 10 mg/kg administration group.

[0170]

1)-8 Analysis of species cross reactivity of CD147 antibody

Using Lipofectamine 2000 (Thermo Fisher SCIENTIFIC K.K., Cat. 11668-019), pcDNA-DEST40-CD147v2 or pCMV3-cynoBSG produced in Example 1)-1 was transfected into CHO-K1 cells (ATCC, CCL-61), and the cells were treated with mouse anti-human CD147 antibody (LN22R8) or rat anti-human CD147 antibody (2P1A6, 2P1B7, 2P3G8, 2P2D10, 2P8C12, 2P10F2, or 2P2D6) at 10 µg/ml one day later, thereby enabling fluorescence detection of binding of each antibody to CD147 expressing CHO-K1 cells using anti-mouse IgG-FITC (MP Biomedicals, Inc., Cat. 554936) or anti-rat IgG-PE (BD Biosciences, Cat. 550767). Fluorescence detection of human and cynomolgus monkey CD147 expression in CHO-K1 cells was enabled by binding of commercially available anti-CD147 antibody (MEM-M6/1, AbD Serotec, Cat. MCA28822). The aforementioned cells were measured using a flow cytometer (CantoII, BD Biosciences), and Figures 2-1 to 2-3 summarize the results. In the graphs, the vertical axis represents the number of cells, and the horizontal axis represents the intensity of fluorescence signals.

[0171]

A commercially available anti-CD147 antibody (MEM-M6/1), a mouse anti-human CD147 antibody (LN22R8), and rat anti-CD147 antibodies (2P1A6, 2P1B7, 2P3G8, 2P2D10, 2P8C12, 2P10F2, and 2P2D6) all exhibited a binding activity to human CD147 expressing CHO-K1 cells (Figures 2-1 to 2-3).

[0172]

The commercially available anti-CD147 antibody (MEM-M6/1) exhibited a binding activity to cynomolgus monkey CD147 expressing CHO-K1 cells, but none of the mouse anti-human CD147 antibody (LN22R8) and the rat anti-human CD147 antibodies (2P1A6, 2P1B7, 2P3G8, 2P2D10, 2P8C12, 2P10F2, and 2P2D6) exhibited a binding activity to cynomolgus monkey CD147 expressing CHO-K1 cells (Figure 2-1 to 2-3).

[0173]

None of the anti-CD147 antibodies exhibited a binding activity to mouse CD147 expressing CHO-K1 cells (data not shown).

[0174]

1)-9 Epitope analysis

Production of human CD147 expressing allogeneic vectors for epitope analysis

As a result of a BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), the amino acid sequences of cynomolgus monkey and human CD147 were calculated to be 81% identical. Therefore, estimation of antitumor epitopes using mutants in which different amino sequences were partially grafted between the species was performed, as it was assumed that limited differences in the amino acid sequences would directly affect recognition of CD147 antibody binding epitopes. Amino acid sequences contained in hCD147v1 and v2 which were in

common were subjected to a sequence comparison between cynomolgus monkey and human CD147 and were classified into 9 different amino acid regions between the species to form mu1 to mu9 (Figure 3). To produce CD147 mutant expression vectors and to confirm the expression on a cell surface, a cDNA sequence of human CD147 variant2 with a FLAG sequence introduced into the N-terminus was artificially synthesized in order to produce a plasmid which was then introduced into a pcDNA3.1 vector, Signal-N-Flag-hCD147v2_pcDNA3.1 (by GenScript Biotech Corporation). Further, 9 types of human-cynomolgus monkey chimeric CD147 expression vectors, hCD147-mu1_pcDNA3.1, hCD147-mu2_pcDNA3.1, hCD147-mu3_pcDNA3.1, hCD147-mu4_pcDNA3.1, hCD147-mu5_pcDNA3.1, hCD147-mu6_pcDNA3.1, hCD147-mu7_pcDNA3.1, hCD147-mu8_pcDNA3.1, and hCD147-mu9_pcDNA3.1, which were formed by introducing DNA encoding cynomolgus monkey CD147 amino acid sequences mu1 to mu9 as amino acid substitution mutations by DNA replacement into the human CD147 gene of the same plasmid, were produced (by GenScript Biotech Corporation).

[0175]

1)-10 Identification of antitumor epitope regions using mutants

The 9 types of expression vectors of human CD147, cynomolgus monkey CD147, or human-cynomolgus monkey chimeric CD147 expression vectors were introduced into CHO-K1 cells (ATCC, CCL-61) using Lipofectamine 2000

(Thermo Scientific, Cat. 11668-019), followed by treatment with an anti-human CD147 mouse antibody (LN22R8) or a rat anti-human CD147 antibody (2P1A6, 2P1B7, 2P3G8, 2P2D10, 2P8C12, 2P10F2, or 2P2D6) at 10 µg/ml one day later, and binding of the anti-CD147 antibody to CD147 expressing CHO-K1 cells was investigated using anti-mouse IgG-PE (DAKO, Cat. R480) or anti-rat IgG-PE (Becton, Dickinson and Company, #550767). Expression of CD147 protein was confirmed using a commercially available anti-FLAG antibody (anti-Flag M2, SIGMA, Cat. F4049-.2MG). Measurements using a flow cytometer (CantoII, BD Biosciences) were taken, and Table 3 summarizes the results. Samples with an increase in fluorescence signals of 10 times or more as compared with those in the control cells that were not treated with primary antibodies were determined to be binding positive (+). Samples with a partial increase in fluorescence signals of less than 10 times were determined to be binding slightly positive (±). Samples with no increase in fluorescence signals as compared with those in the control cells that were not treated with primary antibodies were determined to be binding negative (-). [0176]

All the antibodies 2P3G8, 2P10F2, 2P2D10, and LN22R8 having an antitumor efficacy of 40% or more observed in Example 1)-7 had lost the binding activity to CD147 with

mu3 mutation. This suggested that an important epitope for the antitumor efficacy was the m3 region.

[0177]

[Table 3]

	in vivo anti-tumor effect	mock	pCMV3-cynoBSG	Signal -N-Flag-hCD147V2-pcDN A3.1	hCD1 47-mu1-pcDN A3.1	hCD1 47-mu2-pcDN A3.1	hCD1 47-mu3-pcDN A3.1	hCD1 47-mu4-pcDN A3.1	hCD1 47-mu5-pcDN A3.1	hCD1 47-mu6-pcDN A3.1	hCD1 47-mu7-pcDN A3.1	hCD1 47-mu8-pcDN A3.1	hCD147-mu9-pcDNA3.1
anti-Flag M2	ND	-	-	+	+	+	+	+	+	+	+	+	+
2P1A6	26%	-	±	+	+	+	±	+	+	+	+	+	+
2P1B7	24%	-	-	+	+	+	±	+	+	+	+	+	+
2P3G8	45%	-	-	+	+	+	-	+	+	+	+	+	+
2P10F2	58%	-	-	+	+	+	-	+	+	+	+	+	+
2P8C12	8%	-	-	+	+	+	+	+	+	+	+	+	+
2P2D10	40%	-	-	+	+	+	-	+	+	+	+	+	+
LN22R8	50%	-	-	+	±	+	-	+	+	+	+	+	±
MEM-M6/1	ND	-	+	+	+	+	±	+	+	+	+	+	+

[0178]

1)-11 Determination of the nucleotide sequence of cDNA encoding the variable region of LN22R8 and 2P10F2 antibodies

1)-11-1 Determination of the nucleotide sequence of cDNA encoding the variable region of the LN22R8 antibody

1)-11-1-1 Preparation of total RNA of LN22R8 antibody producing hybridoma

For amplifying a cDNA encoding the variable region of the LN22R8 antibody, total RNA was prepared using a TRIzol Reagent (Ambion) from hybridomas producing LN22R8 antibody.

[0179]

1)-11-1-2 Amplification of cDNA encoding the LN22R8 antibody light chain variable region by 5'-RACE PCR and determination of its sequence

A cDNA encoding the light chain variable region was amplified using about 1 µg of the total RNA prepared in Example 1)-11-1-1 and a SMARTer RACE 5'/3' Kit (Clontech Laboratories, Inc). As primers for amplifying the cDNA encoding the variable region of the light chain gene of the LN22R8 antibody by PCR, UPM (Universal Primer A Mix: attached to the SMARTer RACE 5'/3' Kit) and a primer designed from the sequence of the constant region of a known mouse light chain were used.

[0180]

The cDNA encoding the light chain variable region amplified by 5'-RACE PCR was cloned into a plasmid, and then the nucleotide sequence of the cDNA encoding the light chain variable region was analyzed.

[0181]

SEQ ID NO: 7 represents the nucleotide sequence of the cDNA encoding the LN22R8 antibody light chain variable region thus determined, and SEQ ID NO: 8 represents the amino acid sequence thereof. SEQ ID NOs: 11, 12, and 13 respectively represent CDRL1, CDRL2, and CDRL3 of the LN22R8 antibody light chain variable region.

[0182]

1)-11-1-3 Amplification of cDNA encoding the LN22R8 antibody heavy chain variable region by 5'-RACE PCR and determination of its sequence

A cDNA encoding the heavy chain variable region was amplified using about 1 µg of the total RNA prepared in Example 1)-11-1-1 and a SMARTer RACE 5'/3' Kit (Clontech Laboratories, Inc.). As primers for amplifying the cDNA encoding the variable region of the heavy chain gene of the LN22R8 antibody by PCR, UPM (Universal Primer A Mix: attached to the SMARTer RACE 5'/3' Kit) and a primer designed from the sequence of the constant region of a known mouse heavy chain were used.

[0183]

The cDNA encoding the heavy chain variable region amplified by 5'-RACE PCR was cloned into a plasmid, and then the nucleotide sequence of the cDNA encoding the heavy chain variable region was analyzed.

[0184]

SEQ ID NO: 9 represents the nucleotide sequence of the cDNA encoding the LN22R8 antibody heavy chain

variable region thus determined, and SEQ ID NO: 10 represents the amino acid sequence thereof. SEQ ID NOs: 14, 15, and 16 respectively represent CDRH1, CDRH2, and CDRH3 of the LN22R8 antibody heavy chain variable region. [0185]

1)-11-2 Determination of the nucleotide sequence of cDNA encoding the variable region of the 2P10F2 antibody

The same methods as in Example 1)-11-1 were employed. However, UPM (Universal Primer A Mix: attached to the SMARTer RACE 5'/3' Kit) and a primer designed from the sequence of the constant region of a known rat light chain were used as primers for amplifying the cDNA encoding the variable region of the light chain gene by PCR, and UPM (Universal Primer A Mix: attached to the SMARTer RACE 5'/3' Kit) and a primer designed from the sequence of the constant region of a known rat heavy chain were used as primers for amplifying the cDNA encoding the variable region of the heavy chain gene by PCR.

[0186]

SEQ ID NO: 17 represents the nucleotide sequence of the cDNA encoding the 2P10F2 antibody light chain variable region thus determined, and SEQ ID NO: 18 represents the amino acid sequence thereof. SEQ ID NOs: 21, 22, and 23 respectively represent CDRL1, CDRL2, and CDRL3 of the 2P10F2 antibody light chain variable region. SEQ ID NO: 19 represents the nucleotide sequence of the

cDNA encoding the heavy chain variable region, and SEQ ID NO: 20 represents the amino acid sequence thereof. SEQ ID NOs: 24, 25, and 26 respectively represent CDRH1, CDRH2, and CDRH3 of the 2P10F2 antibody heavy chain variable region.

[0187]

1)-12 Production of human chimeric antibody expression vectors of LN22R8

1)-12-1 Construction of human chimeric and humanized light chain expression vector pCMA-LK

About 5.4 kb of a fragment obtained by digesting a plasmid pcDNA3.3-TOPO/LacZ (Invitrogen Corp.) with restriction enzymes XbaI and PmeI was bound to a DNA fragment containing the human light chain signal sequence represented by SEQ ID NO: 27 and the DNA sequence encoding the human κ chain constant region using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.) to produce pcDNA3.3/LK.

[0188]

Neomycin expressing units were removed from pcDNA 3.3/LK, thereby constructing pCMA-LK.

[0189]

1)-12-2 Construction of human chimeric and humanized IgG1-type heavy chain expression vector pCMA-G1

A DNA fragment from which the light chain signal sequence and the human κ chain constant region were removed by digesting pCMA-LK with XbaI and PmeI was bound

to a DNA fragment containing the human heavy chain signal sequence represented by SEQ ID NO: 28 and the DNA sequence encoding the amino acids in the human IgG1 constant region using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.) to construct pCMA-G1.

[0190]

1)-12-3 Construction of human chimeric and humanized IgG2-type heavy chain expression vector pCMA-G2

Using a DNA fragment containing the human heavy chain signal sequence represented by SEQ ID NO: 29 and the DNA sequence encoding the amino acids in the human IgG2 constant region, pCMA-G2 was constructed in the same manner as in Example 1)-12-2.

[0191]

1)-12-4 Construction of human chimeric LN22R8 light chain expression vector

Using the cDNA encoding the LN22R8 light chain variable region obtained in Example 1)-11-1-2 as a template, PCR was performed using a primer designed for In-fusion cloning, thereby amplifying a DNA fragment containing the cDNA encoding the light chain variable region. The amplified DNA fragment was inserted at the site where pCMA-LK was digested with restriction enzyme BsiWI, using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), thereby constructing a human chimeric LN22R8 light chain expression vector. SEQ ID NO: 30 and SEQ ID NO: 31 respectively represent the

nucleotide sequence of human chimeric LN22R8 light chain and the amino acid sequence of the light chain.

[0192]

1)-12-5 Construction of IgG1-type human chimeric LN22R8 heavy chain expression vector

Using the cDNA encoding the LN22R8 heavy chain variable region obtained in 1)-11-1-3 as a template, PCR was performed using a primer designed for In-fusion cloning, thereby amplifying a DNA fragment containing the cDNA encoding the heavy chain variable region. Using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the amplified DNA fragment was inserted at the site where pCMA-G1 was digested with restriction enzyme BlnI, thereby constructing an IgG1-type human chimeric LN22R8 heavy chain expression vector. SEQ ID NO: 32 and SEQ ID NO: 33 respectively represent the nucleotide sequence of the IgG1-type human chimeric LN22R8 heavy chain and the amino acid sequence of the heavy chain.

[0193]

1)-12-6 Construction of IgG2-type human chimeric LN22R8 heavy chain expression vector

Using the cDNA encoding the LN22R8 heavy chain variable region obtained in Example 1)-11-1-3 as a template, PCR was performed using a primer designed for In-fusion cloning, thereby amplifying a DNA fragment containing the cDNA encoding the heavy chain variable region. Using an In-Fusion HD PCR cloning kit (Clontech

Laboratories, Inc.), the amplified DNA fragment was inserted at the site where pCMA-G2 was digested with restriction enzyme BspI, thereby constructing an IgG2-type human chimeric LN22R8 heavy chain expression vector. SEQ ID NO: 34 and SEQ ID NO: 35 respectively represent the nucleotide sequence of the IgG2-type heavy chain of human chimeric LN22R8 and the amino acid sequence of the heavy chain.

[0194]

1)-12-7 Construction of IgG4P-type human chimeric LN22R8 heavy chain expression vector

A DNA fragment containing the DNA sequence encoding the amino acid sequence of the IgG4P-type heavy chain of human chimeric LN22R8 represented by SEQ ID NO: 36 was synthesized (Geneart AG). Using the synthesized DNA fragment, an IgG4P-type human chimeric LN22R8 heavy chain expression vector was constructed in the same manner as in Example 1)-12-2. SEQ ID NO: 37 represents the amino acid sequence of the IgG4P-type heavy chain of human chimeric LN22R8.

[0195]

1)-13 Production of human chimeric antibody expression vectors of 2P10F2

1)-13-1 Construction of human chimeric and humanized IgG1LALA-type heavy chain expression vector pCMA-G1LALA

Using the human heavy chain signal sequence represented by SEQ ID NO: 38 and a DNA fragment

containing the DNA sequence encoding the amino acids of the human IgG1LALA constant region, pCMA-G1LALA was constructed in the same manner as in Example 1)-12-2.

[0196]

1)-13-2 Construction of human chimeric and humanized IgG4P-type heavy chain expression vector pCMA-G4P

Using the human heavy chain signal sequence represented by SEQ ID NO: 39 and a DNA fragment containing the DNA sequence encoding the amino acids of the human IgG4P constant region, pCMA-G4P was constructed in the same manner as in Example 1)-12-2.

[0197]

1)-13-3 Construction of human chimeric 2P10F2 light chain expression vector

Using the cDNA encoding the 2P10F2 light chain variable region obtained in Example 1)-11-2 as a template, a human chimeric 2P10F2 light chain expression vector was constructed in the same manner as in Example 1)-12-4. SEQ ID NO: 40 and SEQ ID NO: 41 respectively represent the nucleotide sequence of the human chimeric 2P10F2 light chain and the amino acid sequence of the light chain.

[0198]

1)-13-4 Construction of IgG1LALA-type human chimeric 2P10F2 heavy chain expression vector

Using the cDNA encoding the 2P10F2 heavy chain variable region obtained in Example 1)-11-2 as a template,

PCR was performed using a primer designed for In-fusion cloning, thereby amplifying a DNA fragment containing the cDNA encoding the heavy chain variable region. Using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the amplified DNA fragment was inserted at the site where pCMA-G1LALA was digested with restriction enzyme BspI, thereby constructing an IgG1LALA-type human chimeric 2P10F2 heavy chain expression vector. SEQ ID NO: 42 and SEQ ID NO: 43 respectively represent the nucleotide sequence of the IgG1LALA-type heavy chain of human chimeric 2P10F2 and the amino acid sequence of the heavy chain.

[0199]

1)-13-5 Construction of IgG2-type human chimeric 2P10F2 heavy chain expression vector

Using the cDNA encoding the 2P10F2 heavy chain variable region obtained in Example 1)-11-2 as a template, an IgG2-type human chimeric 2P10F2 heavy chain expression vector was constructed in the same manner as in Example 1)-12-6. SEQ ID NO: 44 and SEQ ID NO: 45 respectively represent the nucleotide sequence of the IgG2-type heavy chain of human chimeric 2P10F2 and the amino acid sequence of the heavy chain.

[0200]

1)-13-6 Construction of IgG4P-type human chimeric 2P10F2 heavy chain expression vector

Using the cDNA encoding the 2P10F2 heavy chain variable region obtained in Example 1)-11-2 as a template, PCR was performed using a primer designed for In-fusion cloning, thereby amplifying a DNA fragment containing the cDNA encoding the heavy chain variable region. Using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the amplified DNA fragment was inserted at the site where pCMA-G4P was digested with restriction enzyme BlnI, thereby constructing an IgG4P-type human chimeric 2P10F2 heavy chain expression vector. SEQ ID NO: 46 and SEQ ID NO: 47 respectively represent the nucleotide sequence of the IgG4P-type heavy chain of human chimeric 2P10F2 and the amino acid sequence of the heavy chain.

[0201]

1)-14 Production and preparation of human chimeric antibodies of LN22R8 and 2P10F2

1)-14-1 Production of human chimeric antibodies of LN22R8 and 2P10F2

FreeStyle 293F cells (Invitrogen Corp.) were subcultured and cultured according to the manual. The 1.2×10^9 FreeStyle 293F cells (Invitrogen Corp.) in the logarithmic growth phase were seeded in a 3-L Fernbach Erlenmeyer Flask (Corning Incorporated) and diluted with a FreeStyle293 expression medium (Invitrogen Corp.) to 2.0×10^6 cells/mL. 0.24 mg of a heavy chain expression vector, 0.36 mg of a light chain expression vector, and 1.8 mg of Polyethyleneimine (Polyscience #24765) were

added to 40 mL of an Opti-Pro SFM medium (Invitrogen Corp.), followed by gentle stirring and further standing for 5 minutes. Thereafter, the mixture was added to FreeStyle 293F cells. After shaking culture at 90 rpm in an incubator at 37°C and 8% CO₂ for 4 hours, 600 mL of an EX-CELL VPRO medium (SAFC Biosciences, Sigma-Aldrich Corporation), 18 mL of GlutaMAX I (Gibco), and 30 mL of Yeastolate Ultrafiltrate (Gibco) were added thereto, followed by shaking the culture at 90 rpm in an incubator at 37°C and 8% CO₂ for 7 days, and the obtained culture supernatant was filtered with a Disposable Capsule Filter (Advantec #CCS-045-E1H).

[0202]

A human chimeric antibody of LN22R8 obtained by the combination of the IgG1-type human chimeric LN22R8 heavy chain expression vector and the human chimeric LN22R8 light chain expression vector was named "LN22R8chIgG1". A human chimeric antibody of LN22R8 obtained by the combination of the IgG2-type human chimeric LN22R8 heavy chain expression vector and the human chimeric LN22R8 light chain expression vector was named "LN22R8chIgG2". A human chimeric antibody of LN22R8 obtained by the combination of the IgG4P-type human chimeric LN22R8 heavy chain expression vector and the human chimeric LN22R8 light chain expression vector was named "LN22R8chIgG4P". A human chimeric antibody of L2P10F2 obtained by the combination of the IgG1LALA-type human chimeric 2P10F2

heavy chain expression vector and the human chimeric 2P10F2 light chain expression vector was named "2P10F2chIgG1LALA". A human chimeric antibody of L2P10F2 obtained by the combination of the IgG2-type human chimeric 2P10F2 heavy chain expression vector and the human chimeric 2P10F2 light chain expression vector was named "2P10F2chIgG2". A human chimeric antibody of L2P10F2 obtained by the combination of the IgG4P-type human chimeric 2P10F2 heavy chain expression vector and the human chimeric 2P10F2 light chain expression vector was named "2P10F2chIgG4P".

[0203]

1)-14-2 Purification of human chimeric antibodies of LN22R8 and 2P10F2

Each antibody was purified from the obtained culture supernatant in Example 1)-14-1 by a one-step process of rProtein A affinity chromatography. After the culture supernatant was applied to a column equilibrated with PBS and filled with MabSelectSuRe (available from GE Healthcare Bioscience), the column was washed with PBS an amount twice or more the column volume. Next, elution with a 2M arginine hydrochloride solution (pH 4.0) was performed to collect a fraction containing the antibody. The fraction was subjected to buffer replacement with HBSor (25mM Histidine/5% Sorbitol/pH 6.0) by dialysis (Thermo Scientific, Slide-A-Lyzer Dialysis Cassette). The antibody was concentrated using a Centrifugal UF

Filter Device VIVASPIN20 (fraction molecular weight UF10K, Sartorius AG) to adjust the IgG concentration to 1 mg/mL or more. Finally, filtration was performed using a Minisart-Plus filter (Sartorius AG) to give a purified sample.

[0204]

1)-15 ADCC activity of human chimeric antibodies

The ADCC activity of each human chimeric antibody was evaluated using human peripheral blood mononuclear cells (PBMC) as effector cells and human pancreatic line MIA PaCa-2 as ADCC target cells. After MIA PaCa-2 cells labeled with radioisotope ^{51}Cr were treated with mouse antibody (LN22R8), rat antibody (2P10F2), or human chimeric antibodies (LN22R8chIgG1, LN22R8chIgG2, LN22R8chIgG4P, 2P10F2chIgG1LALA, or 2P10F2chIgG4P) at a concentration of 0.5 or 5 $\mu\text{g}/\text{ml}$ at 4°C for 30 minutes, PBMC separated from human peripheral blood was added thereto in a proportion of 20 times that of the MIA PaCa-2 cells, followed by culture for 4 hours at 37°C in the presence of 5% CO_2 . Using TopCount NXT v2.53, ^{51}Cr released into the supernatant was measured to obtain a total release value. Using the measured value of ^{51}Cr released when MIA PaCa-2 cells labeled with ^{51}Cr were treated with Triton-100, as a maximum release value, and the measured value of ^{51}Cr released when antibody treated cells without the addition of PBMC were treated, as a spontaneous release value, a % specific release was

calculated from the following formula. Figure 6 summarizes the results. As a negative control sample, a sample obtained by treating human IgG (hIgG, ChromPure Human IgG, Jackson ImmunoResearch Laboratories, Inc., Cat. 009-000-003) was measured in the same manner, and the results are shown together. The measurement was performed three times, and the averages and the standard deviations were calculated and shown.

[0205]

% specific release = (Total release - Spontaneous release)/Maximum release

While human IgG (hIgG) and mouse antibody of LN22R8 exhibited no ADCC activity, LN22R8chIgG1 exhibited an ADCC activity of 17.4% at 0.5 µg/ml and 18.1% at 5 µg/ml. The ADCC activities of LN22R8chIgG2 and LN22R8chIgG4P were lower than that of LN22R8chIgG1 and were respectively 3.0% and 2.2% even at 5 µg/ml.

[0206]

2P10F2 rat antibody exhibited an ADCC activity of 4.8% at 0.5 µg/ml and 8.4% at 5 µg/ml. 2P10F2chIgG1LALA exhibited the ADCC activities of 4.7% at 0.5 µg/ml and 2.9% at 5 µg/ml. 2P10F2chIgG4P exhibited the ADCC activities of 3.4% at 0.5 µg/ml and 1.1% at 5 µg/ml, which were lower than those of 2P10F2 rat antibody and 2P10F2chIgG1LALA. As reported in the literature (Bruggemann et al., J.Exp. Med., 1351-1361, 1987), human

chimeric antibodies using an IgG1 subtype exhibited the highest ADCC activity.

[0207]

1)-16 CDC activity of human chimeric antibodies

The complement-dependent cell killing activity (CDC activity) of each anti-human CD147 antibody was evaluated using human pancreatic line MIA PaCa-2 as target cells. A commercially available rabbit complement (Low Tox-M Rabbit Complement, CEDARLANE LABORATORIES LIMITED, Cat. CL3051) was used as a complement. A mouse antibody (LN22R8), a rat antibody (2P10F2), or a human chimeric antibodies (LN22R8chIgG1, LN22R8chIgG2, LN22R8chIgG4P, 2P10F2chIgG1LALA, or 2P10F2chIgG4P) were used as the anti-human CD147 antibody. As a CDC activity negative control antibody, human IgG (hIgG, ChromPure Human IgG, Jackson ImmunoResearch Laboratories, Inc., Cat. 009-000-003) was used. After the antibody was treated at 4°C and at a concentration of 0, 0.1, 1, or 10 µg/ml for one hour, the rabbit complement was added therein to a final concentration of 7.5%, followed by heating at 37°C in the presence of 5% CO₂ for three hours. Thereafter, intercellular ATP contained in living cells was measured using a CellTiter-Glo Luminescent Cell Viability Assay (Promega Corp., Cat. G7572). Luminescence signals obtained using CellTiter-Glo Luminescent Cell Viability Assay were quantified using an EnVision 2104 Multilabel Reader (PerkinElmer Co., Ltd). The measurement was

performed three times, and the averages and the standard deviations were calculated. Luminescence signals obtained from non-treated cells were taken as 100%, and any antibody- and complement-dependent reduced luminescence signals were taken as CDC activity. Figure 7 summarizes the results.

[0208]

Only in the mouse antibody (LN22R8) and the rat antibody (2P10F2), was a concentration-dependent CDC activity observed as compared with the negative control hIgG. In LN22R8, living cells at 10 µg/ml decreased to 41.1% at maximum. In 2P10F2, living cells at 10 µg/ml decreased to 53.5% at maximum.

[0209]

In the human chimeric antibodies (LN22R8chIgG1, LN22R8chIgG2, LN22R8chIgG4P, 2P10F2chIgG1LALA, and 2P10F2chIgG4P), no clear CDC activity was observed as compared with the negative control hIgG.

[0210]

1)-17 ADCP activity of human chimeric antibodies

It has been reported that human IgG antibodies exhibit a cell killing activity of cancer cells by inducing phagocytosis (ADCP) by antibody-dependent monocytes or macrophages via interaction with mouse Fcγ receptors (Overdijk et al., Journal of Immunology, 1-9, 2012). The ADCP activity of each human chimeric antibody was evaluated using RAW264.7 (ATCC, TIB-71) as effector

cells and human pancreatic line PANC-1 or MIA PaCa-2 as ADCP target cells. After ADCP target cells labeled with a PKH67 Green Fluorescent Cell Linker Mini Kit for General Cell Membrane Labeling (SIGMA, Cat. MINI67-1KIT) and the human chimeric antibodies (LN22R8chIgG1, LN22R8chIgG2, or LN22R8chIgG4P) were treated at a concentration of 20 $\mu\text{g/ml}$ at 4°C for one hour, RAW264.7 cells labeled with a PKH26 Red Fluorescent Cell Linker Lit for General Cell Membrane Labeling (SIGMA, Cat. PKH26GL-1KT) were added thereto at 5 times the amount of the ADCP target cells, followed by heating at 37°C in the presence of 5% CO₂ for three hours. Using a flow cytometer (Becton, Dickinson and Company, CantoII), the ratio of PKH26-positive cells that transferred to PKH67 signal-positive cells by phagocytosis was measured. As a negative control sample, a sample obtained by treating human IgG (hIgG, ChromPure Human IgG, Jackson ImmunoResearch Laboratories, Inc., Cat. 009-000-003) was measured in the same manner. The measurement was performed three times, and the averages and the standard deviations were calculated. Figure 8(a) shows the results for PANC-1, and Figure 8(b) shows the results for MIA PaCa-2, respectively.

[0211]

In the case of using PANC-1 cells as ADCP target cells, LN22R8chIgG1 exhibited a high ADCP activity of 9.2%, and LN22R8chIgG4P exhibited a high ADCP activity of

9.0%, as compared with human IgG (5.5%). LN22R8chIgG2 did not exhibit ADCP activity at 5.9%.

[0212]

Also in the case of using MIA PaCa-2 cells as ADCP target cells, a similar tendency was shown, in which LN22R8chIgG1 exhibited a high ADCP activity of 6.6%, and LN22R8chIgG4P exhibited a high ADCP activity of 6.1%, as compared with human IgG (3.6%). LN22R8chIgG2 did not exhibit ADCP activity at 3.6%.

[0213]

1)-18 Measurement of in-vivo antitumor efficacy of human chimeric antibodies

5×10^6 cells of human pancreatic line MIA PaCa-2 were suspended in PBS containing 50% GFR-Matrigel (Corning Inc., Cat. 354230), and the suspension was inoculated subcutaneously into the axilla of 4 to 5 week-old female NOD-scid mice (NOD. CB17-Prkdc^{scid}/J, purchased from CHARLES RIVER LABORATORIES JAPAN, INC). Grouping was performed based on tumor volume 5 to 7 days after the inoculation, and the mouse antibody (LN22R8) and the three types of human chimeric antibodies (LN22R8chIgG1, LN22R8chIgG2, and LN22R8chIgG4P) of the anti-human CD147 antibody LN22R8 were administered intraperitoneally to cancer-bearing mice at 1 mg/kg, 3 mg/kg, or 10 mg/kg (n = 5). The rat antibody (2P10F2) and the two types of human chimeric antibodies (2P10F2chIgG2 and 2P10F2chIgG4P) of the anti-human CD147

antibody 2P10F2 were administered intraperitoneally to cancer-bearing mice at 10 mg/kg (n = 5 to 6). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0214]

Tumor volume (mm³) = 1/2 × minor axis (mm) × minor axis (mm) × major axis (mm)

Figures 9-1(a) to (d) and Figures 9-2(e) to (g) show the results. The graphs also show the averages and the standard errors of changes in tumor volume.

[0215]

In both the mouse antibody and the three types of human chimeric antibodies, LN22R8 exhibited a dose-dependent antitumor efficacy. In the human chimeric antibody LN22R8chIgG4P 10 mg/kg administration group, complete tumor regression was observed 18 days after the inoculation in 5 mice out of 5 mice, and tumor regrowth was not found even at 41 days after the inoculation at the time of the completion of the experiment. In the other LN22R8 antibody administration groups, tumor regrowth was observed in some or all of the mice.

[0216]

In the rat antibody and the two types of human chimeric antibodies, the antitumor efficacy of 2P10F2 were observed. In the 2P10F2chIgG4P 10 mg/kg

administration group, complete tumor regression was observed 21 days after the inoculation in 6 mice out of 6 mice.

[0217]

It was suggested that the antitumor efficacy was exerted by a new mechanism of action on CD147, independent of the mouse immunity, because the mouse antibody LN22R8 and the rat antibody 2P10F2 that are anti-human CD147 antibodies configured to recognize the same epitope site maintain an antitumor efficacy of 90% or more not only in human chimeric antibody chIgG1 having effector functions dependent on the mouse immune system, such as ADCC activity, ADCP, and CDC activity, or human chimeric antibody chIgG4P having ADCP activity but also in human chimeric antibody chIgG2 that hardly shows any effector functions.

[0218]

1)-19 Antitumor efficacy of CD147 human chimeric antibody in NOG mice

NOG (NOD/Shi-scid, IL-2R γ null) mice obtained by crossbreeding a IL-2 receptor γ chain knockout, which is a common cytokine receptor domain, with NOD-scid mice lacking mouse T cells and B cells lack the activities of NK cells and complement in addition to mouse T and B cells and have reduced functions of macrophages and dendritic cells, thus being in an exceptionally severe state of immune failure (Ito, Blood, 3175-3182, 2002).

Whether the antitumor efficacy of the CD147 antibody is affected by such a severe state of failure of the mouse immune system was examined using a MIA PaCa-2 subcutaneous implantation model.

[0219]

5 × 10⁶ cells of human pancreatic line MIA PaCa-2 were suspended in PBS containing 50% GFR-Matrigel (Corning Inc., Cat. 354230), and the suspension was inoculated subcutaneously into the axilla of 7 week-old female NOG mice (NOD/Shi-scid, IL-2RγKO Jic, purchased from In-Vivo Science Inc.). Grouping was performed based on tumor volume 6 days after the inoculation, and the anti-CD147 human chimeric antibody (LN22R8chIgG4P) was administered intraperitoneally to cancer-bearing mice at 10 mg/kg (n = 5). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0220]

Tumor volume (mm³) = 1/2 × minor axis (mm) × minor axis (mm) × major axis (mm)

Figure 10 shows the results. The graphs also show the averages and the standard errors of changes in tumor volume.

[0221]

According to the results of the human chimeric antibody LN22R8chIgG4P shown in Figure 10, the tumor growth inhibition rate 17 days after the inoculation was 99% in the 10 mg/kg administration group, and complete tumor regression was observed in 3 mice out of 5 mice.

[0222]

A possibility of exhibiting an antitumor efficacy independent of mouse immune cells was suggested, because the anti-CD147 human chimeric antibody (LN22R8chIgG4P) exhibited a strong antitumor efficacy also against pancreatic cancer tumors formed in NOG mice, which lack activities of NK cells and complement in addition to mouse T and B cells.

[0223]

(Example 2) Production of monkey-cross-reactive rat antibody by immunization with CD147 protein

The anti-human CD147 antibodies exhibiting a strong antitumor efficacy obtained in Example 1 did not exhibit cross-reactivity with mouse, rat, and cynomolgus monkey CD147s. An attempt to obtain a CD147 antibody exhibiting cross-reactivity with cynomolgus monkey CD147 was made using each antibody obtained in Example 1.

[0224]

2)-1 Immunization

Female WKY/Izm rats (Japan SLC, Inc.) were used for immunization. A mixture of a Recombinant Human BSG, His tagged (available from Creative BioMart) antigen protein

and a Freund's Complete Adjuvant (available from Wako Pure Chemical Industries, Ltd.) was administered to the base of the tail of the rat, and the lymph nodes and spleen of the rat were collected and used for producing hybridomas.

[0225]

2)-2 Production of hybridomas

The lymph node cells or spleen cells were electrofused with mouse myeloma SP2/0-ag14 cells (ATCC, No. CRL-1581) using a LF301 Cell Fusion Unit (BEX CO., LTD.), and the fused cells were diluted with a ClonaCell-HY Selection Medium D (StemCell Technologies Inc.) and cultured. Emerging hybridoma colonies were collected, thereby producing monoclonal hybridomas. The hybridoma colonies collected were each cultured, and anti-CD147 antibody producing hybridomas were screened using the obtained hybridoma culture supernatant.

[0226]

2)-3 Antibody screening by flow cytometry

In order to select antibody producing hybridomas configured to bind to human cancer cells and exhibiting a binding activity to human CD147 and cynomolgus monkey CD147, screening for antibody binding activity was performed using a flow cytometer. A CD147 positive human pancreatic line MIA PaCa-2 was used as human cancer cells. CHO-K1 cells (CHO-K1-hCD147v2 and CHO-K1-cynoCD147) expressing human or cynomolgus monkey CD147 were used in

the same manner as in Example 1)-8 for confirming the binding activity to human or cynomolgus monkey CD147. An equal amount of the hybridoma culture supernatant was added to the suspension of MIA PaCa-2, CHO-K1-hCD147v2, or CHO-K1-cynoCD147, followed by reaction at 4°C for one hour or more. Thereafter, the cells were washed with PBS containing 5% FBS to enable fluorescence detection of binding of each antibody to the cells using anti-rat IgG-PE (BD Biosciences, Cat. 550767). Fluorescence signals of the cells were measured using a flow cytometer (CantoII, BD Biosciences), and the ratio of the fluorescence signals to those in the negative control sample (cells without addition of the hybridoma culture solution) was calculated. Table 2 summarizes a part of the results.

[0227]

Using a commercially available rat antibody isotyping kit (Bio-Rad Laboratories, RMT1), the isotype of the antibody contained in the culture supernatant was determined. Table 4 shows the results.

[0228]

The 2P10F2 obtained in Example 1 exhibited a binding activity to MIA PaCa-2 and CHO-K1-hCD147v2, and exhibited no binding activity to CHO-K1-cynoCD147. Rat_CD147_#84 (which may be referred to as r#84 in this description), rat_CD147_#131 (which may be referred to as r#131 in this description), rat_CD147_#110 (which may be referred to as

r#110 in this description), and rat_CD147_#101 (which may be referred to as r#101 in this description) exhibited a binding activity to MIA PaCa-2, CHO-K1-hCD147v2, and CHO-K1-cynoCD147. Thus, anti-human CD147 rat antibodies exhibiting cross-reactivity with cynomolgus monkey CD147 have been obtained.

[0229]

[Table 4]

Clone ID	Isotype	MIA PaCa-2	CHO-K1-hCD147 v2	CHO-K1-cyno CD147
2P10F2	Rat IgG2b/κ	17.3	33.0	0.0
r#84	Rat IgG2b/κ	9.1	9.9	19.0
r#131	Rat IgG1/κ	4.3	7.8	7.3
r#110	Rat IgG1/κ	2.7	4.9	4.6
r#101	Rat IgG2a/κ	10.2	23.1	8.8

[0230]

2)-4 Preparation of rat monoclonal antibody using low IgG serum

Anti-human CD147 monoclonal antibodies exhibiting cross-reactivity with cynomolgus monkey CD147 were purified from the hybridoma culture supernatant. First, antibody producing hybridomas of rat_CD147_#131 were grown to a sufficient amount with a ClonaCell-HY Selection Medium E, and thereafter the medium was replaced with a Hybridoma SFM (Life Technologies) to which Ultra Low IgG FBS (Life Technologies) was added at 20%, followed by culture for 7 days. This culture supernatant was collected and passed through a 0.45 μm filter for sterilization.

[0231]

2)-5 Preparation of rat monoclonal antibody by high-density culture

The hybridomas of rat_CD147_#84, rat_CD147_#101, or rat_CD147_#110 were cultured using a CL-1000 flask (Becton, Dickinson and Company) to prepare a hybridoma culture supernatant containing each monoclonal antibody.

[0232]

2)-6 Purification of monoclonal antibody

In the same manner as in Example 1)-6, the antibody was purified from the culture supernatant produced in Example 2)-4 and Example 2)-5.

[0233]

2)-7 Screening for antibody by measuring in-vivo antitumor efficacy

5×10^6 cells of human pancreatic line MIA PaCa-2 were suspended in PBS containing 50% GFR-Matrigel (Corning Inc., Cat. 354230), and the suspension was inoculated subcutaneously into the axilla of 5 week-old female NOD-scid mice (NOD. CB17-Prkdc^{scid}/J, purchased from CHARLES RIVER LABORATORIES JAPAN, INC.). Grouping was performed based on tumor volume 6 to 8 days after the inoculation, and the cynomolgus monkey-cross-reactive anti-CD147 rat antibody #84, #101, or #110 was administered intraperitoneally to cancer-bearing mice at 10 mg/kg 8 days and 15 days after the inoculation (n = 5). In the same manner, PBS was administered

intraperitoneally to the mice of the control group. The cynomolgus monkey-cross-reactive anti-CD147 rat antibody #131 was administered intraperitoneally to cancer-bearing mice at 10 mg/kg 6 days after the inoculation (n = 5). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0234]

Tumor volume (mm³) = 1/2 × minor axis (mm) × minor axis (mm) × major axis (mm)

Figures 11(a) to (d) show the results. The graphs also show the averages and the standard errors of changes in tumor volume. The tumor growth inhibition rate 28 days after the inoculation of r#84 was 95% in the 10 mg/kg administration group (Figure 11(a)). The tumor growth inhibition rate 15 days after the inoculation of r#101 was 37% in the 10 mg/kg administration group, but tumor regrowth was observed 28 days after the inoculation (Figure 11(b)). The tumor growth inhibition rate 15 days after the inoculation of r#110 was 51% in the 10 mg/kg administration group, but tumor regrowth was observed 28 days after the inoculation (Figure 11(c)). The tumor growth inhibition rate 16 days after the inoculation of r#131 was 50% in the 10 mg/kg administration group (Figure 11(d)). Thus rat antibody r#84 that strongly inhibits tumor growth in MIA PaCa-2 and rat antibodies

r#101, r#110, and r#131 that partially inhibit the tumor growth were obtained.

[0235]

Figures 11(a) to (d) show the results. The graphs also show the averages and the standard errors of changes in tumor volume. The tumor growth inhibition rate 28 days after the transplantation of r#84 was 95% in the 10 mg/kg administration group (Figure 11(a)). The tumor growth inhibition rate 15 days after the transplantation of r#101 was 37% in the 10 mg/kg administration group, but tumor regrowth was observed 28 days after the transplantation (Figure 11(b)). The tumor growth inhibition rate 15 days after the transplantation of r#110 was 51% in the 10 mg/kg administration group, but tumor regrowth was observed 28 days after the transplantation (Figure 11(c)). The tumor growth inhibition rate 16 days after the transplantation of r#131 was 50% in the 10 mg/kg administration group (Figure 11(d)).

[0236]

2)-8 Epitope analysis: 2P10F2-competitive ELISA

For the purpose of epitope analysis of the monkey-cross-reactive rat CD147 antibodies, whether each antibody inhibited the binding activity to a CD147 recombinant protein of 2P10F2chIgG4P was investigated by competitive ELISA. A human CD147-Fc fusion protein (Sino Biological Inc., 10186-H02H) was dissolved in PBS to 20

µg/ml, and 50 µl of the solution was added to a 96-well plate (Thermo Fisher SCIENTIFIC K.K., Cat. 43454), followed by storage at 4°C. After the protein solution was removed, 300 µl of PBS containing 1% BSA was added thereto, followed by heating at room temperature for one hour. Twenty five µl of the CD147 rat antibodies r#84, r#101, r#110, r#131, or 2P10F2 diluted with PBS containing 1% BSA at 20 or 60 µg/ml, or PBS containing 1% BSA were added as competitive antibodies to the 96-well plate, followed by heating at room temperature for two hours. Twenty five µl of 2P10F2chIgG4P diluted with PBS containing 1% BSA at 20 ng/ml was added to the 96-well plate, followed by heating at room temperature for two hours. The 96-well plate was washed with PBS containing 0.05% Tween 20 (BIO RAD, Cat. 170-6531) twice. 50 µl of Mouse monoclonal HP6025 Anti-Human IgG4 Fc (HRP) (Abcam plc., Cat. ab99823) diluted 2000 times with PBS containing 1% BSA was added to the 96-well plate, followed by heating at room temperature for one hour. The 96-well plate was washed with PBS containing 0.05% Tween 20 (BIO RAD, Cat. 170-6531) three times. 50 µl of Super AquaBlue ELISA Substrate (eBioscience, Thermo Fisher SCIENTIFIC K.K., 00-4203-58) was added to the 96-well plate, followed by heating at room temperature for 20 minutes. The absorbance at 405 nm of the 96-well plate was measured using an EnVision 2104 Multilabel Reader (PerkinElmer Co., Ltd). The measured value of a

well containing no competitive antibodies was used as the control, and the absorbance decreased by each competitive antibody was calculated in terms of %. Figure 12 shows the results. The measurement was performed for three wells, and the average was shown.

[0237]

Similarly to the 2P10F2 rat antibody, r#84, r#101, and r#131 inhibited the binding activity of 2P10F2chIgG4P by 90% or more, and thus it was suggested that their antibody recognition sites were close to that of 2P10F2. Meanwhile, r#110 did not inhibit the binding of the 2P10F2 rat antibody. It is conceivable that the binding of the 2P10F2 rat antibody could not be inhibited because its antibody recognition site was distant from that of 2P10F2, or its binding activity was weak.

[0238]

(Example 3) Cloning of monkey-cross-reactive rat antibody and production of human chimeric antibody

3)-1 Determination of the cloning nucleotide sequence of cDNA encoding the variable region of rat anti-CD147 antibodies

3)-1-1 Determination of the nucleotide sequence of cDNA encoding the variable region of the rat_CD147_#84 antibody

The determination was conducted in the same manner as in Example 1)-11-2. SEQ ID NO: 48 represents the nucleotide sequence of the cDNA encoding the determined

rat_CD147_#84 antibody light chain variable region, and SEQ ID NO: 49 represents the amino acid sequence thereof. SEQ ID NO: 50 represents the nucleotide sequence of the cDNA encoding the heavy chain variable region thereof, and SEQ ID NO: 51 represents the amino acid sequence thereof. SEQ ID NOs: 52, 53, and 54 respectively represent CDRL1, CDRL2 and CDRL3 of the rat_CD147_#84 antibody light chain variable region. SEQ ID NOs: 55, 56, and 57 respectively represent CDRH1, CDRH2, and CDRH3 of the rat_CD147_#84 antibody heavy chain variable region. [0239]

3)-1-2 Determination of the nucleotide sequence of cDNA encoding the variable region of the rat_CD147_#101 antibody

The determination was conducted in the same manner as in Example 1)-11-2. SEQ ID NO: 58 represents the nucleotide sequence of the cDNA encoding the determined rat_CD147_#101 antibody light chain variable region, and SEQ ID NO: 59 represents the amino acid sequence thereof. SEQ ID NO: 60 represents the nucleotide sequence of the cDNA encoding the heavy chain variable region, and SEQ ID NO: 61 represents the amino acid sequence thereof. SEQ ID NOs: 62, 63, and 64 respectively represent CDRL1, CDRL2 and CDRL3 of the rat_CD147_#101 antibody light chain variable region. SEQ ID NOs: 65, 66, and 67 respectively represent CDRH1, CDRH2, and CDRH3 of the rat_CD147_#101 antibody heavy chain variable region.

[0240]

3)-1-3 Determination of the nucleotide sequence of cDNA encoding the variable region of the rat_CD147_#110 antibody

The determination was conducted in the same manner as in Example 1)-11-2. SEQ ID NO: 68 represents the nucleotide sequence of the cDNA encoding the determined rat_CD147_#110 antibody light chain variable region, and SEQ ID NO: 69 represents the amino acid sequence thereof. SEQ ID NO: 70 represents the nucleotide sequence of the cDNA encoding the heavy chain variable region, and SEQ ID NO: 71 represents the amino acid sequence thereof. SEQ ID NOs: 72, 73, and 74 respectively represent CDRL1, CDRL2 and CDRL3 of the rat_CD147_#110 antibody light chain variable region. SEQ ID NOs: 75, 76, and 77 respectively represent CDRH1, CDRH2, and CDRH3 of the rat_CD147_#110 antibody heavy chain variable region.

[0241]

3)-1-4 Determination of the nucleotide sequence of cDNA encoding the variable region of the rat_CD147_#131 antibody

The determination was conducted in the same manner as in Example 1)-11-2. SEQ ID NO: 78 represents the nucleotide sequence of the cDNA encoding the determined rat_CD147_#131 antibody light chain variable region, and SEQ ID NO: 79 represents the amino acid sequence thereof. SEQ ID NO: 80 represents the nucleotide sequence of the

cDNA encoding the heavy chain variable region, and SEQ ID NO: 81 represents the amino acid sequence thereof. SEQ ID NOs: 82, 83, and 84 respectively represent CDRL1, CDRL2 and CDRL3 of the rat_CD147_#131 antibody light chain variable region. SEQ ID NOs: 85, 86, and 87 respectively represent CDRH1, CDRH2, and CDRH3 of the rat_CD147_#131 antibody heavy chain variable region.

[0242]

3)-2 Production of human chimeric antibody expression vectors

3)-2-1 Production of human chimeric antibody expression vector of rat_CD147_#84

3)-2-1-1 Construction of human chimeric and humanized IgG4PFALA-type heavy chain expression vector pCMA-G4PFALA

Using the human heavy chain signal sequence represented by SEQ ID NO: 88 and a DNA fragment containing the DNA sequence encoding the amino acids in the human IgG4PFALA constant region, pCMA-G4PFALA was constructed in the same manner as in Example 1)-12-2.

[0243]

3)-2-1-2 Construction of human chimeric rat_CD147_#84 light chain expression vector

Using the cDNA encoding the rat_CD147_#84 light chain variable region obtained in Example 3)-1-1 as a template, a human chimeric rat_CD147_#84 light chain expression vector was constructed in the same manner as in Example 1)-12-4. SEQ ID NO: 89 and SEQ ID NO: 90

respectively represent the nucleotide sequence of the human chimeric rat_CD147_#84 light chain and the amino acid sequence of the light chain.

[0244]

3)-2-1-3 Construction of IgG1-type human chimeric rat_CD147_#84 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#84 heavy chain variable region obtained in Example 3)-1-1 as a template, an IgG1-type human chimeric rat_CD147_#84 heavy chain expression vector was constructed in the same manner as in Example 1)-12-5. SEQ ID NO: 91 and SEQ ID NO: 92 respectively represent the nucleotide sequence of the IgG1-type heavy chain of human chimeric rat_CD147_#84 and the amino acid sequence of the heavy chain.

[0245]

3)-2-1-4 Construction of IgG2-type human chimeric rat_CD147_#84 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#84 heavy chain variable region obtained in Example 3)-1-1 as a template, an IgG2-type human chimeric rat_CD147_#84 heavy chain expression vector was constructed in the same manner as in Example 1)-12-6. SEQ ID NO: 93 and SEQ ID NO: 94 respectively represent the nucleotide sequence of the IgG2-type heavy chain of human chimeric rat_CD147_#84 and the amino acid sequence of the heavy chain.

[0246]

3)-2-1-5 Construction of IgG4P-type human chimeric
rat_CD147_#84 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#84 heavy chain variable region obtained in Example 3)-1-1 as a template, an IgG4P-type human chimeric rat_CD147_#84 heavy chain expression vector was constructed in the same manner as in Example 1)-13-6. SEQ ID NO: 95 and SEQ ID NO: 96 respectively represent the nucleotide sequence of the IgG4P-type heavy chain of human chimeric rat_CD147_#84 and the amino acid sequence of the heavy chain.

[0247]

3)-2-1-6 Construction of IgG1LALA-type human chimeric
rat_CD147_#84 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#84 heavy chain variable region obtained in Example 3)-1-1 as a template, an IgG1LALA-type human chimeric rat_CD147_#84 heavy chain expression vector was constructed in the same manner as in Example 1)-13-4. SEQ ID NO: 97 and SEQ ID NO: 98 respectively represent the nucleotide sequence of the IgG1LALA-type heavy chain of human chimeric rat_CD147_#84 and the amino acid sequence of the heavy chain.

[0248]

3)-2-1-7 Construction of IgG4PFALA-type human chimeric
rat_CD147_#84 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#84 heavy chain variable region obtained in Example 3)-1-1 as a template, PCR was performed using a primer designed for In-fusion cloning, thereby amplifying a DNA fragment containing the cDNA encoding the heavy chain variable region. Using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the amplified DNA fragment was inserted at the site where pCMA-G4PFALA was digested with restriction enzyme BspI, thereby constructing an IgG4PFALA-type human chimeric rat_CD147_#84 heavy chain expression vector. SEQ ID NO: 99 and SEQ ID NO: 100 respectively represent the nucleotide sequence of the IgG4PFALA-type heavy chain of human chimeric rat_CD147_#84 and the amino acid sequence of the heavy chain.

[0249]

3)-2-2 Production of human chimeric antibody expression vector of rat_CD147_#101

3)-2-2-1 Construction of human chimeric rat_CD147_#101 light chain expression vector

Using the cDNA encoding the rat_CD147_#101 light chain variable region obtained in Example 3)-1-2 as a template, a human chimeric rat_CD147_#101 light chain expression vector was constructed in the same manner as in Example 1)-12-4. SEQ ID NO: 101 and SEQ ID NO: 102 respectively represent the nucleotide sequence of the

human chimeric rat_CD147_#101 light chain and the amino acid sequence of the light chain.

[0250]

3)-2-2-2 Construction of IgG2-type human chimeric rat_CD147_#101 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#101 heavy chain variable region obtained in Example 3)-1-2 as a template, an IgG2-type human chimeric rat_CD147_#101 heavy chain expression vector was constructed in the same manner as in Example 1)-12-6. SEQ ID NO: 103 and SEQ ID NO: 104 respectively represent the nucleotide sequence of the IgG2-type heavy chain of human chimeric rat_CD147_#101 and the amino acid sequence of the heavy chain.

[0251]

3)-2-2-3 Construction of IgG4P-type human chimeric rat_CD147_#101 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#101 heavy chain variable region obtained in Example 3)-1-2 as a template, an IgG4P-type human chimeric rat_CD147_#101 heavy chain expression vector was constructed in the same manner as in Example 1)-13-6. SEQ ID NO: 105 and SEQ ID NO: 106 respectively represent the nucleotide sequence of the IgG4P-type heavy chain of human chimeric rat_CD147_#101 and the amino acid sequence of the heavy chain.

[0252]

3)-2-2-4 Construction of IgG4PFALA-type human chimeric
rat_CD147_#101 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#101 heavy chain variable region obtained in Example 3)-1-2 as a template, an IgG4PFALA-type human chimeric rat_CD147_#101 heavy chain expression vector was constructed in the same manner as in Example 3)-2-1-7. SEQ ID NO: 107 and SEQ ID NO: 108 respectively represent the nucleotide sequence of the IgG4PFALA-type heavy chain of human chimeric rat_CD147_#101 and the amino acid sequence of the heavy chain.

[0253]

3)-2-3 Production of human chimeric antibody expression
vector of rat_CD147_#110

3)-2-3-1 Construction of human chimeric rat_CD147_#110
light chain expression vector

Using the cDNA encoding the rat_CD147_#110 light chain variable region obtained in Example 3)-1-3 as a template, a human chimeric rat_CD147_#110 light chain expression vector was constructed in the same manner as in Example 1)-12-4. SEQ ID NO: 109 and SEQ ID NO: 110 respectively represent the nucleotide sequence of the human chimeric rat_CD147_#110 light chain and the amino acid sequence of the light chain.

[0254]

3)-2-3-2 Construction of IgG2-type human chimeric
rat_CD147_#110 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#110 heavy chain variable region obtained in Example 3)-1-3 as a template, an IgG2-type human chimeric rat_CD147_#110 heavy chain expression vector was constructed in the same manner as in Example 1)-12-6. SEQ ID NO: 111 and SEQ ID NO: 112 respectively represent the nucleotide sequence of the IgG2-type heavy chain of human chimeric rat_CD147_#110 and the amino acid sequence of the heavy chain.

[0255]

3)-2-3-3 Construction of IgG4P-type human chimeric rat_CD147_#110 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#110 heavy chain variable region obtained in Example 3)-1-3 as a template, an IgG4P-type human chimeric rat_CD147_#110 heavy chain expression vector was constructed in the same manner as in Example 1)-13-6. SEQ ID NO: 113 and SEQ ID NO: 114 respectively represent the nucleotide sequence of the IgG4P-type heavy chain of human chimeric rat_CD147_#110 and the amino acid sequence of the heavy chain.

[0256]

3)-2-3-4 Construction of IgG4PFALA-type human chimeric rat_CD147_#110 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#110 heavy chain variable region obtained in Example 3)-1-3 as a template, an IgG4PFALA-type human chimeric rat_CD147_#110

heavy chain expression vector was constructed in the same manner as in Example 3)-2-1-7. SEQ ID NO: 115 and SEQ ID NO: 116 respectively represent the nucleotide sequence of the IgG4PFALA-type heavy chain of human chimeric rat_CD147_#110 and the amino acid sequence of the heavy chain.

[0257]

3)-2-4 Production of human chimeric antibody expression vector of rat_CD147_#131

3)-2-4-1 Construction of human chimeric rat_CD147_#131 light chain expression vector

Using the cDNA encoding the rat_CD147_#131 light chain variable region obtained in Example 3)-1-4 as a template, a human chimeric rat_CD147_#131 light chain expression vector was constructed in the same manner as in Example 1)-12-4. SEQ ID NO: 117 and SEQ ID NO: 118 respectively represent the nucleotide sequence of the human chimeric rat_CD147_#131 light chain and the amino acid sequence of the light chain.

[0258]

3)-2-4-2 Construction of IgG2-type human chimeric rat_CD147_#131 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#131 heavy chain variable region obtained in Example 3)-1-4 as a template, an IgG2-type human chimeric rat_CD147_#131 heavy chain expression vector was constructed in the same manner as in Example 1)-12-6. SEQ ID NO: 119 and SEQ ID

NO: 120 respectively represent the nucleotide sequence of the IgG2-type heavy chain of human chimeric rat_CD147_#131 and the amino acid sequence of the heavy chain.

[0259]

3)-2-4-3 Construction of IgG4P-type human chimeric rat_CD147_#131 heavy chain expression vector

Using the cDNA encoding the rat_CD147_#131 heavy chain variable region obtained in Example 3)-1-4 as a template, an IgG4P-type human chimeric rat_CD147_#131 heavy chain expression vector was constructed in the same manner as in Example 1)-13-6. SEQ ID NO: 121 and SEQ ID NO: 122 respectively represent the nucleotide sequence of the IgG4P-type heavy chain of human chimeric rat_CD147_#131 and the amino acid sequence of the heavy chain.

[0260]

3)-3 Preparation of human chimeric antibody

3)-3-1 Production of human chimeric antibody from monkey-cross-reactive rat antibody

The antibody was produced in the same manner as in Example 1)-14-1.

[0261]

A human chimeric antibody of rat_CD147_#84 obtained by the combination of the IgG1-type human chimeric rat_CD147_#84 heavy chain expression vector and the human chimeric rat_CD147_#84 light chain expression vector was

named "#84chIgG1". A human chimeric antibody of rat_CD147_#84 obtained by the combination of the IgG2-type human chimeric rat_CD147_#84 heavy chain expression vector and the human chimeric rat_CD147_#84 light chain expression vector was named "#84chIgG2". A human chimeric antibody of rat_CD147_#84 obtained by the combination of the IgG4P-type human chimeric rat_CD147_#84 heavy chain expression vector and the human chimeric rat_CD147_#84 light chain expression vector was named "#84chIgG4P". A human chimeric antibody of rat_CD147_#84 obtained by the combination of the IgG1LALA-type human chimeric rat_CD147_#84 heavy chain expression vector and the human chimeric rat_CD147_#84 light chain expression vector was named "#84chIgG1LALA". A human chimeric antibody of rat_CD147_#84 obtained by the combination of the IgG4PFALA-type human chimeric rat_CD147_#84 heavy chain expression vector and the human chimeric rat_CD147_#84 light chain expression vector was named "#84chIgG4PFALA".

[0262]

A human chimeric antibody of rat_CD147_#101 obtained by the combination of the IgG2-type human chimeric rat_CD147_#101 heavy chain expression vector and the human chimeric rat_CD147_#101 light chain expression vector was named "#101chIgG2". A human chimeric antibody of rat_CD147_#101 obtained by the combination of the IgG4P-type human chimeric rat_CD147_#101 heavy chain

expression vector and the human chimeric rat_CD147_#101 light chain expression vector was named "#101chIgG4P". A human chimeric antibody of rat_CD147_#101 obtained by the combination of the IgG4PFALA-type human chimeric rat_CD147_#101 heavy chain expression vector and the human chimeric rat_CD147_#101 light chain expression vector was named "#101chIgG4PFALA".

[0263]

A human chimeric antibody of rat_CD147_#110 obtained by the combination of the IgG2-type human chimeric rat_CD147_#110 heavy chain expression vector and the human chimeric rat_CD147_#110 light chain expression vector was named "#101chIgG2". A human chimeric antibody of rat_CD147_#110 obtained by the combination of the IgG4P-type human chimeric rat_CD147_#110 heavy chain expression vector and the human chimeric rat_CD147_#110 light chain expression vector was named "#110chIgG4P". A human chimeric antibody of rat_CD147_#110 obtained by the combination of the IgG4PFALA-type human chimeric rat_CD147_#110 heavy chain expression vector and the human chimeric rat_CD147_#110 light chain expression vector was named "#110chIgG4PFALA".

[0264]

A human chimeric antibody of rat_CD147_#131 obtained by the combination of the IgG2-type human chimeric rat_CD147_#131 heavy chain expression vector and the human chimeric rat_CD147_#131 light chain expression

vector was named "#131chIgG2". A human chimeric antibody of rat_CD147_#131 obtained by the combination of the IgG4P-type human chimeric rat_CD147_#131 heavy chain expression vector and the human chimeric rat_CD147_#131 light chain expression vector was named "#131chIgG4P".

[0265]

3)-3-2 Purification of human chimeric antibody of monkey-cross-reactive rat antibodies

The antibodies were purified from the culture supernatant obtained in Example 3)-3-1 in the same manner as in Example 1)-14-2.

[0266]

(Example 4) In-vivo antitumor efficacy of human chimeric antibodies

5×10^6 cells of human pancreatic line MIA PaCa-2 were suspended in PBS containing 50% GFR-Matrigel (Corning Inc., Cat. 354230), and the suspension was inoculated subcutaneously into the axilla of 5 to 6 week-old female NOD-scid mice (NOD. CBL7-Prkdc^{scid}/J, purchased from CHARLES RIVER LABORATORIES JAPAN, INC). Grouping was performed based on tumor volume 5 to 6 days after the inoculation, and the cynomolgus monkey-cross-reactive anti-CD147 human chimeric antibody (#84chIgG1, #84chIgG1LALA, #84chIgG2, #84chIgG4P, or #84chIgG4PFALA) was administered intraperitoneally to cancer-bearing mice at 1, 3, or 10 mg/kg on the day after the grouping (n = 5). The cynomolgus monkey-cross-reactive anti-CD147

human chimeric antibody (#101chIgG2, #101chIgG4P, #101chIgG4PFALA, #110chIgG2, #110chIgG4P, #110chIgG4PFALA, #131chIgG2, or #131chIgG4P) was administered intraperitoneally to cancer-bearing mice at 3 or 10 mg/kg on the day after the grouping (n = 5). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0267]

Tumor volume (mm³) = 1/2 × minor axis (mm) × minor axis (mm) × major axis (mm)

Figures 13-1(a) to (d), Figures 13-2(e) to (h), Figures 13-3 (i) to (l), and Figures 13-4 (m) and (n) show the results. The graphs also show the averages and the standard errors of changes in tumor volume.

[0268]

Human chimeric antibody #84 exhibited a dose-dependent antitumor efficacy in all IgG subtypes. In the #84chIgG4P 10 mg/kg administration group in which the strongest antitumor efficacy was observed, complete tumor regression was observed 20 days after the inoculation in 5 mice out of 5 mice.

[0269]

Human chimeric antibody #101 exhibited a dose-dependent antitumor efficacy in all IgG subtypes. In the 101chIgG4P 10 mg/kg administration group in which the

strongest antitumor efficacy was observed, complete tumor regression was observed 20 days after the inoculation in 4 mice out of 5 mice.

[0270]

Human chimeric antibody #110 exhibited a dose-dependent antitumor efficacy in all IgG subtypes. In the 110chIgG4P 10 mg/kg administration group in which the strongest antitumor efficacy was observed, complete tumor regression was observed 22 days after the inoculation in 3 mice out of 5 mice.

[0271]

Human chimeric antibody #131 exhibited a dose-dependent antitumor efficacy in the IgG4P subtype. There was no difference in antitumor efficacy observed in the 3 mg/kg and 10 mg/kg administration groups of #131chIgG2.

[0272]

All human chimeric antibodies exhibited tumor growth inhibition effects of 68 to 100% at a dose of 10 mg/kg, irrespective of the human IgG subtype of each antibody. It was suggested that the cynomolgus monkey-cross-reactive anti-human CD147 human chimeric antibodies #084, #101, #110, and #131 were also independent of the mouse immune system, similar to the anti-human CD147 antibody LN22R8 or 2P10F2 obtained from cell immunization, and exhibited antitumor efficacy by a new mechanism of action on CD147.

[0273]

(Example 5) Evaluation of binding activity of human chimeric antibody to CD147

The dissociation constant for binding of #84chIgG1, #84chIgG2, #84chIgG4P, #84chIgG1LALA, #84chIgG4PFALA, #101chIgG4P, or #110chIgG4P produced in Example 3)-3-1 to human CD147 was measured using a Biacore T200 (available from GE Healthcare Bioscience). Anti-Human IgG (Fc) antibody was immobilized on a sensor chip by using a Human Antibody Capture Kit (available from GE Healthcare Bioscience) followed by capturing human chimeric antibody as a ligand and associating an antigen as an analyte. HBS-EP+ (available from GE Healthcare Bioscience) as a running buffer and CM5 (available from GE Healthcare Bioscience) as a sensor chip were used. After 1 $\mu\text{g/mL}$ or 2 $\mu\text{g/mL}$ of the human chimeric antibody was added onto the chip at 10 $\mu\text{L/minute}$ over 60 seconds, a serial dilution of a CD147 protein as an antigen (0.25 to 4 $\mu\text{g/mL}$ with respect to #131chIgG4P or 0.5 to 8 $\mu\text{g/mL}$ with respect to #84chIgG1, #84chIgG2, #84chIgG4P, #84chIgG1LALA, #84chIgG4PFALA, #101chIgG4P, or #110chIgG4P) was associated at a flow rate of 30 $\mu\text{L/minute}$ over 120 seconds, followed by subsequent monitoring for a dissociation phase of 120 seconds, 300 seconds, or 600 seconds. Here, the CD147 protein used was expressed in *Escherichia coli*, purified in 2 steps of Ni affinity and SEC, and then the tag was cleaved. 3M magnesium chloride (available from GE Healthcare Bioscience) was added at a

flow rate 20 $\mu\text{L}/\text{minute}$ over 30 seconds as a regenerating solution. Using a 1:1 binding model, the binding rate constant k_a , the dissociation rate constant k_d , and the dissociation constant (K_D ; $K_D = k_d/k_a$) were calculated.

Table 5 shows the results.

[0274]

[Table 5]

Dissociation constant for binding of human chimeric antibody to human CD147

	Name	K_D (nM)
1	#84chIgG1	393
2	#84chIgG2	334
3	#84chIgG4P	381
4	#84chIgG1LALA	371
5	#84chIgG4PFALA	358
6	#101chIgG4P	10.5
7	#110chIgG4P	9.94
8	#131chIgG4P	3.45

[0275]

(Example 6) Production of humanized antibody

6)-1 Design of humanized antibody

6)-1-1 Molecular modeling of variable region

A method known as homology modeling (Methods in Enzymology, 203, 121-153, (1991)) was used. Using a commercially available protein three-dimensional structure analysis program, Discovery Studio (available from Dassault Systemes), the homologous sequences of the variable regions were identified from the registered structures in Protein Data Bank (Nuc. Acids Res. 35, D301-D303 (2007)). Three-dimensional models were created

using the identified heavy chains, light chains, and heavy and light chain interface structures as templates. [0276]

6)-1-2 Method for designing humanized antibody

The antibody was humanized by CDR grafting (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). Having a high homology, the consensus sequence of the human kappa chain subgroup 1 and the consensus sequence of the human gamma chain subgroup 3 defined in KABAT et al. (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, available from Panasonic Corporation Institutes of Health, Bethesda, MD. (1991)) were selected as acceptors for the framework regions of light and heavy chains of rat_CD147_#84, respectively. Having a high homology, the consensus sequence of the human kappa chain subgroup 1 defined in KABAT et al., and IGHV3-30*05 and IGHJ3*01 of the human gamma chain defined in IMGT (THE INTERNATIONAL IMMUNOGENETICS INFORMATION SYSTEM (R)) were selected as acceptors for the framework regions of light chain and heavy chains of rat_CD147_#101, respectively. Having a high homology, IGKV1-39*01 and IGKJ4*01 of the human kappa chain defined in IMGT and IGHV1-2*02 and IGHJ6*01 of the human gamma chain defined in IMGT were selected as acceptors for the light and heavy chains of rat_CD147_#110, respectively. Having a high homology, IGKV1-39*01 and IGKJ2*01 of the human kappa chain defined in IMGT and IGHV3-30*05 and IGHJ6*01 of the human gamma

chain defined in IMGT were selected as acceptors for the light and heavy chains of rat_CD147_#131, respectively. Donor residues to be imported into the acceptors were selected by analyzing the three-dimensional models while referring to the criteria given by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)) or others. [0277]

6)-1-3 Humanization of rat_CD147_#84 heavy chain

The amino acid residues of the acceptor shown in Figure 14(a) were imported into the variable region of the rat_CD147_#84 heavy chain, thereby designing the variable region of humanized antibody heavy chain #84H1h. [0278]

Humanized antibody heavy chains were designed by connecting the variable region designed above with the gamma chain constant regions of human IgG2 and IgG4P, and named #84H1hIgG2 and #84H1hIgG4P, respectively. SEQ ID NO: 123 represents the full-length amino acid sequence of #84H1hIgG2. SEQ ID NO: 124 represents a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 123. SEQ ID NO: 125 represents the full-length amino acid sequence of #84H1hIgG4P. SEQ ID NO: 126 represents a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 125. [0279]

6)-1-4 Humanization of rat_CD147_#84 light chain

The amino acid residues of the acceptor shown in Figure 14(b) were imported into the variable region of the rat_CD147_#84 light chain, thereby designing a variable region of humanized antibody light chain #84L2h.

[0280]

A humanized antibody light chain was constructed by connecting the variable region designed above with the human κ chain constant region, and named #84L2h. SEQ ID NO: 127 represents the full-length amino acid sequence of the light chain #84L2h. SEQ ID NO: 128 represents a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 127.

[0281]

6)-1-5 Humanized antibody #84

A humanized antibody #84H1L2hIgG2 was designed by combining the heavy chain #84H1hIgG2 and the light chain #84L2h designed as above. Further, a humanized antibody #84H1L2hIgG4P was designed by combining the heavy chain #84H1hIgG4P and the light chain #84L2h.

[0282]

6)-1-6 Humanization of rat_CD147_#101 heavy chain

The amino acid residues of the acceptor shown in Figure 15(a) were imported into the variable region of the rat_CD147_#101 heavy chain, thereby designing a variable region of humanized antibody heavy chain #101H1h. Humanized antibody heavy chains were constructed by connecting the variable region designed above with the

gamma chain constant regions of human IgG2 and IgG4P and named #101H1hIgG2 and #101H1hIgG4P, respectively. SEQ ID NO: 129 represents the full-length amino acid sequence of #101H1hIgG2. SEQ ID NO: 130 represents a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 129. SEQ ID NO: 131 represents the full-length amino acid sequence of #101H1hIgG4P. SEQ ID NO: 132 represents a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 131.

[0283]

6)-1-7 Humanization of rat_CD147_#101 light chain

The amino acid residues of the acceptor shown in Figure 15(b) were imported into the variable region of the rat_CD147_#101 light chain, thereby designing a variable region of humanized antibody light chain #101L2h. In the variable region designed above, a humanized antibody light chain connected with human κ chain constant region was designed and named #101L2h. SEQ ID NO: 133 represents the full-length amino acid sequence of light chain #101L2h. SEQ ID NO: 134 represents a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 133.

[0284]

6)-1-8 Humanized antibody #101

A humanized antibody #101H1L2hIgG2 was designed by combining the heavy chain #101H1hIgG2 and the light chain #101L2h designed as above. Further, a humanized antibody

#101H1L2hIgG4P was designed by combining the heavy chain #101H1hIgG4P and the light chain #101L2h.

[0285]

6)-1-9 Humanization of rat_CD147_#110 heavy chain

The amino acid residues of the acceptors shown in Figures 16(a) and (b) were each imported into the variable region of rat_CD147_#110 heavy chain, thereby designing variable regions of humanized antibody heavy chains #110H1h and #110H13h.

[0286]

Humanized antibody heavy chains were constructed by connecting the variable region designed above with the human gamma chain constant region of IgG4P and named #110H1hIgG4P and #110H13hIgG4P, respectively. SEQ ID NO: 135 represents the full-length amino acid sequence of #110H1hIgG4P, and SEQ ID NO: 147 represents the full-length amino acid sequence of #110H13hIgG4P. SEQ ID NO: 136 represents a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 135, and SEQ ID NO: 148 represents a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 147.

[0287]

6)-1-10 Humanization of rat_CD147_#110 light chain

The amino acid residues of the acceptors shown in Figures 16(c), (d), and (e) were each imported into the variable region of the rat_CD147_#110 light chain,

thereby designing variable regions of humanized antibody light chains #110L4h, #110L2h, and #110L12h.

[0288]

Humanized antibody light chains were constructed by connecting the variable region designed above with the human κ chain constant region and named #110L4h, #110L2h, and #110L12h, respectively. SEQ ID NO: 137 represents the full-length amino acid sequence of light chain #110L4h, SEQ ID NO: 149 represents the full-length amino acid sequence of light chain #110L2h, and SEQ ID NO: 151 represents the full-length amino acid sequence of light chain #110L12h. SEQ ID NO: 138 represents a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 137, SEQ ID NO: 150 represents a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 149, and SEQ ID NO: 152 represents a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 151.

[0289]

6)-1-11 Humanized antibody #110

A humanized antibody #110H1L4hIgG4P was designed by combining the heavy chain #110H1hIgG4P and the light chain #110L4h designed as above. Further, a humanized antibody #110H13L2hIgG4P was designed by combining the heavy chain #110H13hIgG4P and the light chain #110L2h, and a humanized antibody #110H13L12hIgG4P was designed by combining the heavy chain #110H13hIgG4P and the light chain #110L12h.

[0290]

6)-1-12 Humanization of rat_CD147_#131 heavy chain

The amino acid residues of the acceptor shown in Figure 17(a) were imported into the variable region of the rat_CD147_#131 heavy chain, thereby designing a variable region of humanized antibody heavy chain #131H2h.

[0291]

A humanized antibody heavy chain was constructed by connection the variable region designed above with the gamma chain constant region of human IgG2 and named #131H2hIgG2. SEQ ID NO: 139 represents the full-length amino acid sequence of #131H2hIgG2. SEQ ID NO: 140 represents a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 139.

[0292]

6)-1-13 Humanization of rat_CD147_#131 light chain

The amino acid residues of the acceptor shown in Figure 17(b) were imported into the variable region of the rat_CD147_#131 light chain, thereby designing a variable region of humanized antibody light chain #131L2h.

[0293]

A humanized antibody light chain was constructed by connecting the variable region designed above with the human κ chain constant region and named #131L2h. SEQ ID NO: 141 represents the full-length amino acid sequence of light chain #131L2h. SEQ ID NO: 142 represents a

nucleotide sequence encoding the amino acid sequence of
SEQ ID NO: 141.

[0294]

6)-1-14 Humanized antibody #131

A humanized antibody #131H2L2hIgG2 was designed by combining the heavy chain #131H2hIgG2 and the light chain #131L2h as designed above.

[0295]

6)-2 Construction of humanized antibody light chain
expression vectors

6)-2-1 Construction of #84L2h expression vector

A DNA fragment represented by nucleotide positions 37 to 402 of the nucleotide sequence of #84L2h represented by SEQ ID NO: 128 was synthesized (Geneart AG). Using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the synthesized DNA fragment was inserted into the site where pCMA-LK was digested with restriction enzyme BsiWI, thereby constructing a #84L2h expression vector.

[0296]

6)-2-2 Construction of #101L2h expression vector

A DNA fragment represented by nucleotide positions 37 to 402 of the nucleotide sequence of #101L2h represented by SEQ ID NO: 134 was synthesized (Geneart AG). A #101L2h expression vector was constructed in the same manner as in Example 6)-2-1.

[0297]

6)-2-3 Construction of #110L4h, #110L2h, and #110L12h expression vectors

DNA fragments represented by nucleotide positions 37 to 402 of the nucleotide sequences of #110L4h, #110L2h, and #110L12h represented by SEQ ID NO: 138, SEQ ID NO: 150 and SEQ ID NO: 152 were synthesized (GeneArt AG). #110L4h, #110L2h, and #110L12h expression vectors were constructed in the same manner as in Example 6)-2-1.

[0298]

6)-2-4 Construction of #131L2h expression vector

A DNA fragment represented by nucleotide positions 37 to 402 of the nucleotide sequence of #131L2h represented by SEQ ID NO: 142 was synthesized (GeneArt AG). A #131L2h expression vector was constructed in the same manner as in Example 6)-2-1.

[0299]

6)-3 Construction of humanized antibody heavy chain expression vectors

6)-3-1 Construction of #84H1hIgG2 expression vector

A DNA fragment represented by nucleotide positions 36 to 437 of the nucleotide sequence of #84H1hIgG2 represented by SEQ ID NO: 124 was synthesized (GeneArt AG). Using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the synthesized DNA fragment was inserted into the site where pCMA-G2 was digested with restriction enzyme BspI, thereby constructing a #84H1hIgG2 expression vector.

[0300]

6)-3-2 Construction of #84H1hIgG4P expression vector

A DNA fragment represented by nucleotide positions 36 to 437 of the nucleotide sequence of #84H1hIgG4P represented by SEQ ID NO: 126 was synthesized (GeneArt AG). Using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the synthesized DNA fragment was inserted into the site where pCMA-G4P was digested with restriction enzyme BspI, thereby constructing a #84H1hIgG4P expression vector.

[0301]

6)-3-3 Construction of #101H1hIgG2 expression vector

A DNA fragment represented by nucleotide positions 36 to 428 of the nucleotide sequence of #101H1hIgG2 represented by SEQ ID NO: 130 was synthesized (GeneArt AG). A #101H1hIgG2 expression vector was constructed in the same manner as in Example 6)-3-1.

[0302]

6)-3-4 Construction of #101H1hIgG4P expression vector

A DNA fragment represented by nucleotide positions 36 to 428 of the nucleotide sequence of #101H1hIgG4P represented by SEQ ID NO: 132 was synthesized (GeneArt AG). A #101H1hIgG4P expression vector was constructed in the same manner as in Example 6)-3-2.

[0303]

6)-3-5 Construction of #110H1hIgG4P and #110H13hIgG4P expression vectors

DNA fragments represented by nucleotide positions 36 to 425 of the nucleotide sequences of #110H1hIgG4P and #110H13hIgG4P represented by SEQ ID NO: 136 and SEQ ID NO: 148 were synthesized (GeneArt AG). #110H1hIgG4P and #110H13hIgG4P expression vectors were constructed in the same manner as in Example 6)-3-2.

[0304]

6)-3-6 Construction of #131H2hIgG2 expression vector

A DNA fragment represented by nucleotide positions 36 to 431 of the nucleotide sequence of #131H2hIgG2 represented by SEQ ID NO: 140 was synthesized (GeneArt AG). A #131H2hIgG2 expression vector was constructed in the same manner as in Example 6)-3-1.

[0305]

6)-4 Preparation of humanized antibody

6)-4-1 Production of humanized antibody

The antibody was produced in the same manner as in Example 1)-14-1. A series of humanized antibodies was obtained by combining an H chain expression vector and an L chain expression vector corresponding to the combinations of an H chain and an L chain shown in Example 6)-1-5, Example 6)-1-8, Example 6)-1-11, and Example 6)-1-14.

[0306]

6)-4-2 One-step purification of humanized antibody

An antibody was purified from the culture supernatant obtained in Example 6)-4-1 by a one-step

process of rProtein A affinity chromatography. After the culture supernatant was applied to a column equilibrated with PBS and filled with MabSelectSuRe (available from GE Healthcare Bioscience), the column was washed with PBS an amount twice or more the column volume. Next, elution with a 2M arginine hydrochloride solution (pH 4.0) was performed to collect a fraction containing the antibody. The fraction was subjected to buffer replacement with PBS by dialysis (Thermo Scientific, Slide-A-Lyzer Dialysis Cassette). The antibody was concentrated using a Centrifugal UF Filter Device VIVASPIN20 (fraction molecular weight UF10K, Sartorius AG) to adjust the IgG concentration to 1 mg/mL or more. Finally, filtration was performed using a Minisart-Plus filter (Sartorius AG) to give a purified sample.

[0307]

6)-4-3 Two-step purification of humanized antibody

The culture supernatant obtained in Example 6)-4-1 was purified by a two-step process of rProtein A affinity chromatography and ceramic hydroxyapatite. After the culture supernatant was applied to a column equilibrated with PBS and filled with MabSelectSuRe (available from GE Healthcare Bioscience), the column was washed with PBS in an amount twice or more the column volume. Next, the antibody was eluted with a 2M arginine hydrochloride solution (pH 4.0). A fraction containing the antibody was subjected to buffer replacement with PBS by dialysis

(Thermo Scientific, Slide-A-Lyzer Dialysis Cassette), followed by 5-fold dilution with a 5 mM sodium phosphate/50 mM MES/pH 7.0 buffer, and was applied to a ceramic hydroxyapatite column (Japan Bio-Rad Laboratories, Inc., Bio-Scale CHT Type-1 Hydroxyapatite Column) equilibrated with a 5 mM NaPi/50 mM MES/30 mM NaCl/pH 7.0 buffer. Linear gradient elution with sodium chloride was performed to collect a fraction containing the antibody. The fraction was subjected to buffer replacement with HBSor (25 mM Histidine/5% Sorbitol/pH 6.0) by dialysis (Thermo Scientific, Slide-A-Lyzer Dialysis Cassette). The antibody was concentrated using a Centrifugal UF Filter Device VIVASPIN20 (fraction molecular weight UF10K, Sartorius AG) to adjust the IgG concentration to 25 mg/mL. Finally, filtration was performed using a Minisart-Plus filter (Sartorius AG) to give a purified sample.

[0308]

(Example 7) Measurement of activities of humanized antibody

7)-1 Evaluation of the binding activity of humanized antibody to CD147

The dissociation constant for binding of humanized anti-human CD147 antibody #84H1L2hIgG2, #84H1L2hIgG4P, #101H1L2hIgG2, #101H1L2hIgG4P, #110H1L4hIgG4P, or #131H2L2hIgG2 produced in Example 6)-4-3 to human CD147 was measured using a Biacore T200 (available from GE Healthcare Bioscience). Anti-Human IgG (Fc) antibody was

immobilized on a sensor chip by using a Human Antibody Capture Kit (available from GE Healthcare Bioscience) followed by capturing humanized antibody as a ligand and associating an antigen as an analyte. HBS-EP+ (available from GE Healthcare Bioscience) as a running buffer and CM5 (available from GE Healthcare Bioscience) as a sensor chip were used. After 1 $\mu\text{g/mL}$ of a humanized antibody was added onto the chip at 10 $\mu\text{L/minute}$ over 60 seconds, the serial dilution of the antigen used in Example 5) was associated at a flow rate of 30 $\mu\text{L/minute}$ over 120 seconds (0.0625 to 1 $\mu\text{g/mL}$ with respect to #101H1L2hIgG2 and #101H1L2hIgG4P, and 0.25 to 4 $\mu\text{g/mL}$ with respect to #84H1L2hIgG2, #84H1L2hIgG4P, #110H1L4hIgG4P, #110H13L2hIgG4P, #110H13L12hIgG4P, and #131H2L2hIgG2), followed by subsequent monitoring for a dissociation phase of 300 seconds. 3M magnesium chloride (available from GE Healthcare Bioscience) as a regenerating solution was added thereto at a flow rate of 20 $\mu\text{L/minute}$ over 30 seconds. The binding rate constant k_a , the dissociation rate constant k_d , and the dissociation constant (K_D ; $K_D = k_d/k_a$) were calculated using a 1:1 binding model.

[0309]

Table 6 shows the results.

[0310]

[Table 6]

Dissociation constant for binding of humanized antibody to human CD147

Name	KD (nM)
#84H1L2hIgG2	227
#84H1L2hIgG4P	242
#101H1L2hIgG2	10.5
#101H1L2hIgG4P	10.5
#110H1L4hIgG4P	8.60
#110H13L2hIgG4P	58.9
#110H13L12hIgG4P	154
#131H2L2hIgG2	5.84

[0311]

7)-2 Antitumor effect of humanized CD147 antibodies in pancreatic xenograft model

5 × 10⁶ cells of human pancreatic line MIA PaCa-2 were suspended in PBS containing 50% GFR-Matrigel (Corning Inc., Cat. 354230), and the suspension was inoculated subcutaneously into the axilla of 4 week-old female NOD-scid mice (NOD. CB17-Prkdc^{scid}/J, purchased from CHARLES RIVER LABORATORIES JAPAN, INC). Grouping was performed based on tumor volume, the humanized CD147 antibody (#84H1L2hIgG2, #84H1L2Ig4P, #101H1L2hIg2, #101H1L2hIg4P, #110H1L4hIg4P, or #131H2L2hIg2) produced in Example 6)-4-2 was administered intraperitoneally to mice bearing tumor at 3 mg/kg or 10 mg/kg 7 days after the inoculation (n = 5). As a control drug, gemcitabine (purchased from Eli Lilly Japan K.K.) that is a standard therapeutic agent for pancreatic cancer was administered intraperitoneally to cancer-bearing mice at 400 mg/kg 7 and 14 days after the inoculation (n = 5). The major

axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0312]

Figures 18(a) to (d) show the results. The graphs also show the averages and the standard errors of changes in tumor volume.

[0313]

While the tumor growth inhibition rate of the control drug was 71%, and no tumor disappearance was recognized, all the humanized CD147 antibodies exhibited a more potent antitumor efficacy in the 3 or 10 mg/kg administration group than gemcitabine. In the 10 mg/kg administration group of #84H1L2hIg4P, #101H1L2hIg2, #101H1L2hIg4P, or #110H1L4hIg4P, a potent antitumor efficacy with tumor disappearance was observed.

[0314]

(Example 8) In-vitro activation of p38MAPK signal by CD147 antibody

8)-1 Induction of p38MAPK phosphorylation by anti-human CD147 human chimeric antibody in pancreatic cancer cells PANC-1

It has been reported that CD147 activation promotes p38MAPK phosphorylation (Lim et al., FEBS Letters, 88-92, 1998) (Li et al., J. Hepatology, 1378-1389, 2015). In order to investigate the influence of an anti-CD147

antibody exhibiting an antitumor efficacy on the p38MAPK signal, p38MAPK phosphorylation in PANC-1 cells treated with the CD147 human chimeric antibody LN22R8chIgG4P at 10 µg/ml for 15 minutes was evaluated using Simple Western assays (ProteinSimple Japan K.K., Wes). As a control sample, PANC-1 cells treated with human IgG (Jackson ImmunoResearch Inc., 009-000-003) at 10 µg/ml in the same manner were analyzed. In order to detect p38MAPK, p38 MAPK rabbit mAb (Cell Signaling Technology, Inc., Cat. #9212) was used. In order to detect phosphorylated p38MAPK, P-p38 MAPK (T180/Y182) (D3F9) XP rabbit mAb (Cell Signaling Technology, Inc., #4511S) was used. Figure 19 shows the ratio of the phosphorylated p38MAPK signal to the detected p38MAPK signal. The antibody treated samples were measured twice, and the graph shows the averages.

[0315]

In the LN22R8chIgG4P antibody treated group, an increase in the phosphorylated MAPK signal was observed.

[0316]

8)-2 Anti-CD147 antibody mediated phosphorylation of HSP27 which is a molecule downstream of the p38 signal

It has been reported that HSP27 is phosphorylated by activation of p38MAPK (Landry et al., Biochem. Cell Biol., 703-707, 1995). In order to investigate whether p38 phosphorylation induced by the CD147 human chimeric antibody LN22R8 in fact promotes activation of the p38

signal, phosphorylation in the antibody treated samples of 8)-1 was evaluated using Simple Western assays (ProteinSimple Japan K.K., Wes). In order to detect HSP27, an anti-HSP27 antibody (R&D systems, Cat. AF15801) was used. In order to detect phosphorylated HSP27, a Phospho-HSP27 (Ser82) antibody (Cell Signaling Technology, Inc., 2401S) or an Anti-HSP27 (phospho Ser15) antibody (Abcam plc., Cat. ab76313) was used.

[0317]

Figure 20(a) or Figure 20(b) shows the ratio of the Ser82 or Ser15 phosphorylated HSP27 signal to the detected HSP27 signal, respectively. The antibody treated samples were evaluated in duplicate, and the graphs show the averages.

[0318]

In the LN22R8chIgG4P antibody treated group, an increase in phosphorylated HSP27 signal was observed. Since the phosphorylation and activation of HSP27 is downstream and is induced by p38 phosphorylation, which in turn is induced by the CD147 human chimeric antibody, it was found that the anti-human CD147 human chimeric antibody LN22R8IgG4P induced the activation of p38MAPK signal. Likewise, the activation of p38MAPK signal was observed also in pancreatic cancer cells MIA PaCa-2.

[0319]

(Example 9) Activation of the p38 signal by CD147 antibody in xenograft tumor tissue

Whether the activation of the p38MAPK signal by the CD147 antibody observed in vitro also occurs within tumors formed subcutaneously in mice was investigated in the subcutaneous tumors of MIA PaCa-2 mice. 5×10^6 cells of human pancreatic line MIA PaCa-2 were suspended in PBS containing 50% GFR-Matrigel (Corning Inc., Cat. 354230), and the suspension was inoculated subcutaneously into the axilla of 5 week-old female NOD-scid mice (NOD.CB17-Prkdc^{scid}/J, purchased from CHARLES RIVER LABORATORIES JAPAN, INC). Grouping was performed based on tumor volume 5 days after the inoculation, and the anti-human CD147 human chimeric antibody LN22R8chIgG4P was administered intraperitoneally to mice bearing tumor at 10 mg/kg on the day after the grouping. Tumors were sampled 6, 24, 48, 72 hours after the antibody administration, frozen using dry ice, and stored frozen. In order to prepare samples from the frozen tumor tissues (n = 3), a gentleMACS Octo Dissociator with Heaters (Miltenyi Biotec K.K.) was used. The prepared tumor lysates were analyzed using Simple Western assays (ProteinSimple Japan K.K., Wes).

[0320]

Figure 21(a) shows the averages of the detected p38MAPK signal together with the standard errors. Figure 21(b) shows the averages of the detected phosphorylated p38MAPK signal together with the standard errors. Figure 21(c) shows the averages of the ratio of the

phosphorylated p38MAPK signal to the p38MAPK signal together with the standard errors. In LN22R8chIgG4P, phosphorylated P38MAPK increased from 6 hours to 72 hours after the administration. The ratio of the phosphorylated p38MAPK signal was at its peak at 6 hours after administration and then decreased as p38MAPK increased from 24 hours to 72 hours.

[0321]

Since an increase in p38 phosphorylation and an increase in HSP27 phosphorylation due to administration of the CD147 chimeric antibody were also observed in the subcutaneous tumors of mice, it was found that the p38 signal was activated.

[0322]

(Example 10) Induction of molecules such as CXCL8 downstream of p38MAPK

It has been reported that activation of p38MAPK causes induction of CXCL8 via mRNA stabilization (Hoffmann et al., J. Leukoc. Biol., 847-855, 2002) and activation of the SMAD3/4 signal (Leovonen et al., PLOS ONE, e57474, 2013). RNA extracted from mouse subcutaneous tumors after administration of the antibody was investigated by quantitative PCR to determine whether CXCL8 gene expression was induced within MIA PaCa-2 tumors after administration of the antibody. Likewise, the RHOB gene (Vasilaki et al., FASEB Journal, 891-905, 2010) which has been reported to be induced by activation

of SMAD signaling was also investigated for changes due to administration of the CD147 antibody. As endogenous control genes, expression of the importin (ipo8) gene and the TATA box binding protein (tbp) gene were measured. After the anti-human CD147 human chimeric antibody was administered, and MIA PaCa-2 tumors were sampled 72 hours later and treated with RNA later (Qiagen N.V., Cat. 76104), RNA was extracted using an RNeasy Mini Kit (250) (Qiagen N.V., Cat. 74106). As the anti-human CD147 human chimeric antibody, LN22R8chIgG1, LN22R8chIgG2, or LN22R8chIgG4P produced in Example 1)-14 was used. For quantitative RT-PCR, an EXPRESS One-Step SuperScript qRT-PCR kit Universal (ThermoFisher Scientific, Cat. 11781-01K) was used, and importin (ipo8) (Thermo Fisher Scientific, Cat. Hs00183533_m1), TATA box binding protein (tbp) (Thermo Fisher Scientific, Cat. Hs00427621_m1), rashomolog family member B (rhoB) (Thermo Fisher Scientific, Cat. hs00269660_s1), and interleukin 8 (Thermo Fisher Scientific, Cat. Hs00174103_m1) were used as gene quantitative probes. Gene-specific increases in fluorescence signals following PCR reaction were measured using ABIPrism 7500 (Applied Biosystems). Figure 22(a) shows the averages ($n = 3$) of the ipo8/tbp gene expression ratio together with the standard deviations. Figure 22(b) shows the averages ($n = 3$) of the cxcl8/tbp gene expression ratio together with the standard deviations. Figure 22(c) shows the averages ($n = 3$) of

the rhoB/tbp gene expression ratio together with the standard deviations.

[0323]

After the administration of LN22R8chIgG1, LN22R8chIgG2, or LN22R8chIgG4P, ipo8 gene expression did not vary, but induction of cxcl8 and rhoB expression in the anti-human CD147 human chimeric antibody administration group was observed. The induction of cxcl8 and rhoB expression was in keeping with the magnitude of the antitumor efficacy of the human chimeric antibody shown in Example 1)-18, suggesting that the induction of expression of both genes may possibly correlate with the antitumor efficacy.

[0324]

(Example 11) Induction of cxcl8 and rhoB genes by enhancing antitumor efficacy through conversion of anti-human CD147 rat antibody into human chimeric antibody

The antitumor efficacy of the anti-human CD147 rat antibody rat_CD147_#110 was enhanced by human chimerization as shown in Example 2 and Example 7. For the induction of cxcl8 and rhoB observed in tumors after the administration of the human chimeric antibody LN22R8chIgG1, LN22R8chIgG2, or LN22R8chIgG4P, rat_CD147_#110 was compared with the chimeric antibody #110chIgG4P in the same manner as in 14)-2.

[0325]

Figure 23(a) shows the averages ($n = 3$) of the ipo8/tbp gene expression ratio together with the standard deviations.

[0326]

Figure 23(b) shows the averages ($n = 3$) of the cxcl8/tbp gene expression ratio together with the standard deviations.

[0327]

Figure 23(c) shows the averages ($n = 3$) of the rhoB/tbp gene expression ratio together with the standard deviations.

[0328]

The ipo8 gene expression did not vary due to the administration of the antibody. While no change was observed in cxcl8 and rhoB gene expression due to the administration of rat_CD147_#110, as in the antibody non-administered group, induction of cxcl8 and rhoB by #110chIgG4P was observed. It was suggested that the induction of both genes was a parameter correlated with the antitumor efficacy by the CD147 antibody.

[0329]

(Example 12) Evaluation using SMAD4-negative pancreatic cancer-bearing model

The transcription factor, SMAD4, is known as one of the molecules important for activation of SMAD signaling (Zang, et al., Current Biology, 270-276, 1997). It is known that SMAD signaling is partially impaired due to

genetic defects of SMAD4 in some pancreatic cancers (Hahn, et al., Science, 350-353, 1996). Whether the CD147 antibody exhibits an antitumor efficacy in pancreatic cancer cell line BxPC-3 lacking SMAD4 and with SMAD signaling partially inhibited (Yasutome et al., Clin. Exp. Metastasis, 461-473, 2005) was investigated. 2.5×10^6 cells of human pancreatic line BxPC-3 (ATCC, Cat. CRL-1687) were suspended in PBS containing 100% Matrigel (Corning Inc., Cat. 354234), and the suspension was inoculated subcutaneously into the axilla of 6 week-old female BALB/c-nu mouse (CAnN. Cg-Foxn1nu/CrlCrlj, purchased from CHARLES RIVER LABORATORIES JAPAN, INC). Grouping was performed based on tumor volume 8 days after the inoculation, and the mouse anti-human CD147 antibody LN22R8R8 or the rat anti-human CD147 antibody 2P10F2 was administered intraperitoneally to cancer-bearing mice at 10 mg/kg 8, 15, and 22 days after the inoculation (n = 5). As a control drug, gemcitabine (Eli Lilly Japan K.K., Gemzar (R)) that is a therapeutic agent for pancreatic cancer was administered to mice bearing tumor via the tail vein at 200 mg/kg 8, 15, 22 days after the inoculation (n = 5). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0330]

Tumor volume (mm³) = 1/2 × minor axis (mm) × minor axis (mm) × major axis (mm)

Figure 24 shows the results. The graphs also show the averages and the standard errors of changes in tumor volume. The BxPC-3 tumor was resistant to LN22R8 antibody, 2P10F2 antibody, and gemcitabine.

[0331]

(Example 13) Acquisition of CD147 antibody sensitivity by restoration of SMAD4

It has been reported that the SMAD signal recovers by restoration of SMAD4 in some pancreatic cancer cell lines. Whether the SMAD signal which was recovered by restoration of SMAD4 causes an increase in CD147 antibody sensitivity was investigated.

[0332]

13)-1 Generation of SMAD4 stably expressing cells

Using a Retro-X™ Q vector kit, SMAD4 stably expressing cell line BxPC-3 was generated. A human SMAD4 gene produced by artificial synthesis was introduced into a cloning site of pQCXIP contained in the kit as a retroviral vector (Takara Bio Inc., Retro-X™ Q Vector Set, Cat. 631516) to form a SMAD4 expressing retroviral vector. Using a Retro-X Universal Packaging System (Takara Bio Inc., Cat. 631530), the SMAD4 expressing retroviral vector was introduced into BxPC-3, and BxPC-3 cells in which a retrovirus was incorporated into a chromosome by viral infection and which thus became

puromycin resistant and SMAD4 positive were selected using puromycin (Takara Bio Inc., Cat. 631306) to serve as SMAD4-positive BxPC-3 cells, BxPC-3-SMAD4. The retroviral vector pQCXIP was introduced in the same manner, and BxPC-3 cells which became puromycin resistant served as BxPC-3-mock. The retrovirus infection experiment was performed twice to produce lot. 1 and lot. 2 of BxPC-3-mock and BxPC-3-SMAD4.

[0333]

13)-2 Confirmation of CD147 and SMAD4 expression

BxPC-3 (ATCC, Cat. CRL-1687), and BxPC-3-mock and BxPC-3-SMAD4 produced in Example 13)-1 were analyzed using Simple Western assays (ProteinSimple Japan K.K., Wes). As a SMAD4-positive control sample, MIA PaCa-2 was used. For detecting SMAD4, an anti-SMAD4 antibody (R&D systems, Cat. AF2097) was used. For detecting GAPDH, an anti-GAPDH antibody (Abfrontier, Cat. LF-MA0026) was used. For detecting CD147, an anti-CD147 antibody (Abcam, Cat. Ab108317) was used.

[0334]

Figure 25(a) shows the results. MIA PaCa-2 was SMAD4 positive and CD147 positive. BxPC-3 was SMAD4 negative and CD147 positive. BxPC-3-mock was SMAD4 negative and CD147 positive with no influence of retrovirus infection observed. Both lot. 1 and lot. 2 of BxPC-3-SMAD4 with the SMAD4 expressing retroviral vector introduced were SMAD4 positive. The SMAD4 expression of

Lot. 2 was high, and thus lot. 2 was used as BxPC-3-SMAD4 in the subsequent experiments.

[0335]

13)-3 Sensitivity of SMAD4 stably expressing BxPC-3 tumor to CD147 human chimeric antibody

2.5 × 10⁶ cells of BxPC-3-mock or BxPC-3-SMAD4 were suspended in PBS containing 100% Matrigel (Corning Inc., Cat. 354234), and the suspension was inoculated subcutaneously into the axilla of 5 week-old female BALB/c-nu mice (CAnN. Cg-Foxn1nu/CrlCrlj, purchased from CHARLES RIVER LABORATORIES JAPAN, INC). Grouping was performed based on tumor volume 6 days after the inoculation of BxPC-3-mock, and grouping of BxPC-3-SMAD4 was performed 3 days after the inoculation, respectively, the human chimeric anti-human CD147 antibody LN22R8R8chIgG2LN22R8R8 or LN22R8R8chIgG4PLN22R8R8 was administered intraperitoneally to cancer-bearing mice at 10 mg/kg on the day, and 7, 14, 21, 28 days after the grouping (n = 5). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0336]

Tumor volume (mm³) = 1/2 × minor axis (mm) × minor axis (mm) × major axis (mm)

Figure 25(b) and Figure 25(c) show the results. The graphs also show the averages and the standard errors of changes in tumor volume. The BxPC-3-mock tumor was resistant or exhibited low sensitivity to the LN22R8R8chIgG2 or LN22R8R8chIgG4P antibody. The BxPC-3-SMAD4 tumor exhibited a partial sensitivity to the LN22R8R8chIgG2 or LN22R8R8chIgG4P antibody.

[0337]

13)-4 Intratumoral changes in p38 signal due to SMAD4 expression

It has been reported that the p38 signal is enhanced by expressing SMAD4 in SMAD4-negative pancreatic cancer cells (Chen et al., B. M. C., 1471-2407, 2014). Changes in p38MAPK and phosphorylated p38MAPK in the BxPC-3-SMAD4 tumor due to the administration of the anti-human CD147 human chimeric antibody (72 hours after the administration of the antibody) were analyzed using Simple Western assays (ProteinSimple Japan K.K., Wes) in the same manner as in Example 13)-1. In the same manner as in Example 1)-18, LN22R8chIgG4P as the anti-human CD147 human chimeric antibody was administered to mice bearing a MIA PaCa-2 subcutaneous tumor at 10 mg/kg. For sample preparation (n = 3) from tumor tissues, a gentleMACS Octo Dissociator with Heaters (Miltenyi Biotec K.K.) was used.

[0338]

Figure 26(a) shows the averages of the measured values of three tumor samples together with the standard errors of the measured values of p38 MAPK in the form of graphs. Figure 26(b) shows the averages of the measured values of three tumor samples together with the standard errors of the measured values of phosphorylated p38 MAPK in the form of graphs.

[0339]

In the BxPC-3-SMAD4 tumors, p38 and phosphorylated p38 increased twofold. 72 hours after the administration of the CD147 human chimeric antibody, a partial reduction in p38 and a partial increase in phosphorylated p38 were observed.

[0340]

It was found that the p38 signal was increased due to SMAD4 expression in the tumors of pancreatic cancer cells BxPC-3. There is a possibility that the increase in the SMAD4-dependent p38 signal contributed to the increase in sensitivity to the CD147 human chimeric antibody LN22R8chIgG4P.

[0341]

(Example 14) Antitumor efficacy of chimeric CD147 antibody in gemcitabine-resistant pancreatic cancer

The antitumor efficacy of the CD147 antibody in a gemcitabine-resistant pancreatic cancer tumor model was investigated. 5×10^6 cells of human pancreatic line MIA PaCa-2 were suspended in PBS containing 50% GFR-Matrigel

(Corning Inc., Cat. 354230), and the suspension was inoculated subcutaneously into the axilla of 5 week-old female NOD-scid mice (NOD. CB17-Prkdc^{scid}/J, CHARLES RIVER LABORATORIES JAPAN, INC). Gemcitabine (Eli Lilly Japan K.K.), which is a standard therapeutic agent for pancreatic cancer, was intraperitoneally administered at 400 mg/kg 6 days after the inoculation, and grouping of mice in which gemcitabine-resistant tumors were confirmed to grow was performed based on tumor size one week later. Gemcitabine was intraperitoneally administered to the control group at 400 mg/kg (n = 5) 13 days (grouping date) and 20 days after the inoculation. As a CD147 antibody and gemcitabine combined administration group (n = 5), the CD147 human chimeric antibody LN22R8chIgG4P was intraperitoneally administered at 10 mg/kg 13 days (grouping date) and 20 days after the inoculation, in addition to gemcitabine intraperitoneally administered at 400 mg/kg 13 days (grouping date) and 20 days after the inoculation. The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0342]

The graph of Figure 27 showing the results shows the averages and the standard errors of changes in tumor volume. The graph also shows the tumor growth of the

non-administered group (non treatment) without administration of gemcitabine and antibody (n = 5).

[0343]

While the average tumor size of the gemcitabine administration group, as the control drug, was 1269 mm³ 28 days after the inoculation, and no regressing tumors were observed, the average tumor size of the group to which the CD147 human chimeric antibody LN22R8chIgG4P was administered in combination was 15 mm³, and tumor regression was observed in 3 out of 5 mice. These results showed that there was a possibility for CD147 antibody sensitivity to be exhibited in growing pancreatic cancer tumors resistant to gemcitabine.

[0344]

(Example 15) Antitumor efficacy of humanized CD147 antibody in a liver cancer model

15)-1 Expression of CD147 and SMAD4 in Hep G2 cells

CD147 and SMAD4 expression in liver cancer cell line HepG2 cells (ATCC, Cat. HB-8065) was investigated in the same manner as in Example 13)-2. As to the control specimens that were CD147 positive, MIA PaCa-2 and BxPC-3 (ATCC, Cat. CRL-1687) were used. MIA PaCa-2 was used as the control specimen that was SMAD4 positive, and BxPC-3 was used as the control specimen that was SMAD4 negative.

[0345]

The ratio of the detected signal to GAPDH was determined, and the results are summarized as follows.

The Hep G2 cells were found to be CD147 and SMAD4 positive.

[0346]

Signal ratio of CD147/GAPDH

MIA PaCa-2	0.481
------------	-------

BxPC-3	0.481
--------	-------

Hep G2	2.944
--------	-------

[0347]

Signal ratio of SMAD4/GAPDH

MIA PaCa-2	0.329
------------	-------

BxPC-3	0.003
--------	-------

Hep G2	0.723
--------	-------

[0348]

15)-2 Confirmation of expression of CD147 in Hep G2 cells using a flow cytometer

CD147 expressed on the cell surface of HepG2 cells (ATCC, Cat. HB-8065) was analyzed using a flow cytometer. In order to confirm human CD147 expression, an anti-human CD147 mouse IgG1 antibody MEM-M6/1-APC labeled with APC (Thermofisher, Cat. MA1-10104) was used as a commercially available anti-human CD147 antibody. As a mouse IgG1 Isotype control antibody, mIgG1-APC (Miltenyi Biotec K.K., Cat. 130-092-214) was used. MEM-M6/1-APC was added to a suspension of HepG2 cells in an amount of 1/10, followed by treatment at 4°C for 30 minutes. The cells were washed with a PBS buffer solution containing 5% FBS,

followed by measurement using a flow cytometer (CantoII, BD Biosciences). Figure 28(a) summarizes the results.

[0349]

It was found that the Hep G2 cells stained with MEM-M6/1-APC exhibited a strong fluorescence signal, thus they expressed CD147.

[0350]

15)-3 Activation of p38 by humanized CD147 antibody in liver cancer cells

In order to investigate the influence of the anti-CD147 antibody on p38MAPK in liver cancer cells HepG2, P38 phosphorylation in HepG2 cells (ATCC, Cat. HB-8065) treated with the anti-human CD147 human chimeric antibody (LN22R8chIgG4P) or the anti-human CD147 humanized antibody (#84H1L2hIgG2, #84H1L2hIg4P, #101H1L2hIgG2, #101H1L2hIgG4P, #110H1L4hIg4P, or #131H2L2hIgG2) produced in Example 6)-4-2 at 10 µg/ml for 15 minutes were evaluated using Simple Western assays (ProteinSimple Japan K.K., Wes). As a control sample, HepG2 cells treated with human IgG (hIgG, Jackson ImmunoResearch Inc., 009-000-003) at 10 µg/ml in the same manner were analyzed. For detecting p38MAPK, p38 MAPK rabbit mAb (Cell Signaling Technology, Inc., Cat. #9212) was used. For detecting phosphorylated p38MAPK, P-p38 MAPK (T180/Y182) (D3F9) XP rabbit mAb (Cell Signaling Technology, Inc., #4511S) was used. Figure 28(b) shows the ratio of the

phosphorylated p38MAPK signal to the detected p38MAPK signal.

[0351]

An increase in the phosphorylated p38 MAPK signal twice or more than that of the human IgG treated group due to the treatment with LN22R8chIgG4P, #84H1L2hIgG2, #84H1L2hIg4P, #101H1L2hIgG2, #101H1L2hIg4P, #110H1L4hIg4P or #131H2L2hIgG2 was observed, and it was found that p38MAPK phosphorylation was induced by the anti-human CD147 antibody in liver cancer cells, as well as in pancreatic cancer cells.

[0352]

15)-4 Comparison of antitumor efficacy between humanized CD147 antibody and sorafenib in liver cancer

The antitumor efficacy of the anti-human CD147 human chimeric antibody and the humanized antibody in a human liver cell line HepG2 (ATCC, Cat. HB-8065) which was CD147 and SMAD4 positive and in which p38 phosphorylation due to the anti-human CD147 antibody was observed were examined.

[0353]

5×10^6 cells of the human liver cell line HepG2 were suspended in PBS containing 50% Matrigel (Corning Inc., Cat. 354234), and the suspension was inoculated subcutaneously into the axilla of 4 week-old female NOD-scid mice (NOD. CB17-Prkdc^{scid}/J, purchased from CHARLES RIVER LABORATORIES JAPAN, INC). Grouping was

performed based on tumor volume 9 days after the inoculation, and the human chimeric CD147 antibody (LN22R8chIgG4P) or the humanized CD147 antibody (#84H1L2hIgG2, #84H1L2hIg4P, or #110H1L4hIg4P) produced in Example 6)-4-2 was administered intraperitoneally to cancer-bearing mice at 1 mg/kg or 10 mg/kg (n = 5). As a control drug, sorafenib (Nexavar tablets 200 mg, Bayer AG), which is a therapeutic agent for liver cancer, was dissolved in a PEG-35 castor oil (Cremophor EL, NACALAI TESQUE, INC., Cat. 09727-14) ethanol solvent according to the reference (Chang et.al., Cancer Chem.Thera.Pharm., 2007) attached to Nexavar tablets, and the solution was orally administered to cancer-bearing mice at 30 mg/kg or 90 mg/kg 9, 10, 11, 12, 13, 16, 17, and 18 days after the inoculation (n = 5). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0354]

Tumor volume (mm³) = 1/2 × minor axis (mm) × minor axis (mm) × major axis (mm)

Table 7 and Figures 29(a) to (d) show the results. The graphs also show the averages and the standard errors of changes in tumor volume. As a result of the administration of sorafenib, partial inhibition of tumor growth was observed, but tumor disappearance was not

observed. All of LN22R8chIgG4P, #84H1L2hIgG2, #84H1L2hIg4P, and #110H1L4hIg4P exhibited potent antitumor efficacy with tumor disappearance.

[0355]

[Table 7]

Group configuration/Administration (n=5)	Average tumor volume (mm ³)	Tumor growth inhibition rate (%)	Number of examples of complete tumor regression
Non-treated	1740	0	0/5
Vehicle, qD1x9 po	1576	9	0/5
Sorafenib 30mg/kg, qD1x9 po	1425	18	0/5
Sorafenib 90mg/kg, qD1x9 po	1252	28	0/5
LN22R8chIgG4P, 1 mg/kg, Single dose ip	664	62	0/5
LN22R8chIgG4P, 10 mg/kg, Single dose ip	13	99	2/5
#84H1L2hIgG2, 1 mg/kg, Single dose ip	456	74	0/5
#84H1L2hIgG2, 10 mg/kg, Single dose ip	339	81	4/5
#84H1L2hIgG4P, 1 mg/kg, Single dose ip	471	73	0/5
#84H1L2hIgG4P, 10 mg/kg, Single dose ip	6	100	4/5
#110H1L4hIgG4P, 1 mg/kgSingle dose ip	410	76	0/5
#110H1L4hIgG4P, 10 mg/kg, Single dose ip	257	85	2/5

[0356]

(Example 16) Effect on T cells and PBMC

It has been reported that expression of CD147 increases in CD4 positive and CD8 positive T cells with T cell activation (Hu et al., J. Cell. Mol. Med., 2132-2143, 2010), and some CD147 antibodies have an effect of inducing T cell activation and inhibiting growth (Koch et al., Int. Immunology, 777-786, 1999; Chiampanichayakul et al., Immunology 167-178, 2006). The influence of the anti-human CD147 antibody, exhibiting a potent antitumor efficacy that is independent of an effector function, on

peripheral blood lymphocytes (PBL) containing T cells was investigated.

[0357]

16)-1 Increase in CD147 expression by CD3/CD28 stimulation of PBMC

Whether CD147 expression increases with T cell activation was investigated using human PBMC. Human PBMC was cultured in an RPMI1640 medium containing 10% FBS at 37°C in the presence of 5% CO₂. Growth was induced by adding Dynabeads Human T-Activator CD3/CD28 (CD3/CD28 beads, Thermo Fisher SCIENTIFIC K.K., Cat. 1131D) during culture, and analysis was performed using a flow cytometer 4 days later to investigate changes in CD147 expression. In order to confirm human CD147 expression, anti-human CD147 mouse IgG1 antibody MEM-M6/1-APC labeled with APC (CD147-APC, Thermofisher, Cat. MA1-10104) was used as a commercially available anti-human CD147 antibody. As a mouse IgG1 Isotype control antibody, mIgG1-APC (Miltenyi Biotec K.K., Cat. 130-092-214) was used. In order to detect CD3, CD4, and CD8 T cells contained in human PBMC, APC/Fire™ 750 anti-human CD3 Antibody (available from BioLegend, Inc., Cat. 344840), PerCP/Cy5.5 anti-human CD4 (available from BioLegend, Inc., Cat. 344608), and Brilliant Violet 510 anti-human CD8 (available from BioLegend, Inc., Cat. 344732) were used.

[0358]

Figure 30 summarizes the results of CD147-APC binding in CD3 and CD4-positive cells and CD3 and CD8-positive cells. In the case where the CD3 and CD4-positive cells and CD3 and CD8-positive cells were stimulated by CD3/CD28 beads, an increase in CD147 expression was confirmed, and an increase in CD147 expression on the membrane surface of T cells with T cell activation was confirmed.

[0359]

16)-2 Evaluation of the action of anti-human CD147 antibody on the growth of human peripheral blood mononuclear cells

The action of anti-human CD147 antibody on the growth of human peripheral blood mononuclear cells (PBMC) was analyzed. As an anti-human CD147 antibody, 2P10F2chIgG4P was used. After fluorescence labeling of PBMC using a CellVue Claret Far Red Fluorescent Cell Linker Kit (sigma, Cat. MIDCLARET-1KT), cells were cultured in an RPMI1640 medium containing 10% FBS at 37°C in the presence of 5% CO₂. 2P10F2chIgG4P (10 µg/ml) was added during culture when growth was induced by adding IL-2 and Dynabeads Human T-Activator CD3/CD28 (CD3/CD28 beads, Thermo Fisher SCIENTIFIC K.K., Cat. 1131D) to investigate the influence on the growth. PBMC cell fluorescence signals that decreased due to cell division were measured using a flow cytometer (CantoII, BD

Biosciences) 3 days and 5 days after the culture. Figure 31 summarizes the results.

[0360]

PBMC cell fluorescence signals that decreased due to cell division were observed 3 days and 5 days after the culture because of the addition of CD3/CD28 beads. In the case of adding IL-2, 2P10F2chIgG4P, or IL-2 and 2P10F2chIgG4P during culture, there was no change in the decrease of the fluorescence signals observed. It was suggested that the anti-human CD147 human chimeric antibody 2P10F2chIgG4P that exhibited a potent antitumor efficacy in the mouse tumor model of human pancreatic cancer shown in Example 1)-18 has no influence on the growth of PBMC.

[0361]

16)-3 Evaluation of anti-human CD147 antibody on cytokine production by human peripheral blood lymphocytes

As an anti-human CD147 antibody, the human chimeric antibody #84chIgG1, #84chIgG2, #84chIgG4P, #84chIgG1LALA, #84chIgG4PFALA, #101chIgG4P, or #110chIgG4P was used. PBL was prepared from human peripheral blood using Ficoll-Paque PLUS (GE Healthcare Japan). A 96-well plate was coated with 10 µg/ml of the human chimeric antibody. Human IgG (hIgG, Jackson ImmunoResearch Inc., 009-000-003) as a negative control antibody and a Dynabeads Human T-Activator CD3/CD28 (CD3/CD28-beads, Thermo Fisher SCIENTIFIC K.K., Cat. 1131D) as a positive control

antibody that induces T cell activation and cytokine induction were used. 1×10^6 cells of PBL were added to the wells coated with the antibody, and cytokines (IL2, TNF α , and INF γ) in the medium were measured 24 hours later. A Dynabeads Human T-Activator CD3/CD28 was directly added to the wells with PBL added, and cytokines (IL2, TNF α , and INF γ) in the medium were measured 24 hours later in the same manner. Quantikine ELISA Human IL-2 (R&D systems, Cat. D2050) was used for measuring IL2. Amersham TNF- α Human, Biotrak Easy ELISA (GE Healthcare Japan, Cat. RPN5967) was used for measuring TNF α . A Human IFN- γ ELISA development kit (Mabtech, Cat. 3420-1H-6) was used for measuring INF γ . Each measurement was performed three times, and the averages and the standard deviations of the detected absorbance were calculated. Figure 32 summarizes the results.

[0362]

It was confirmed that all cytokines (IL2, TNF α , and INF γ) measured and produced from PBL increased only in culture in the wells with CD3/CD28-beads serving as a positive control added, and cytokines did not increase in culture in the wells coated with any one of the anti-human CD147 human chimeric antibodies, as per hIgG which was serving as a negative control.

[0363]

(Example 17) X-ray crystal structure analysis of humanized #110Fab'-CD147 complex

17)-1 Crystallization of complex

Humanized #110H1L4hIgG4P was cleaved by Pepsin, and the obtained Fab'2 was reduced with Dithiothreitol, followed by alkylation with Iodoacetamide to obtain a Fab' fragment. A mixture of the Fab' fragment and hCD147v2 (22-205) used in Example 5) was subjected to gel filtration chromatography using a Superdex 10/300GL Increase column (GE Healthcare) to obtain a complex fraction. The complex was replaced with a buffer solution (10mM Tris HCl pH 7.5, 50mM NaCl) using AmiconUltra15 MWCO 10K (available from Millipore Corporation) to be concentrated to 13g/L. The complex solution was crystallized by vapor diffusion. A solution formed by adding an equal amount of a precipitant solution (0.1 M NaMalonate, pH 7.0, 12% (w/v) Polyethylene Glycol 3350) to 0.5 μ L of a protein solution was put into a closed container containing 0.05 mL of a precipitant solution so that the two solutions were not in contact with each other, followed by standing at 25°C. A crystal of 0.15 mm $\times$ 0.15 mm $\times$ 0.3 mm obtained about one week later was immersed in a precipitant solution with Polyethylene Glycol 400 added to give 30% (w/v), followed by freezing with liquid nitrogen. X-ray diffraction data was collected using beam line PF-BL17A of Photon Factory, High Energy Accelerator Research Organization (KEK) (Ibaraki, Japan). The diffraction intensity was quantified from the diffraction image

obtained using the software, mosflm (CCP4: Collaborative Computational Project No. 4) to determine the crystal structure factor. The crystal space group was P21, and the crystal unit cell was ($a = 64.96 \text{ \AA}$, $b = 93.37 \text{ \AA}$, $c = 98.31 \text{ \AA}$, $\alpha = \gamma = 90$, and $\beta = 90.89$).

[0364]

17)-2 Structural analysis of complex

Molecular replacement was performed using the three-dimensional structure coordinate of the obtained structure factor and the homology model of the Fab' fragment, and a known structure of the human CD147 (PDBID: 3b5h) to determine the phase. For calculation, a software phaser (CCP4: Collaborative Computational Project No.4) was used. The crystal contained two complexes in the asymmetric unit. The structure was refined using the software, Refmac5 (CCP4: Collaborative Computational Project No.4), and the model was corrected using the software, coot. This operation was repeated to obtain a final R value of 23% and a free R value of 28% with a resolution of $2.3 \text{ \AA}$. The final model included two Fab' fragments of humanized #110H1L4, and hCD147v2 binding to each of them. For one hCD147v2, an electronic density corresponding to amino acid residues 23-203 was observed, but for the other, an electronic density corresponding to domain 1 was not clear, and only an electronic density corresponding to amino acid residues 103-202 was observed.

[0365]

17)-3 Identification of epitope

Amino acid residues of hCD147v2 that were present within 4 Å from the Fab' fragment binding surface of humanized #110H1L4 in the two complexes contained in the asymmetric unit in common were as follows: Arg106, Lys108, Ala109, Val110, Lys127, Ser128, Glu129, Ser130, Val131, Pro132, Pro133, Val134, Gln164, and Gly165. Figure 41 shows the ribbon model and the entire surface of the complexes, and Figure 42 shows the interaction between hCD147v2 and humanized #110H1L4.

[0366]

(Example 18) Antitumor effect of humanized CD147 antibody in a gastric cancer model

The antitumor efficacy of the anti-human CD147 human chimeric antibody and the humanized antibody on human gastric cancer cell line KATO III cells (ATCC, Cat. HTB-103) confirmed to be CD147 positive by a flow cytometer were evaluated.

[0367]

5×10^6 cells of human gastric cancer cell line KATO III were suspended in 100% Matrigel (Corning Inc., Cat. 354234), and the suspension was inoculated subcutaneously into the axilla of 5-week old female NOD-scid mice (NOD.CB17-Prkdc^{scid}/J, purchased from CLEA Japan, Inc). Grouping was performed based on tumor volume 3 days after the inoculation, and the human chimeric CD147 antibody

(LN22R8chIgG4P) or the humanized CD147 antibody (#110H1L4hIgG4P) produced in Example 6)-4-2 was administered intraperitoneally to cancer-bearing mice at 10 mg/kg every 7 days after the grouping (n = 6). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0368]

Tumor volume (mm³) = 1/2 × minor axis (mm) × minor axis (mm) × major axis (mm)

Figure 43 shows the results. The graphs also show the averages and the standard errors of changes in tumor volume. While the average tumor volume of the mice 24 days after the inoculation was 290 mm³ in the untreated group, it was 199 mm³ in the LN22R8chIgG4p administration group and 134 mm³ in the #110H1L4hIgG4P administration group. The calculated antitumor efficacy was 31% in the LN22R8chIgG4p administration group and 54% in the #110H1L4hIgG4P administration group.

[0369]

(Example 19) Antitumor effect of humanized CD147 antibody in a chronic myeloid leukemia model

The antitumor efficacy of anti-human CD147 humanized antibody on human chronic myeloid leukemia cell line KU812 cells (ATCC, Cat. CRL-2099) confirmed to be CD147 positive by a flow cytometer was examined.

[0370]

5 × 10⁶ cells of chronic myeloid leukemia cell line KU812 were suspended in PBS containing 50% Matrigel (Corning Inc., Cat. 354234), and the suspension was inoculated subcutaneously into the axilla of 5-week old female NOD-scid mice (NOD. CB17-Prkdc^{scid}/J, purchased from CLEA Japan, Inc). Grouping was performed based on tumor volume 3 days after the inoculation, and the humanized CD147 antibody (#110H1L4hIg4P) produced in Example 6)-4-2 was administered intraperitoneally to mice bearing tumor at 10 mg/kg every 7 days after the grouping (n = 5). As a control drug, imatinib (AstaTech, Inc., Cat. #63168), which is a standard therapeutic agent for chronic myeloid leukemia, adjusted with distilled water to a 9 mg/ml solution was orally administered to cancer-bearing mice at 90 mg/kg (daily administration except Saturdays and Sundays; administration 4, 7, 8, 9, 10, 11, 14, 15, 16, 17, 18, 21, 22, 23, 24 days after the inoculation). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0371]

Tumor volume (mm³) = 1/2 × minor axis (mm) × minor axis (mm) × major axis (mm)

Figure 44 shows the results. The graph shows the averages and the standard errors of changes in tumor volume. While the average tumor volume of the mice in the untreated group was 627 mm³ 25 days after the inoculation, it was 328 mm³ in the imatinib administration group and 6 mm³ in the #110H1L4hIgG4P administration group. The calculated antitumor efficacy was 48% in the imatinib administration group and 97% in the #110H1L4hIgG4P administration group. In the #110H1L4hIgG4P administration group alone, complete tumor regression was observed in 4 examples out of 5 examples.

[0372]

(Example 20) Antitumor effect of humanized CD147 antibody in a colon cancer model

The antitumor efficacy of each humanized antibody on human colon cancer cell line SW620 cells (ATCC, Cat. CCL-227) confirmed to be CD147 positive by a flow cytometer was evaluated.

[0373]

5 × 10⁶ cells of human colon cancer cell line SW620 were suspended in 100% Matrigel (Corning Inc., Cat. 354234), and the suspension was inoculated subcutaneously into the axilla of 5-week old female NOD-scid mice (NOD.CB17-Prkdc^{scid}/J, purchased from CLEA Japan, Inc). Grouping was performed based on tumor volume 3 days after the inoculation, and the human chimeric CD147 antibody (LN22R8chIgG4P) or the humanized CD147 antibody

(#084H1L2hIg4P or #110H1L4hIg4P) produced in Example 6)-4-2 was administered intraperitoneally to cancer-bearing mice at 10 mg/kg every 7 days after the grouping (n = 5). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0374]

Tumor volume (mm³) = $1/2 \times \text{minor axis (mm)} \times \text{minor axis (mm)} \times \text{major axis (mm)}$

Figure 45 shows the results. The graph shows the averages and the standard errors of changes in tumor volume. While the average tumor volume of the mice in the untreated group 21 days after the inoculation was 1302 mm³, it was 709 mm³ in the #084H1L2hIg4P administration group and 403 mm³ in the #110H1L4hIgG4P administration group. The calculated antitumor efficacy was 46% in the #084H1L2hIg4P administration group and 69% in the #110H1L4hIgG4P administration group.

[0375]

(Example 21) Antitumor efficacy of humanized CD147 antibody in the renal cancer 786-O model

The antitumor efficacy of each humanized antibody on human renal cancer 786-O confirmed to be CD147 positive by a flow cytometer was evaluated. 5×10^6 cells of human renal cancer 786-O were suspended in 50% Matrigel (Corning Inc., Cat. 354234), and the suspension was

inoculated subcutaneously into the axilla of 5-week old female NOD-scid mice (NOD. CB17-Prkdc^{scid}/J, purchased from CLEA Japan, Inc). Grouping was performed based on tumor volume 3 days after the inoculation, and the human chimeric CD147 antibody (LN22R8chIgG4P) or the humanized CD147 antibody (#084H1L2hIg4P or #110H1L4hIg4P) produced in Example 6)-4-2 was administered intraperitoneally to cancer-bearing mice at 10 mg/kg every 7 days after the grouping a total of 4 times (n = 6). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0376]

Tumor volume (mm³) = 1/2 × minor axis (mm) × minor axis (mm) × major axis (mm)

Figure 46 shows the results. The graph shows the averages and the standard errors of changes in tumor volume. While the average tumor volume of the mice in the untreated group 31 days after the inoculation was 918 mm³, it was 224 mm³ in the #084H1L2hIg4P administration group and 379 mm³ in the #110H1L4hIgG4P administration group. The calculated antitumor efficacy was 76% in the #084H1L2hIg4P administration group and 59% in the #110H1L4hIgG4P administration group.

[0377]

(Example 22) Antitumor effect of humanized CD147 antibody in an acute myeloid leukemia (AML) model

The antitumor efficacy of the humanized antibody on human AML cell line OCI-AML3 cells (DSMZ, Cat. ACC 582) confirmed to be CD147 positive by a flow cytometer was evaluated.

[0378]

5×10^6 cells of human AML cell line OCI-AML3 cell were suspended in 50% GFR-Matrigel (Corning Inc., Cat. 354230), and the suspension was inoculated subcutaneously into the axilla of 5-week old female NOD-scid mice (NOD. CB17-Prkdc<scid>/J, purchased from CLEA Japan, Inc). Grouping was performed based on tumor volume 3 days after the inoculation, and the humanized CD147 antibody (#110H1L4hIg4P) produced in Example 6)-4-2 was administered via a tail vein injection to mice bearing tumor at 10 mg/kg every 7 days after the grouping (n = 6). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0379]

Tumor volume (mm³) = $1/2 \times$ minor axis (mm) $\times$ minor axis (mm) $\times$ major axis (mm)

Figure 47 shows the results. The graph shows the averages and the standard errors of changes in tumor volume. While the average tumor volume of the mice in

the untreated group 21 days after the inoculation was 1533 mm³, it was 394 mm³ in the #110H1L4hIgG4P administration group. The calculated antitumor efficacy was 74% in the #110H1L4hIgG4P administration group.

[0380]

(Example 23) Measurement of efficacies of humanized anti-CD147 antibodies having different binding activities

5 × 10⁶ cells of human pancreatic line MIA PaCa-2 were suspended in PBS containing 50% GFR-Matrigel (Corning Inc., Cat. 354230), and the suspension was inoculated subcutaneously into the axilla of 4-week old female Nude mice (BALB/c Slc-nu/nu, purchased from Japan SLC, Inc). Grouping was performed based on tumor volume, and three types of humanized CD147 antibodies (#110H1L4hIgG4P, #110H13L02hIgG4P, and #110H13L12hIgG4P; Table 6 shows their binding activities) having different binding activities to the CD147 protein produced in Example 6)-4-2 were each administered to mice bearing tumor via the tail vein at 10 mg/kg 7 days after the inoculation (n = 6). As a control drug, gemcitabine (purchased from Eli Lilly Japan K.K.), which is a standard therapeutic agent for pancreatic cancer, was administered to cancer-bearing mice via the tail vein at 400 mg/kg 3 and 10 days after the inoculation (n = 6). The major axis and the minor axis of the inoculated tumor were measured twice a week using an electronic digital

caliper (available from MITUTOYO CORPORATION) to calculate the tumor volume by the following formula.

[0381]

Figure 48 shows the results. The graph shows the averages and the standard errors of changes in tumor volume.

[0382]

While the tumor growth inhibition rate of gemcitabine as a control drug was 66%, all the humanized CD147 antibodies exhibited a more potent antitumor efficacy in the 10 mg/kg administration group than gemcitabine.

[0383]

(Example 24) Comparison of CD147 antibodies

24)-1 Evaluation of binding activity of existing anti-CD147 antibodies to antigen

Purified antibodies were prepared based on the sequences of 4A5, 5F6 of WO2010/036460 and PPAT-082-03 of WO2017/061602. The dissociation constant for binding of 4A5, 5F6 or PPAT-082-03 to the CD147 protein was measured using a Biacore T200 (available from GE Healthcare Bioscience). Anti-Human IgG (Fc) antibody was immobilized on a sensor chip by using a Human Antibody Capture Kit (available from GE Healthcare Bioscience) followed by capturing human chimeric antibody as a ligand and associating an antigen as an analyte. HBS-EP+ (available from GE Healthcare Bioscience) as a running

buffer and CM5 (available from GE Healthcare Bioscience) as a sensor chip were used. After 1 $\mu\text{g/mL}$ of a competitive antibody was added onto the chip at 10 $\mu\text{L/minute}$ over 60 seconds, a serial dilution of the antigen used in Example 2)-5 (0.5 to 8 $\mu\text{g/mL}$) was added thereto at a flow rate of 30 $\mu\text{L/minute}$ over 120 seconds, followed by subsequent monitoring for a dissociation phase of 300 seconds. 3M magnesium chloride (available from GE Healthcare Bioscience) as a regenerating solution was added thereto at a flow rate of 20 $\mu\text{L/minute}$ over 30 seconds. The binding rate constant k_a , the dissociation rate constant k_d , and the dissociation constant (K_D ; $K_D = k_d/k_a$) were calculated using a 1:1 binding model for analyzing data. The dissociation constant for binding of the humanized CD147 antibody (#084H1L2hIgG4P or #110H1L4hIgG4P) to CD147 protein was calculated by the method of Example 7)-1.

[0384]

Table 8 shows information on the calculated dissociation constant, the effector function, and the epitope region.

[0385]

[Table 8]

Antibody	Antibody format	KD (nM)	Effector function	Epitope region
#110H1L4h	Human IgG4P	8.60	ADCC(-) CDC(-) ADCP(±)	Arg106 to Gly165 of SEQ ID NO: 3 (variant 2 of human CD147)
#084H1L2h	Human IgG4P	242	ADCC(-) CDC(-) ADCP(+)	In the vicinity from Asp65 to Gln81 of SEQ ID NO: 3 (variant 2 of human CD147)
4A5	Mouse IgG2a	132	ADCC(+) CDC(+) ADCP(+)	Competing with #084H1L2h
5F6	Human IgG1	71.9	ADCC(+) CDC(-) ADCP(+)	Competing with #084H1L2h
PPAT-082-03	Human IgG1	33.2	ADCC(+) CDC(-) ADCP(+)	Competing with #084H1L2h

[0386]

24)-2 Competitive ELISA with existing CD147 antibody

Using humanized CD147 antibody (#084H1L2hIg4P or #110H1L4hIg4P) produced in Example 6)-4-2, the binding activity to Recombinant Human CD147/Fc (Sino Biological Inc., Cat. 10186-H02H) was evaluated by competitive ELISA. As competitive antibodies, 4A5 and 5F6 antibodies prepared in 24)-1 and PPAT-082-03 antibody were used. As a competition negative control antibody, human IgG (Jackson ImmunoResearch Inc., Cat. 130093) was used. As a competition positive control antibody, the #84H1L2hIgG2 antibody was used with respect to the #084H1L2hIg4P antibody and the #110chIgG2 antibody was used with respect to #110H1L4hIg4P.

[0387]

Recombinant Human CD147/Fc diluted with PBS at 2 µg/ml was added to a 96-well plate (Thermo Scientific,

Cat. 43454) at 50 µl/well, followed by overnight storage at 4°C. After removing the protein solution, 300 µl of PBS containing 1% BSA was added thereto, followed by heating at room temperature for one hour. After removing the solution, 25 µl of an existing antibody solution (0, 0.2, 2, or 20 µg/ml) was added thereto, followed by heating at room temperature for two hours. 25 µl of the humanized CD147 antibody (#084H1L2hIg4P or #110H1L4hIg4P) at a concentration of 20 ng/ml was added to each well, followed by heating at room temperature for two hours. After washing with PBS containing 0.05% Tween20 (Bio-Rad Laboratories, Inc., Cat. 170-6531) twice, 50 µl of anti-human IgG4-HRP (Abcam plc., Cat.ab99823) diluted 2000 times with PBS containing 1% BSA was added thereto, followed by heating at room temperature for one hour. After washing with PBS containing 0.05% Tween20 (Bio-Rad Laboratories, Inc., Cat. 170-6531) three times, the washing solution was sufficiently drained, and 50 µl of an HRP substrate solution (eBioscience, Thermo Fisher SCIENTIFIC K.K., Cat.00-4203-58) was added thereto, followed by heating at room temperature for 15 to 20 minutes. Thereafter, the absorbance at 405 nm was measured using a plate reader (model name: EnVision2104, available from PerkinElmer, Inc).

[0388]

Table 8 and Figures 49(a) and (b) show the results.

[0389]

The binding of #084H1L2hIg4P to Recombinant Human CD147/Fc was inhibited by the presence of an existing CD147 antibody at 1 to 10 µg/ml in the presence of hIgG. The binding of #110H1L4hIg4P to Recombinant Human CD147/Fc was not inhibited by the presence of an existing CD147 antibody at 0.1 to 10 µg/ml in the presence of hIgG but was inhibited by the presence of #110chIgG2 antibody. It was found that the epitope of #084H1L2hIg4P competed with the binding of the existing anti-CD147 antibody, but the epitope of #110H1L4hIg4P was not affected by the binding of the existing anti-CD147 antibody. Table 8 shows the epitope information of the H110H1L4h antibody represented by Example 17)-3. The epitope region of #084H1L2h was estimated from the test results of the competitive binding (Example 2)-8) with the 2P10F2 antibody, the epitope of which was estimated by analysis using CD147 mutants of Example 1)-9. Table 8 shows the results.

[0390]

24)-3 Evaluation of ADCC of anti-CD147 antibodies

According to the method of Example 1)-15, the ADCC activity of anti-CD147 antibody was evaluated. Different conditions from those in the method of Example 1)-15 included, evaluation being performed using HepG2 cells (ATCC, Cat. HB-8065) as ADCC target cells and #110H1L4hIgG4P, #084H1L2hIg4P, 4A5, 5F6, and PPAT-082-03 being used as CD147 antibodies at a concentration of 1

µg/ml. The measurement was performed in triplicate, and the averages and the standard deviations were calculated. In the case where ⁵¹Cr was detected in 5% or more of the cells, the ADCC was determined to be positive (+). In the case of less than 5%, the ADCC activity was determined to be negative (-). Table 8 shows the results.

[0391]

The ADCC activity was not detected in #110H1L4hIgG4P and #084H1L2hIg4P, and thus ADCC was determined to be (-). In 4A5, 5F6, and PPAT-082-03, ⁵¹Cr was detected in 5% or more of cells, and thus ADCC was determined to be positive (+). #110H1L4hIgG4P and #084H1L2hIg4P were negative for ADCC and so are expected to induce potentially a lower level of cell death of normal cells, such as blood cells expressing CD147, in the human body than 4A5, 5F6, and PPAT-082-03, which were positive for ADCC activity.

[0392]

24)-4 Evaluation of CDC of anti-CD147 antibodies

According to the method of Example 1)-16, the complement-dependent cell killing activity (CDC activity) by anti-human CD147 antibody was evaluated. Different conditions from those in the method of Example 1)-16 included, measurement being performed by using human liver cell line HepG2 cells (ATCC, Cat. HB-8065) as target cells and #110H1L4hIgG4P, #084H1L2hIg4P, 4A5, 5F6, and PPAT-082-03 as anti-human CD147 antibodies and adding

a rabbit complement to a final concentration of 8%. The measurement was performed in triplicate, and the averages and the standard deviations were calculated. Antibodies in which 30% or more of antibody-dependent CDC activity was observed were determined to have a positive CDC activity and were shown as CDC (+) in the table. Table 8 shows the results. Only 4A5 out of the anti-human CD147 antibodies exhibited a positive CDC activity.

#110H1L4hIgG4P, #084H1L2hIg4P, 5F6, and PPAT-082-03 were negative for CDC and are expected to induce potentially a lower level of cell death of normal cells, such as blood cells expressing CD147, in the human body than 4A5, which had a positive CDC activity.

24)-5 Evaluation of ADCP of anti-CD147 antibodies

According to the method of Example 1)-17, the ADCP activity of anti-CD147 antibody was measured. Different conditions from those in the method of Example 1)-17 included, the ADCP activity being measured by using human liver cell line HepG2 cells (ATCC, Cat. HB-8065) as target cells and adding #110H1L4hIgG4P, #084H1L2hIg4P, 4A5, 5F6, and PPAT-082-03 as anti-CD147 antibodies at a concentration of 1 μ g/ml and an equal amount of labeled RAW264.7 cells to ADCP target cells. The measurement was performed in triplicate, and the averages and the standard deviations were calculated. An increase in ADCP activity of less than 10% of the human IgG treated group was determined to be slightly positive ($\pm$), and an

increase in activity of 10% or more was determined to be positive (+). Table 8 shows the results.

[0393]

#110H1L4hIgG4P had an ADCP activity of less than 10%, and the ADCC activity was determined to be $\pm$. #084H1L2hIgG4P, 4A5, 5F6, and PPAT-082-03 antibodies had an ADCP activity of 10% or more, and the ADCP activity was determined to be +. #110H1L4hIgG4P that recognizes CD147-D2 had a lower ADCP activity than other CD147 antibodies that recognize CD147-D1. After CD147 antibody is bound to CD147, the FC portion of the antibody needs to be recognized by Fc γ receptor expressed on macrophages or mononuclear cells for ADCP. However, there is a possibility that the ADCP activity is low because the epitope of the #110 antibody is close to the surface of the cells, and it is more difficult for Fc γ receptor to recognize the FC portion of the antibody bound to CD147 serving as an antigen than other antibodies that recognize CD147-D1. It is expected that #110H1L4hIgG4P having a slightly positive ADCP activity is less likely to induce cell death of normal cells, such as blood cells expressing CD147, in the human body than #084H1L2hIgG4P, 4A5, 5F6, and PPAT-082-03, which have a positive ADCP activity.

[0394]

(Example 25)

25)-1 Blood system cell aggregation by anti-CD147
antibody

It has been reported that some anti-CD147 antibodies induce aggregation of blood cells (Kasinrerk, et al., Immunology 1999, 96 (2) p184-192). Aggregation of blood cells could potentially cause a serious blood toxicity (Doll, C., et al., 1994, Curr. Opin. Oncol., 345-350) and is a property that is not desirable for a therapeutic antibody. Differences in cell aggregation activity between anti-CD147 antibodies were investigated. As CD147 antibodies, #110H1L4hIgG4P, #084H1L2hIg4P, 4A5, 5F6, and PPAT-082-03 were evaluated. As a negative control antibody, human IgG (hIgG, ChromPure Human IgG, Jackson ImmunoResearch Laboratories, Inc., Cat. 009-000-003) was used. HEL92.1.7 cells (purchased from ATCC, Cat. #TIB-180) with RPMI1640 medium (Thermo Fisher SCIENTIFIC K.K., Cat. 11875-093) containing 10% FBS (HyClone, GE Healthcare, Cat. SH30084.03) were added to a 96-well U bottom plate (Sumitomo Bakelite Co., Ltd., Cat. MS-9096U) at 1600 cells/80 µl per well, followed by culture for 4 hours under conditions of 5% CO₂, a humidity of 95%, and 37°C. 20 µl of an anti-CD147 antibody solution (150 µg/ml, 50 µg/ml) was added to each well to a final concentration of 30 or 10 µg/ml. After culture for 2 days under conditions of 5% CO₂, a humidity of 95%, and 37°C, microscopic observation was conducted.

[0395]

While cell aggregation due to the addition of human IgG or #110H1L4hIgG4P was not observed, cells aggregated in the presence of #084H1L2hIg4P, 4A5, 5F6, or PPAT-082-03 antibody, and cell masses overlapping at the center of the plate were observed. It was revealed that #110H1L4hIgG4P that recognizes CD147-D2 has no blood cell aggregation activity and so was different from other CD147 antibodies that recognize the CD147-D1 domain. Since CD147 antibodies including #084 that have a cell aggregation activity may potentially cause toxicity such as a thrombus via aggregation of blood cells when administered to humans, it is desirable to avoid or reduce such side effects by the combined use of a subcutaneous injection of heparin at a dose used for treating a thrombus or low-molecular weight heparin or anti-platelet drugs.

[0396]

25)-2 Evaluation of the risk of cytokine release syndrome

In some antibodies such as OKT3 and TGN1412, administration of the therapeutic antibodies causes an increase in cytokines in the blood by activating immune cells, thereby causing serious cytokine release syndrome (Gaston, R., *Kidney International*, 1991, 141-148; Suntharalingam, G., et al., *N. Engl. J. Med.* 2006, 1018-1028). It has been reported that some CD147 antibodies act on immune cells, having an action of increasing production of interferon gamma or interleukin-4 (Hu, J.,

et al., J. Cell. Mol. Med., 2010, 2132-2143). The toxicity of antibody drugs due to cytokine release syndrome can be predicted by a cytokine release assay using peripheral blood (Vessillier, S. et al., J. Immunol. Methods, 2015, 43-52). Thus, the risk of cytokine release syndrome was evaluated by a cytokine release assay using human peripheral blood. #110H1L4hIgG4P, #110chIgG4ProFALA, #084H1L2hIg4P, and #084H1L2hIg2 were used as CD147 antibodies, and bevacizumab (Genentech, Inc.), trastuzumab (Roche Pharma AG), alemtuzumab (Sanofi K.K.), and anti-human CD3 antibody (BioLegend Cat. No317326) were used as comparative antibodies. For all evaluated CD147 antibodies, acceleration of cell growth was not observed in human peripheral blood mononuclear cells (6 donors were evaluated for each), and the influence on cytokine release (TNF α , INF- γ , IL-2, IL-6, IL-8, IL-10, and MIP-1 α) was less than in bevacizumab, which has a low risk of cytokine release syndrome. For anti-human CD3 antibody (OKT3), acceleration of cell growth and acceleration of cytokine release (TNF α , INF- γ , IL-2, IL-6, IL-8, IL-10, and MIP-1 α) were observed. It was demonstrated that #110H1L4hIgG4P, #110chIgG4ProFALA, #084H1L2hIg4P, and #084H1L2hIg2 do not induce cytokine release that could potentially cause cytokine release syndrome.

[0397]

25)-3 Evaluation of the safety of anti-CD147 antibody in monkeys

It has been reported that some mouse anti-CD147 antibodies, when administered to mice, inhibit the functions of CD147, induce aggregation of red blood cells in the spleen, and reduce the amount of red blood cells in the peripheral blood, thereby causing anemia (Coste, I. et al., Blood, 2001, 3984-3988). Since the CD147 antibodies obtained in the present invention such as #110H1L4hIgG4P do not exhibit binding to mouse CD147, the safety is not appropriately evaluated in mice. Therefore, the safety was evaluated by administering #110H1L4hIgG4P, which is an anti-CD147 antibody confirmed to have a binding activity to human and monkey CD147 by experiments using a flow cytometer, to cynomolgus monkeys. #110H1L4hIgG4P was intravenously administered to cynomolgus monkeys (one male and one female) at a single dose of 99.2 mg/kg that is the maximum dose. As a result, serious toxicity (changes in body weight and food intake or histopathological changes) was not observed either in the observation period of 15 days from the administration or by histopathological examination at the completion of the observation period. #110H1L4hIgG4P exhibited no toxicity to cynomolgus monkeys, thus indicating that it may be used for human cancer treatment.

[0398]

(Example 26) Influence of KLF5 on sensitivity to CD147 antibody

A lethal EMT signal is known as a signal of SMAD2/SMAD3/SMAD4-dependent cell death in cancer cells, and it has been reported that, in SMAD4-negative cancer cells, there is an increased expression of transcription factor KLF5 protein, which is normally reduced by SMAD signaling, and this reduces the lethal EMT signal in SMAD4-negative cancer cells (David, C., Cell, 2016, 1015-1030). Since the CD147 antibody of the present invention activates SMAD signaling and exhibits an antitumor efficacy on SMAD4-positive cells, it was considered to induce a SMAD signal-dependent cell death. Whether KLF5 is involved in the sensitivity to the CD147 antibody-dependent antitumor efficacy was investigated.

[0399]

26)-1 Production of KLF5 expressing cell line

According to the method of Example 13, KLF5 stably expressing cell line MIA PaCa-2 cells were produced. SEQ ID NOs: 145 and 146 respectively represent the amino acid sequence and the nucleotide sequence of human KLF5. A retroviral vector pQCXIP with a sequence (Ref seq. ID: NM_001730.4) of the KLF5 gene contained in it (GenScript Biotech Corporation, Cat. OHu21278C) was produced and the retroviral vector was used for the production of the retrovirus. The retrovirus was incorporated into a chromosome by viral infection, and MIA PaCa-2 cells that

became resistant to puromycin and positive for KLF5 were selected to form KLF5 positive MIA PaCa-2 cells, MIA PaCa-2-KLF5. A retroviral vector pQCXIP was infected in the same manner to form puromycin resistant MIA PaCa-2 cells, MIA PaCa-2-mock.

[0400]

26)-2 Confirmation of CD147 and SMAD4 expression

CD147 expressions of MIA PaCa-2-mock and MIA PaCa-2-KLF5 were confirmed using a flow cytometer. According to the method of Example 13-2, it was confirmed that the KLF5 expression level in MIA PaCa-2-KLF5 increased from that in MIA PaCa-2-mock. For detecting KLF5, a KLF5 antibody (Cell Signaling Technology, Inc., Cat. #51586) was used.

26)-3 Sensitivity of MIA PaCa-2 tumor expressing KLF5 to humanized CD147 antibody

According to the method of Example 7)-2, the sensitivities of tumors of MIA PaCa-2-KLF5 and MIA PaCa-2-mock to the humanized CD147 antibody #110H1L4hIgG4P were compared. The humanized CD147 antibody (#110H1L4hIg4P) produced in Example 6)-4-2 was administered to mice bearing tumor via the tail vein at 1 mg/kg 3 days after the cell inoculation (n = 6). The antibody was administered 7 days later in the same manner. A PBS buffer solution was administered to mice bearing tumor of the control group via the tail vein in the same manner (n = 6). Figure 50 shows the results.

[0401]

The average volume of tumor of MIA PaCa-2-mock decreased to 9% of that in the control group 14 days after the administration of the humanized CD147 antibody, thus exhibiting a sensitivity to the CD147 antibody. The average tumor volume of MIA PaCa-2-KLF5 was 80% of the control group, thus exhibiting a low sensitivity to the CD147 antibody. It was found that the SMAD signal-dependent antitumor efficacy of the CD147 antibody is suppressed by KLF5 expression.

Industrial Applicability

[0402]

The present invention provides a CD147-specific antibody that activates CD147 and exhibits potent antitumor efficacy. The present invention provides an antibody exhibiting potent antitumor efficacy independent of effector functions. The antibody of the present invention exhibits efficacy in liver cancer cells that is clearly better than that of sorafenib that is used as one of the standard of care drugs for liver cancer. The antibody of the present invention exhibits efficacy in pancreatic cancer cells significantly stronger than that of gemcitabine that is used as one of the standard of care drugs for pancreatic cancer. The antibody of the present invention exhibits efficacy in chronic myeloid leukemia cells significantly stronger than that of

imatinib that is used as one of the standard of care drugs for chronic myeloid leukemia. The antibody of the present invention has antitumor efficacy that is exhibited independent of effector functions and causes fewer concerns in terms of safety evaluation, and thus an anti-CD147 antibody with excellent safety is provided. CD147 is expressed not only in tumor cells, but also in blood cells. However, the antibody of the present invention does not act on T cells and PBMC and is independent of effector functions, so that the antibody has an advantage of causing fewer safety concerns in development thereof as an antitumor agent. The present invention provides a pharmaceutical composition comprising the above antibody, as well as a method for treating tumors using the antibody and/or the pharmaceutical composition.

Sequence Listing Free Text

[0403]

SEQ ID NO: 1: Amino acid sequence of variant 1 of human CD147

SEQ ID NO: 2: Nucleotide sequence of variant 1 of human CD147

SEQ ID NO: 3: Amino acid sequence of variant 2 of human CD147

SEQ ID NO: 4: Nucleotide sequence of variant 2 of human CD147

SEQ ID NO: 5: Amino acid sequence of human SMAD4

SEQ ID NO: 6: Nucleotide sequence of human SMAD4

SEQ ID NO: 7: Nucleotide sequence of the light chain
variable region of LN22R8

SEQ ID NO: 8: Amino acid sequence of the light chain
variable region of LN22R8

SEQ ID NO: 9: Nucleotide sequence of the heavy chain
variable region of LN22R8

SEQ ID NO: 10: Amino acid sequence of the heavy chain
variable region of LN22R8

SEQ ID NO: 11: Amino acid sequence of CDRL1 of LN22R8

SEQ ID NO: 12: Amino acid sequence of CDRL2 of LN22R8

SEQ ID NO: 13: Amino acid sequence of CDRL3 of LN22R8

SEQ ID NO: 14: Amino acid sequence of CDRH1 of LN22R8

SEQ ID NO: 15: Amino acid sequence of CDRH2 of LN22R8

SEQ ID NO: 16: Amino acid sequence of CDRH3 of LN22R8

SEQ ID NO: 17: Nucleotide sequence of the light chain
variable region of 2P10F2

SEQ ID NO: 18: Amino acid sequence of the light chain
variable region of 2P10F2

SEQ ID NO: 19: Nucleotide sequence of the heavy chain
variable region of 2P10F2

SEQ ID NO: 20: Amino acid sequence of the heavy chain
variable region of 2P10F2

SEQ ID NO: 21: Amino acid sequence of CDRL1 of 2P10F2

SEQ ID NO: 22: Amino acid sequence of CDRL2 of 2P10F2

SEQ ID NO: 23: Amino acid sequence of CDRL3 of 2P10F2

SEQ ID NO: 24: Amino acid sequence of CDRH1 of 2P10F2

SEQ ID NO: 25: Amino acid sequence of CDRH2 of 2P10F2

SEQ ID NO: 26: Amino acid sequence of CDRH3 of 2P10F2

SEQ ID NO: 27: DNA comprising DNA sequence coding for human light chain signal sequence and human kappa chain constant region

SEQ ID NO: 28: DNA comprising DNA sequence coding for amino acid of human heavy chain signal sequence and human IgG1 constant region

SEQ ID NO: 29: DNA comprising DNA sequence coding for amino acid of human heavy chain signal sequence and human IgG2 constant region

SEQ ID NO: 30: Nucleotide sequence of the light chain of human chimeric LN22R8

SEQ ID NO: 31: Amino acid sequence of the light chain of human chimeric LN22R8

SEQ ID NO: 32: Nucleotide sequence of the heavy chain IgG1 type of human chimeric LN22R8

SEQ ID NO: 33: Amino acid sequence of the heavy chain IgG1 type of human chimeric LN22R8

SEQ ID NO: 34: Nucleotide sequence of the heavy chain IgG2 type of human chimeric LN22R8

SEQ ID NO: 35: Amino acid sequence of the heavy chain IgG2 type of human chimeric LN22R8

SEQ ID NO: 36: DNA comprising DNA sequence coding for amino acid sequence of human chimera LN22R8_heavy chain_IgG4P type

SEQ ID NO: 37: Amino acid sequence of the heavy chain
IgG4P type of human chimeric LN22R8

SEQ ID NO: 38: DNA comprising DNA sequence coding for
amino acid of human heavy chain signal sequence and human
IgG1LALA constant region

SEQ ID NO: 39: DNA comprising DNA sequence coding for
amino acid of human heavy chain signal sequence and human
IgG4P constant region

SEQ ID NO: 40: Nucleotide sequence of the light chain of
human chimeric 2P10F2

SEQ ID NO: 41: Amino acid sequence of the light chain of
human chimeric 2P10F2

SEQ ID NO: 42: Nucleotide sequence of the heavy chain
IgG1LALA type of human chimeric 2P10F2

SEQ ID NO: 43: Amino acid sequence of the heavy chain
IgG1LALA type of human chimeric 2P10F2

SEQ ID NO: 44: Nucleotide sequence of the heavy chain
IgG2 type of human chimeric 2P10F2

SEQ ID NO: 45: Amino acid sequence of the heavy chain
IgG2 type of human chimeric 2P10F2

SEQ ID NO: 46: Nucleotide sequence of the heavy chain
IgG4P type of human chimeric 2P10F2

SEQ ID NO: 47: Amino acid sequence of the heavy chain
IgG4P type of human chimeric 2P10F2

SEQ ID NO: 48: Nucleotide sequence of the light chain
variable region of rat_CD147_#84(r#84)

SEQ ID NO: 49: Amino acid sequence of the light chain variable region of rat_CD147_#84(r#84)

SEQ ID NO: 50: Nucleotide sequence of the heavy chain variable region of rat_CD147_#84(r#84)

SEQ ID NO: 51: Amino acid sequence of the heavy chain variable region of rat_CD147_#84(r#84)

SEQ ID NO: 52: Amino acid sequence of CDRL1 of rat_CD147_#84(r#84)

SEQ ID NO: 53: Amino acid sequence of CDRL2 of rat_CD147_#84(r#84)

SEQ ID NO: 54: Amino acid sequence of CDRL3 of rat_CD147_#84(r#84)

SEQ ID NO: 55: Amino acid sequence of CDRH1 of rat_CD147_#84(r#84)

SEQ ID NO: 56: Amino acid sequence of CDRH2 of rat_CD147_#84(r#84)

SEQ ID NO: 57: Amino acid sequence of CDRH3 of rat_CD147_#84(r#84)

SEQ ID NO: 58: Nucleotide sequence of the light chain variable region of rat_CD147_#101(r#101)

SEQ ID NO: 59: Amino acid sequence of the light chain variable region of rat_CD147_#101(r#101)

SEQ ID NO: 60: Nucleotide sequence of the heavy chain variable region of rat_CD147_#101(r#101)

SEQ ID NO: 61: Amino acid sequence of the heavy chain variable region of rat_CD147_#101(r#101)

SEQ ID NO: 62: Amino acid sequence of CDRL1 of
rat_CD147_#101(r#101)

SEQ ID NO: 63: Amino acid sequence of CDRL2 of
rat_CD147_#101(r#101)

SEQ ID NO: 64: Amino acid sequence of CDRL3 of
rat_CD147_#101(r#101)

SEQ ID NO: 65: Amino acid sequence of CDRH1 of
rat_CD147_#101(r#101)

SEQ ID NO: 66: Amino acid sequence of CDRH2 of
rat_CD147_#101(r#101)

SEQ ID NO: 67: Amino acid sequence of CDRH3 of
rat_CD147_#101(r#101)

SEQ ID NO: 68: Nucleotide sequence of the light chain
variable region of rat_CD147_#110(r#110)

SEQ ID NO: 69: Amino acid sequence of the light chain
variable region of rat_CD147_#110(r#110)

SEQ ID NO: 70: Nucleotide sequence of the heavy chain
variable region of rat_CD147_#110(r#110)

SEQ ID NO: 71: Amino acid sequence of the heavy chain
variable region of rat_CD147_#110(r#110)

SEQ ID NO: 72: Amino acid sequence of CDRL1 of
rat_CD147_#110(r#110)

SEQ ID NO: 73: Amino acid sequence of CDRL2 of
rat_CD147_#110(r#110)

SEQ ID NO: 74: Amino acid sequence of CDRL3 of
rat_CD147_#110(r#110)

SEQ ID NO: 75: Amino acid sequence of CDRH1 of
rat_CD147_#110(r#110)

SEQ ID NO: 76: Amino acid sequence of CDRH2 of
rat_CD147_#110(r#110)

SEQ ID NO: 77: Amino acid sequence of CDRH3 of
rat_CD147_#110(r#110)

SEQ ID NO: 78: Nucleotide sequence of the light chain
variable region of rat_CD147_#131(r#131)

SEQ ID NO: 79: Amino acid sequence of the light chain
variable region of rat_CD147_#131(r#131)

SEQ ID NO: 80: Nucleotide sequence of the heavy chain
variable region of rat_CD147_#131(r#131)

SEQ ID NO: 81: Amino acid sequence of the heavy chain
variable region of rat_CD147_#131(r#131)

SEQ ID NO: 82: Amino acid sequence of CDRL1 of
rat_CD147_#131(r#131)

SEQ ID NO: 83: Amino acid sequence of CDRL2 of
rat_CD147_#131(r#131)

SEQ ID NO: 84: Amino acid sequence of CDRL3 of
rat_CD147_#131(r#131)

SEQ ID NO: 85: Amino acid sequence of CDRH1 of
rat_CD147_#131(r#131)

SEQ ID NO: 86: Amino acid sequence of CDRH2 of
rat_CD147_#131(r#131)

SEQ ID NO: 87: Amino acid sequence of CDRH3 of
rat_CD147_#131(r#131)

SEQ ID NO: 88: DNA comprising DNA sequence coding for amino acid of human heavy chain signal sequence and human IgG4PFALA constant region

SEQ ID NO: 89: Nucleotide sequence of the light chain of human chimeric rat_CD147_#84

SEQ ID NO: 90: Amino acid sequence of the light chain of human chimeric rat_CD147_#84

SEQ ID NO: 91: Nucleotide sequence of the heavy chain IgG1 type of human chimeric rat_CD147_#84

SEQ ID NO: 92: Amino acid sequence of the heavy chain IgG1 type of human chimeric rat_CD147_#84

SEQ ID NO: 93: Nucleotide sequence of the heavy chain IgG2 type of human chimeric rat_CD147_#84

SEQ ID NO: 94: Amino acid sequence of the heavy chain IgG2 type of human chimeric rat_CD147_#84

SEQ ID NO: 95: Nucleotide sequence of the heavy chain IgG4P type of human chimeric rat_CD147_#84

SEQ ID NO: 96: Amino acid sequence of the heavy chain IgG4P type of human chimeric rat_CD147_#84

SEQ ID NO: 97: Nucleotide sequence of the heavy chain IgG1LALA type of human chimeric rat_CD147_#84

SEQ ID NO: 98: Amino acid sequence of the heavy chain IgG1LALA type of human chimeric rat_CD147_#84

SEQ ID NO: 99: Nucleotide sequence of the heavy chain IgG4PFALA type of human chimeric rat_CD147_#84

SEQ ID NO: 100: Amino acid sequence of the heavy chain IgG4PFALA type of human chimeric rat_CD147_#84

SEQ ID NO: 101: Nucleotide sequence of the light chain of human chimeric rat_CD147_#101

SEQ ID NO: 102: Amino acid sequence of the light chain of human chimeric rat_CD147_#101

SEQ ID NO: 103: Nucleotide sequence of the heavy chain IgG2 of human chimeric rat_CD147_#101

SEQ ID NO: 104: Amino acid sequence of the heavy chain IgG2 of human chimeric rat_CD147_#101

SEQ ID NO: 105: Nucleotide sequence of the heavy chain IgG4P of human chimeric rat_CD147_#101

SEQ ID NO: 106: Amino acid sequence of the heavy chain IgG4P of human chimeric rat_CD147_#101

SEQ ID NO: 107: Nucleotide sequence of the heavy chain IgG4PFALA of human chimeric rat_CD147_#101

SEQ ID NO: 108: Amino acid sequence of the heavy chain IgG4PFALA of human chimeric rat_CD147_#101

SEQ ID NO: 109: Nucleotide sequence of the light chain of human chimeric rat_CD147_#110

SEQ ID NO: 110: Amino acid sequence of the light chain of human chimeric rat_CD147_#110

SEQ ID NO: 111: Nucleotide sequence of the heavy chain IgG2 of human chimeric rat_CD147_#110

SEQ ID NO: 112: Amino acid sequence of the heavy chain IgG2 of human chimeric rat_CD147_#110

SEQ ID NO: 113: Nucleotide sequence of the heavy chain IgG4P of human chimeric rat_CD147_#110

SEQ ID NO: 114: Amino acid sequence of the heavy chain
IgG4P of human chimeric rat_CD147_#110

SEQ ID NO: 115: Nucleotide sequence of the heavy chain
IgG4PFALA of human chimeric rat_CD147_#110

SEQ ID NO: 116: Amino acid sequence of the heavy chain
IgG4PFALA of human chimeric rat_CD147_#110

SEQ ID NO: 117: Nucleotide sequence of the light chain of
human chimeric rat_CD147_#131

SEQ ID NO: 118: Amino acid sequence of the light chain of
human chimeric rat_CD147_#131

SEQ ID NO: 119: Nucleotide sequence of the heavy chain
IgG2 of human chimeric rat_CD147_#131

SEQ ID NO: 120: Amino acid sequence of the heavy chain
IgG2 of human chimeric rat_CD147_#131

SEQ ID NO: 121: Nucleotide sequence of the heavy chain
IgG4P of human chimeric rat_CD147_#131

SEQ ID NO: 122: Amino acid sequence of the heavy chain
IgG4P of human chimeric rat_CD147_#131

SEQ ID NO: 123: Amino acid sequence of #84H1hIgG2

SEQ ID NO: 124: Nucleotide sequence of #84H1hIgG2

SEQ ID NO: 125: Amino acid sequence of #84H1hIgG4P

SEQ ID NO: 126: Nucleotide sequence of #84H1hIgG4P

SEQ ID NO: 127: Amino acid sequence of #84L2h

SEQ ID NO: 128: Nucleotide sequence of #84L2h

SEQ ID NO: 129: Amino acid sequence of #101H1hIgG2

SEQ ID NO: 130: Nucleotide sequence of #101H1hIgG2

SEQ ID NO: 131: Amino acid sequence of #101H1hIgG4P

SEQ ID NO: 132: Nucleotide sequence of #101H1hIgG4P
SEQ ID NO: 133: Amino acid sequence of #101L2h
SEQ ID NO: 134: Nucleotide sequence of #101L2h
SEQ ID NO: 135: Amino acid sequence of #110H1hIgG4P
SEQ ID NO: 136: Nucleotide sequence of #110H1hIgG4P
SEQ ID NO: 137: Amino acid sequence of #110L4h
SEQ ID NO: 138: Nucleotide sequence of #110L4h
SEQ ID NO: 139: Amino acid sequence of #131H2hIgG2
SEQ ID NO: 140: Nucleotide sequence of #131H2hIgG2
SEQ ID NO: 141: Amino acid sequence of #131L2h
SEQ ID NO: 142: Nucleotide sequence of #131L2h
SEQ ID NO: 143: mu3 region of human CD147v1
SEQ ID NO: 144: mu3 region of cynomolgus monkey CD147
SEQ ID NO: 145: Amino acid sequence of human KLF5
SEQ ID NO: 146: Nucleotide sequence of human KLF5
SEQ ID NO: 147: Amino acid sequence of #110H13hIgG4P
SEQ ID NO: 148: Nucleotide sequence of #110H13hIgG4P
SEQ ID NO: 149: Amino acid sequence of #110L2h
SEQ ID NO: 150: Nucleotide sequence of #110L2h
SEQ ID NO: 151: Amino acid sequence of #110L12h
SEQ ID NO: 152: Nucleotide sequence of #110L12h

Claims

[Claim 1]

An antibody against human CD147 or an antigen-binding fragment thereof, which competes with at least one antibody selected from the group consisting of the following (A) to (F) for binding to human CD147 and which activates signal transduction through CD147:

(A) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 71 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 69;

(B) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 51 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 49;

(C) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 61 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 59;

(D) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 81 and a light chain variable region

consisting of an amino acid sequence represented by SEQ ID NO: 79;

(E) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 10 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 8; and

(F) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 20 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 18.

[Claim 2]

An antibody against human CD147 or an antigen-binding fragment thereof, which binds to an epitope to which at least one antibody selected from the group consisting of the following (A) to (F) binds, and which activates signal transduction through CD147:

(A) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 71 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 69;

(B) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 51 and a light chain variable region

consisting of an amino acid sequence represented by SEQ ID NO: 49;

(C) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 61 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 59;

(D) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 81 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 79;

(E) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 10 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 8; and

(F) an antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 20 and a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 18.

[Claim 3]

The antibody against human CD147 or the antigen-binding fragment thereof according to claim 1 or 2, which has decreased ADCC activity or lacks ADCC activity.

[Claim 4]

The antibody against human CD147 or the antigen-binding fragment thereof according to any one of claims 1 to 3, which has decreased CDC activity or lacks CDC activity.

[Claim 5]

The antibody against human CD147 or the antigen-binding fragment thereof according to any one of claims 1 to 4, which has decreased ADCP activity or lacks ADCP activity.

[Claim 6]

The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 5, which binds to an epitope comprising residues of arginine (Arg) at position 106 to glycine (Gly) at position 165 in SEQ ID NO: 3.

[Claim 7]

The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 6, which binds to an epitope comprising residues of arginine (Arg) at position 106, lysine (Lys) at position 108, alanine (Ala) at position 109, valine (Val) at position 110, lysine (Lys) at position 127, serine (Ser) at position 128, glutamic acid (Glu) at position 129, serine (Ser) at position 130, valine (Val) at position 131, proline (Pro) at position 132, proline (Pro) at position 133, valine (Val) at position 134, glutamine (Gln) at position 164 and glycine (Gly) at position 165 in the amino acid sequence set forth in SEQ ID NO: 3.

[Claim 8]

The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 7, wherein:

in the heavy chain sequence, which comprises a variable region having CDRH1, CDRH2 and CDRH3, the CDRH1 consists of an amino acid sequence represented by SEQ ID NO: 75, the CDRH2 consists of an amino acid sequence represented by SEQ ID NO: 76, and the CDRH3 consists of an amino acid sequence represented by SEQ ID NO: 77; and

in the light chain sequence, which comprises a variable region having CDRL1, CDRL2 and CDRL3, the CDRL1 consists of an amino acid sequence represented by SEQ ID NO: 72, the CDRL2 consists of an amino acid sequence represented by SEQ ID NO: 73, and the CDRL3 consists of an amino acid sequence represented by SEQ ID NO: 74.

[Claim 9]

The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 5, which binds to an epitope comprising an amino acid sequence set forth in SEQ ID NO: 143 or an amino acid sequence in which one or several amino acids are deleted, substituted or added in the sequence of SEQ ID NO: 143.

[Claim 10]

The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 5 or 9, wherein:

in the heavy chain sequence, which comprises a variable region having CDRH1, CDRH2 and CDRH3, the CDRH1

consists of an amino acid sequence represented by SEQ ID NO: 55, the CDRH2 consists of an amino acid sequence represented by SEQ ID NO: 56, and the CDRH3 consists of an amino acid sequence represented by SEQ ID NO: 57; and

in the light chain sequence, which comprises a variable region having CDRL1, CDRL2 and CDRL3, the CDRL1 consists of an amino acid sequence represented by SEQ ID NO: 52, the CDRL2 consists of an amino acid sequence represented by SEQ ID NO: 53, and the CDRL3 consists of an amino acid sequence represented by SEQ ID NO: 54.

[Claim 11]

The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 5 or 9, wherein:

in the heavy chain sequence, which comprises a variable region having CDRH1, CDRH2 and CDRH3, the CDRH1 consists of an amino acid sequence represented by SEQ ID NO: 65, the CDRH2 consists of an amino acid sequence represented by SEQ ID NO: 66, and the CDRH3 consists of an amino acid sequence represented by SEQ ID NO: 67; and

in the light chain sequence, which comprises a variable region having CDRL1, CDRL2 and CDRL3, the CDRL1 consists of an amino acid sequence represented by SEQ ID NO: 62, the CDRL2 consists of an amino acid sequence represented by SEQ ID NO: 63, and the CDRL3 consists of an amino acid sequence represented by SEQ ID NO: 64.

[Claim 12]

The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 5 or 9, wherein:

in the heavy chain sequence, which comprises a variable region having CDRH1, CDRH2 and CDRH3, the CDRH1 consists of an amino acid sequence represented by SEQ ID NO: 85, the CDRH2 consists of an amino acid sequence represented by SEQ ID NO: 86, and the CDRH3 consists of an amino acid sequence represented by SEQ ID NO: 87; and

in the light chain sequence, which comprises a variable region having CDRL1, CDRL2 and CDRL3, the CDRL1 consists of an amino acid sequence represented by SEQ ID NO: 82, the CDRL2 consists of an amino acid sequence represented by SEQ ID NO: 83, and the CDRL3 consists of an amino acid sequence represented by SEQ ID NO: 84.

[Claim 13]

The antigen-binding fragment of the antibody according to any one of claims 1 to 12, which is selected from the group consisting of Fab, F(ab')₂, Fab' and Fv.

[Claim 14]

The antibody according to any one of claims 1 to 12, which is scFv.

[Claim 15]

The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 12, which is a chimeric antibody.

[Claim 16]

The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 12, which is humanized.

[Claim 17]

The antibody according to any one of claims 1 to 16, wherein the heavy chain comprises a human immunoglobulin G1 heavy chain constant region, a human immunoglobulin G2 heavy chain constant region or a human immunoglobulin G4 heavy chain constant region, and the light chain comprises a human immunoglobulin κ light chain constant region.

[Claim 18]

The antibody according to claim 17, wherein the heavy chain comprises the human immunoglobulin G4 heavy chain constant region.

[Claim 19]

The antibody according to claim 18, wherein within the human immunoglobulin G4 heavy chain constant region, the serine (Ser) at position 228 as indicated by the EU index is substituted with a proline (Pro).

[Claim 20]

The antibody according to claim 18, wherein within the human immunoglobulin G4 heavy chain constant region, the phenylalanine (Phe) at position 234 as indicated by the EU index is substituted with an alanine (Ala), and the leucine (Leu) at position 235 as indicated by the EU index is substituted with an alanine (Ala).

[Claim 21]

The antibody according to claim 18, wherein within the human immunoglobulin G4 heavy chain constant region, the serine (Ser) at position 228 as indicated by the EU index is substituted with a proline (Pro), the phenylalanine (Phe) at position 234 as indicated by the EU index is substituted with an alanine (Ala), and the leucine (Leu) at position 235 as indicated by the EU index is substituted with an alanine (Ala).

[Claim 22]

The antibody according to claim 17, wherein the heavy chain comprises the human immunoglobulin G2 heavy chain constant region.

[Claim 23]

An antibody against human CD147 or an antigen-binding fragment thereof, which comprises the following (c) and (d), and activates signal transduction through CD147:

(c) a heavy chain variable region selected from the group consisting of the following (c1) to (c4):

(c1) a heavy chain variable region consisting of amino acid residues at positions 20 to 136 in an amino acid sequence represented by SEQ ID NO: 135;

(c2) a heavy chain variable region consisting of amino acid residues at positions 20 to 136 in an amino acid sequence represented by SEQ ID NO: 147;

(c3) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (c1) or (c2); and

(c4) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence according to any one of (c1) to (c3); and

(d) a light chain variable region selected from the group consisting of the following (d1) to (d5):

(d1) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 137;

(d2) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 149;

(d3) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 151;

(d4) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence according to any one of (d1) to (d3); and

(d5) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR

sequence in the sequence according to any one of (d1) to (d4).

[Claim 24]

The antibody or the antigen-binding fragment thereof according to claim 23, which comprises:

the heavy chain variable region consisting of amino acid residues at positions 20 to 136 in the amino acid sequence represented by SEQ ID NO: 135; and

the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 137.

[Claim 25]

The antibody or the antigen-binding fragment thereof according to claim 23, which comprises:

the heavy chain consisting of amino acid residues at positions 20 to 463 in the amino acid sequence represented by SEQ ID NO: 135; and

the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 137.

[Claim 26]

The antibody or the antigen-binding fragment thereof according to claim 23, which comprises:

the heavy chain variable region consisting of amino acid residues at positions 20 to 136 in the amino acid sequence represented by SEQ ID NO: 147; and

the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 149.

[Claim 27]

The antibody or the antigen-binding fragment thereof according to claim 23, which comprises:

the heavy chain consisting of amino acid residues at positions 20 to 463 in the amino acid sequence represented by SEQ ID NO: 147; and

the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 149.

[Claim 28]

The antibody or the antigen-binding fragment thereof according to claim 23, which comprises:

the heavy chain variable region consisting of amino acid residues at positions 20 to 136 in the amino acid sequence represented by SEQ ID NO: 147; and

the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 151.

[Claim 29]

The antibody or the antigen-binding fragment thereof according to claim 23, which comprises:

the heavy chain consisting of amino acid residues at positions 20 to 463 in the amino acid sequence represented by SEQ ID NO: 147; and

the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 151.

[Claim 30]

An antibody against human CD147 or an antigen-binding fragment thereof, which comprises the following (a) and (b), and activates signal transduction through CD147:

(a) a heavy chain variable region selected from the group consisting of the following (a1) to (a4):

(a1) a heavy chain variable region consisting of amino acid residues at positions 20 to 140 in an amino acid sequence represented by SEQ ID NO: 123;

(a2) a heavy chain variable region consisting of amino acid residues at positions 20 to 140 in an amino acid sequence represented by SEQ ID NO: 125;

(a3) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (a1) or (a2); and

(a4) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence according to any one of (a1) to (a3); and

(b) a light chain variable region selected from the group consisting of the following (b1) to (b3):

(b1) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 127;

(b2) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (b1); and

(b3) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence of (b1) or (b2).

[Claim 31]

The antibody or the antigen-binding fragment thereof according to claim 30, which comprises:

the heavy chain variable region consisting of amino acid residues at positions 20 to 140 in the amino acid sequence represented by SEQ ID NO: 123 or the heavy chain variable region consisting of amino acid residues at positions 20 to 140 in the amino acid sequence represented by SEQ ID NO: 125; and

the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 127.

[Claim 32]

The antibody or the antigen-binding fragment thereof according to claim 30, which comprises:

the heavy chain consisting of amino acid residues at positions 20 to 466 in the amino acid sequence represented by SEQ ID NO: 123 or the heavy chain consisting of amino acid residues at positions 20 to 467 in the amino acid sequence represented by SEQ ID NO: 125; and

the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 127.

[Claim 33]

An antibody against human CD147 or an antigen-binding fragment thereof, which comprises the following (e) and (f), and activates signal transduction through CD147:

(e) a heavy chain variable region selected from the group consisting of the following (e1) to (e4):

(e1) a heavy chain variable region consisting of amino acid residues at positions 20 to 137 in an amino acid sequence represented by SEQ ID NO: 129;

(e2) a heavy chain variable region consisting of amino acid residues at positions 20 to 137 in an amino acid sequence represented by SEQ ID NO: 131;

(e3) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (e1) or (e2); and

(e4) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence according to any one of (e1) to (e3); and

(f) a light chain variable region selected from the group consisting of the following (f1) to (f3):

(f1) a light chain variable region consisting of amino acid residues at positions 21 to 128 in an amino acid sequence represented by SEQ ID NO: 133;

(f2) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework region outside each CDR sequence in the sequence of (f1); and

(f3) an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the sequence of a framework region outside each CDR sequence in the sequence of (f1) or (f2).

[Claim 34]

The antibody or the antigen-binding fragment thereof according to claim 33, which comprises:

the heavy chain variable region consisting of amino acid residues at positions 20 to 137 in the amino acid sequence represented by SEQ ID NO: 129 or the heavy chain variable region consisting of amino acid residues at positions 20 to 137 in the amino acid sequence represented by SEQ ID NO: 131; and

the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 133.

[Claim 35]

The antibody or the antigen-binding fragment thereof according to claim 33, which comprises:

the heavy chain consisting of amino acid residues at positions 20 to 463 in the amino acid sequence represented by SEQ ID NO: 129 or the heavy chain consisting of amino acid residues at positions 20 to 464 in the amino acid sequence represented by SEQ ID NO: 131; and

the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 133.

[Claim 36]

An antibody against human CD147 or an antigen-binding fragment thereof, which comprises the following (g) and (h), and activates signal transduction through CD147:

(g) a heavy chain variable region selected from the group consisting of the following (g1) to (g3):

(g1) a heavy chain variable region consisting of amino acid residues at positions 20 to 138 in an amino acid sequence represented by SEQ ID NO: 139;

(g2) an amino acid sequence having at least 95% or more sequence identity with the sequence of a framework

region outside each CDR sequence in the sequence of (g1);
and

(g3) an amino acid sequence in which one or
several amino acids are deleted, substituted, or added in
the sequence of a framework region outside each CDR
sequence in the sequence of (g1) or (g2); and

(h) a light chain variable region selected from the
group consisting of the following (h1) to (h3):

(h1) a light chain variable region consisting of
amino acid residues at positions 21 to 128 in an amino
acid sequence represented by SEQ ID NO: 141;

(h2) an amino acid sequence having at least 95% or
more sequence identity with the sequence of a framework
region outside each CDR sequence in the sequence of (h1);
and

(h3) an amino acid sequence in which one or
several amino acids are deleted, substituted, or added in
the sequence of a framework region outside each CDR
sequence in the sequence of (h1) or (h2).

[Claim 37]

The antibody or the antigen-binding fragment thereof
according to claim 36, which comprises:

the heavy chain variable region consisting of amino
acid residues at positions 20 to 138 in the amino acid
sequence represented by SEQ ID NO: 139; and

the light chain variable region consisting of amino acid residues at positions 21 to 128 in the amino acid sequence represented by SEQ ID NO: 141.

[Claim 38]

The antibody or the antigen-binding fragment thereof according to claim 36, which comprises:

the heavy chain consisting of amino acid residues at positions 20 to 464 in the amino acid sequence represented by SEQ ID NO: 139; and

the light chain consisting of amino acid residues at positions 21 to 234 in the amino acid sequence represented by SEQ ID NO: 141.

[Claim 39]

The antibody against human CD147 or the antigen-binding fragment thereof according to any one of claims 23 to 38, which has decreased ADCC activity or lacks ADCC activity.

[Claim 40]

The antibody against human CD147 or the antigen-binding fragment thereof according to any one of claims 23 to 39, which has decreased CDC activity or lacks CDC activity.

[Claim 41]

The antibody against human CD147 or the antigen-binding fragment thereof according to any one of claims 23 to 40, which has decreased ADCP activity or lacks ADCP activity.

[Claim 42]

A pharmaceutical composition comprising at least one of the antibodies or antigen-binding fragments thereof according to any one of claims 1 to 41.

[Claim 43]

The pharmaceutical composition according to claim 42, which is an antitumor agent.

[Claim 44]

The pharmaceutical composition according to claim 43, wherein the tumor is a tumor expressing CD147.

[Claim 45]

The pharmaceutical composition according to claim 43 or 44, wherein the tumor is pancreatic cancer, liver cancer, gastric cancer, colon cancer, renal cancer, breast cancer, uterine cancer, ovarian cancer, lung cancer, lymphoma, thyroid cancer, skin cancer, head and neck cancer, sarcoma, prostate cancer, bladder cancer, brain tumor, gastrointestinal stromal tumor (GIST), leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), malignant lymphoma, B-cell lymphoma, non-Hodgkin's lymphoma or diffuse large B-cell lymphoma (DLBCL).

[Claim 46]

The pharmaceutical composition according to any one of claims 43 to 45, wherein the tumor is pancreatic cancer, liver cancer, gastric cancer, colon cancer, renal

cancer, leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), malignant lymphoma, B-cell lymphoma, non-Hodgkin's lymphoma or diffuse large B-cell lymphoma (DLBCL).

[Claim 47]

The pharmaceutical composition according to any one of claims 43 to 46, wherein the tumor is a SMAD4-positive tumor or a tumor having a decreased expression level of or lacking the expression of KLF5.

[Claim 48]

The pharmaceutical composition according to any one of claims 42 to 47, further comprising another antitumor agent.

[Claim 49]

A method for treating a tumor, comprising administering the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 41 or the pharmaceutical composition according to any one of claims 42 to 48 to a patient.

[Claim 50]

The method according to claim 49, wherein the tumor is a tumor expressing CD147.

[Claim 51]

The method according to claim 49 or 50, wherein the tumor is pancreatic cancer, liver cancer, gastric cancer, colon cancer, renal cancer, breast cancer, uterine cancer,

ovarian cancer, lung cancer, lymphoma, thyroid cancer, skin cancer, head and neck cancer, sarcoma, prostate cancer, bladder cancer, brain tumor, gastrointestinal stromal tumor (GIST), leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), malignant lymphoma, B-cell lymphoma, non-Hodgkin's lymphoma or diffuse large B-cell lymphoma (DLBCL).

[Claim 52]

The method according to any one of claims 49 to 51, wherein the tumor is pancreatic cancer, liver cancer, gastric cancer, colon cancer, renal cancer, leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), malignant lymphoma, B-cell lymphoma, non-Hodgkin's lymphoma or diffuse large B-cell lymphoma (DLBCL).

[Claim 53]

The method according to any one of claims 49 to 52, wherein the tumor is a SMAD4-positive tumor or a tumor having a decreased expression level of or lacking the expression of KLF5.

[Claim 54]

The method according to any one of claims 49 to 53, wherein the antibody or the antigen-binding fragment thereof, or the pharmaceutical composition is administered in combination with another antitumor agent.

[Claim 55]

A polynucleotide which encodes the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 41.

[Claim 56]

The polynucleotide according to claim 55, which comprises a polynucleotide selected from the group consisting of the following (j1) to (j3):

(j1) a polynucleotide encoding CDRH1 consisting of an amino acid sequence represented by SEQ ID NO: 75, CDRH2 consisting of an amino acid sequence represented by SEQ ID NO: 76 and CDRH3 consisting of an amino acid sequence represented by SEQ ID NO: 77, and a polynucleotide encoding CDRL1 consisting of an amino acid sequence represented by SEQ ID NO: 72, CDRL2 consisting of an amino acid sequence represented by SEQ ID NO: 73 and CDRL3 consisting of an amino acid sequence represented by SEQ ID NO: 74;

(j2) a polynucleotide having at least 95% sequence identity with the nucleotide sequence according to (j1); and

(j3) a polynucleotide in which one to several nucleotides are substituted, deleted or added in the polynucleotide according to (j1) or (j2).

[Claim 57]

The polynucleotide according to claim 55, which comprises a polynucleotide selected from the group consisting of the following (i1) to (i3):

(i1) a polynucleotide encoding CDRH1 consisting of an amino acid sequence represented by SEQ ID NO: 55, CDRH2 consisting of an amino acid sequence represented by SEQ ID NO: 56 and CDRH3 consisting of an amino acid sequence represented by SEQ ID NO: 57, and a polynucleotide encoding CDRL1 consisting of an amino acid sequence represented by SEQ ID NO: 52, CDRL2 consisting of an amino acid sequence represented by SEQ ID NO: 53 and CDRL3 consisting of an amino acid sequence represented by SEQ ID NO: 54;

(i2) a polynucleotide having at least 95% sequence identity with the nucleotide sequence according to (i1); and

(i3) a polynucleotide in which one to several nucleotides are substituted, deleted or added in the polynucleotide according to (i1) or (i2).

[Claim 58]

The polynucleotide according to claim 55, which comprises a polynucleotide selected from the group consisting of the following (k1) to (k3):

(k1) a polynucleotide encoding CDRH1 consisting of an amino acid sequence represented by SEQ ID NO: 65, CDRH2 consisting of an amino acid sequence represented by SEQ ID NO: 66 and CDRH3 consisting of an amino acid

sequence represented by SEQ ID NO: 67, and a polynucleotide encoding CDRL1 consisting of an amino acid sequence represented by SEQ ID NO: 62, CDRL2 consisting of an amino acid sequence represented by SEQ ID NO: 63 and CDRL3 consisting of an amino acid sequence represented by SEQ ID NO: 64;

(k2) a polynucleotide having at least 95% sequence identity with the nucleotide sequence according to (k1); and

(k3) a polynucleotide in which one to several nucleotides are substituted, deleted or added in the polynucleotide according to (k1) or (k2).

[Claim 59]

The polynucleotide according to claim 53, which comprises a polynucleotide selected from the group consisting of the following (m1) to (m3):

(m1) a polynucleotide encoding CDRH1 consisting of an amino acid sequence represented by SEQ ID NO: 85, CDRH2 consisting of an amino acid sequence represented by SEQ ID NO: 86 and CDRH3 consisting of an amino acid sequence represented by SEQ ID NO: 87, and a polynucleotide encoding CDRL1 consisting of an amino acid sequence represented by SEQ ID NO: 82, CDRL2 consisting of an amino acid sequence represented by SEQ ID NO: 83 and CDRL3 consisting of an amino acid sequence represented by SEQ ID NO: 84;

(m2) a polynucleotide having at least 95% sequence identity with the nucleotide sequence according to (m1); and

(m3) a polynucleotide in which one to several nucleotides are substituted, deleted or added in the polynucleotide according to (m1) or (m2).

[Claim 60]

An expression vector comprising the polynucleotide according to any one of claims 55 to 59.

[Claim 61]

A host cell transformed with the expression vector according to claim 60.

[Claim 62]

A method for producing the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 41, comprising a step of culturing the host cell according to claim 61 and collecting a target antibody or an antigen-binding fragment thereof from a culture supernatant.

[Claim 63]

The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 41, wherein the activation of signal transduction through CD147 is an activation of p38MAPK and/or an activation of SMAD4.

[Claim 64]

The antibody or the antigen-binding fragment thereof according to claim 63, wherein the activation of p38MAPK

and/or the activation of SMAD4 is an increase in p38MAPK expression level, the phosphorylation of p38MAPK, the phosphorylation of HSP27, an increase in CXCL8 expression level, an increase in rhoB expression level, a decrease in KLF5 mRNA or a decrease in KLF5 protein expression level.

[Claim 65]

A method for treating a tumor, comprising administering the antibody or the antigen-binding fragment thereof according to claim 63 or 64.

[Claim 66]

A method for predicting responsiveness to cancer treatment, comprising:

detecting the expression of SMAD4 or the expression of KLF5 in a biological sample derived from a cancer patient; and

determining a patient's responsiveness to cancer treatment with the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 41 or the pharmaceutical composition according to any one of claims 42 to 48 to be positive, when SMAD4 expression is detected in the patient, or a decrease in KLF5 expression level or a lack of KLF5 expression is detected in the patient.

[Claim 67]

A method for selecting subjects for cancer treatment, comprising:

detecting the expression of SMAD4 or the expression of KLF5 in a biological sample derived from a cancer patient; and

selecting a patient as a subject of cancer treatment with the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 41 or the pharmaceutical composition according to any one of claims 42 to 48, when SMAD4 expression is detected in the patient or a decrease in KLF5 expression level or a lack of KLF5 expression is detected in the patient.

[Claim 68]

A method for treating cancer, comprising:

using a biological sample derived from a cancer patient to detect the presence or the absence of the expression of SMAD4 or to detect the expression of KLF5 in the biological sample; and

administering the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 41 or the pharmaceutical composition according to any one of claims 42 to 48 to a patient in whom SMAD4 expression is detected or to a patient in whom a decrease in KLF5 expression level or a lack of KLF5 expression is detected.

[Claim 69]

A kit for determining responsiveness to cancer treatment with the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 41 or the pharmaceutical composition according to any one of

claims 42 to 48, the kit comprising at least one means for detecting the expression of SMAD4 or the expression of KLF5 in a biological sample derived from a cancer patient.

[Claim 70]

An antibody-drug complex comprising the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 41 conjugated to another drug.

[Claim 71]

A bispecific antibody comprising: the antigen-binding fragment of the antibody according to any one of claims 1 to 41; and an antigen-binding fragment which binds to an antigen other than CD147.