DESCRIPTION

ANTI-AXL ANTIBODY

Technical Field

The present invention relates to anti-anexelekto (AXL) antibodies, and anticancer agents containing the antibodies as an active ingredient.

Background Art

Anexelekto (also referred to as "AXL", "UFO", "ARK", or "TYRO7"; hereinafter referred to as "AXL") is a receptor tyrosine kinase that exists on the cell membrane (Non-patent Document 1). It is said to be responsible for signal transduction to downstream molecules through its autophosphorylation, which occurs after it binds to the ligand Gas6 (growth arrest specific gene 6) (Non-patent Document 2).

AXL is presumed to have molecular functions involved in cell growth enhancement, suppression of apoptosis, cell migration, and cell adhesion. Experimentally observed phenomena in cells treated with Gas6 protein support this presumption. Reported experimental results include suppression of cell death and enhancement of cell growth in rat vascular smooth muscle (Non-patent Documents 3 and 4), acceleration of cell growth and the suppression of cell death after serum starvation in mouse NIH3T3 cells (Non-patent Documents 5 and 6), promotion of cell growth in mouse cardiac fibroblasts (Non-patent Document 7), enhancement of cell growth in human prostate cancer cells (Non-patent Document 8), enhancement of cell growth and infiltration and suppression of cell death in human gastric carcinoma cells (Non-patent Document 9), enhancement of the migration ability of human and rat vascular smooth muscle cells (Non-patent Document 10), enhancement of the cell migration ability of mouse neurons (Non-patent Document 11), and aggregation of cells highly expressing mouse AXL (Non-patent Document 12).

Similarly, PI3K–Akt pathway and MAPK pathway are said to function as downstream pathways of the signal transduction mediated by AXL based on molecular analyses of intracellular signals after treatment with Gas6 (Non-patent Document 2). An analysis with a yeast two-hybrid method using an AXL intracellular region as bait confirmed direct molecular interaction with these downstream pathways. As a result, GrbB2/PI3K/p55γ/SOCS-1/NcK2/RanBP2/C1-TEN were identified (Non-patent Document 13). The interactions of these molecules suggest the presence of intracellular signal transduction pathways as downstream from the AXL signal. Furthermore, the observed interactions support

the presumption that AXL functions in cell growth enhancement, suppression of apoptosis, cell migration, and cell adhesion. AXL has also been identified as a gene highly expressed when TNFα-induced cell death of mouse fibroblasts is suppressed by IL-15. The suppression of TNFα-induced cell death was abolished by suppressing AXL expression, and the phosphorylation of IL-15 receptors and AXL was enhanced by treatment with IL-15. These experimental findings also suggest that signal transduction is mediated by the complex of AXL and IL-15 receptors (Non-patent Document 14).

Tumorigenicity of nude mice has been reported to disappear as a result of inhibiting Gas6-dependent phosphorylation of AXL in human glioma lines overexpressing the AXL dominant negative form (Non-patent Document 15). However, there were no reports or suggestions of anti-AXL antibody that inhibits phosphorylation and its existence remained unclear.

AXL is a single-pass transmembrane receptor tyrosine kinase, and the extracellular region is composed of two immunoglobulin-like domains (referred to as IgD1 and IgD2) and two fibronectin type III domains (referred to as FND1 and FND2) from the N-terminal side (Non-patent Document 1). Although FND is known to be expressed in molecules such as neural cell adhesion molecules and nephrins involved in intercellular adhesion, detailed functions of FND in AXL are unclear (Non-patent Document 16).

AXL has been identified as an oncogene that retains an inherent ability to transform cells, and has been studied as a carcinogenesis-related molecule. Many analyses of AXL expression have been reported on the protein and mRNA. The high expression of AXL protein has been reported in human cancer tissues and cancer cells, including lung cancer (Non-patent Document 17), breast cancer (Non-patent Document 18), ovarian cancer (Non-patent Document 19), thyroid cancer (Non-patent Document 20), melanoma (Non-patent Document 20), renal cancer (Non-patent Document 21), gastric cancer (Non-patent Document 9), and glioma (Non-patent Document 22). Furthermore, the high expression of AXL protein is suggested by the high level of AXL mRNA in esophageal cancer (Non-patent Document 23), colon cancer (Non-patent Document 24), and acute myeloid leukemia (Non-patent Document 25). There are also reports of inhibition of lumen formation *via* suppression of AXL by RNAi in HUVEC (Non-patent Document 26), reduced tumor-forming ability of human breast cancer cells in mice resulting from constitutive suppression of AXL (Non-patent Document 26), and reduced tumor-forming ability of human glioma cells in mice resulting from a constitutive high expression of dominant negative mutants (Non-patent Document 22). The involvement of AXL molecular functions in tumor growth is strongly suggested.

Polyclonal antibodies to the extracellular domain of AXL have been reported to have a migration inhibitory action on highly invasive breast cancer cell lines (Patent Document 1).

However, non-clinical studies showed that drugs demonstrating cancer-cell-migration-inhibitory action do not necessarily demonstrate antitumor activity. For example, matrix metalloproteinase (hereinafter abbreviated to "MMP") has been known to promote tumor infiltration and migration. Thus, as candidates of anticancer agents, attention has been focused on various matrix metalloproteinase inhibitors that inhibit the enzyme activity of MMP, and clinical studies have been conducted on various pharmaceutical agents such as Batimastat, Marimastat, and Prinomastat. However, antitumor effects have not been observed in clinical trials (Non-patent Document 27).

Accordingly, there have been no reports or suggestions and it remains unknown whether antibodies that bind to a specific region of AXL have antitumor effects particularly *in vivo*, whether they can reduce AXL expression levels, and whether they can suppress cancer.

Prior Art References

Patent Documents

Patent Document 1: WO 2004/008147
Patent Document 2: WO 2007/114319
Patent Document 3: WO 2009/041643

Non-patent Documents

Non-patent Document 1: O'Bryan *et al.*, Mol Cell Biol 1991; 11: 5016-5031
Non-patent Document 2: Varnum *et al.*, Nature 1995; 373: 623-626
Non-patent Document 3: Nakano *et al.*, FEBS Lett 1996; 387: 78-80
Non-patent Document 4: Nakano *et al.*, J Biol Chem 1995; 270: 5702-5705
Non-patent Document 5: Goruppi *et al.*, Mol Cell Biol 1997; 17: 4442-4453
Non-patent Document 6: Bellosta *et al.*, Oncogene 1997; 15: 2387-2397
Non-patent Document 7: Stenhoff *et al.*, Biochem Biophys Res Commun 2004; 319: 871-878
Non-patent Document 8: Sainaghi *et al.*, J Cell Physiol 2005; 204: 36-44
Non-patent Document 9: Sawabu *et al.*, Mol Carcinog 2007; 46: 155-164
Non-patent Document 10: Fridell *et al.*, J Biol Chem 1998; 273: 7123-7126
Non-patent Document 11: Allen *et al.*, Mol Cell Biol 2002; 22: 599-613
Non-patent Document 12: McCloskey *et al.*, J Biol Chem 1997; 272: 23285-23291
Non-patent Document 13: Hafizi *et al.*, Biochem Biophys Res Commun 2002; 299: 793-800
Non-patent Document 14: Budagian *et al.*, Embo J 2005; 24: 4260-4270
Non-patent Document 15: Vajkoczy P *et al.*, Proc Natl Acad Sci USA 2006; 103: 5799-5804
Non-patent Document 16: Yamagata *et al.*, Curr. Opin. Cell Biol 2003; 15: 621-632
Non-patent Document 17: Shieh *et al.*, Neoplasia 2005; 7: 1058-1064
Non-patent Document 18: Meric *et al.*, Clin Cancer Res 2002; 8: 361-367
Non-patent Document 19: Sun *et al.*, Oncology 2004; 66: 450-457

Non-patent Document 20: Ito *et al.*, Thyroid 2002; 12: 971-975
Non-patent Document 21: Chung *et al.*, DNA Cell Biol 2003; 22: 533-540
Non-patent Document 22: Vajkoczy *et al.*, Proc Natl Acad Sci USA 2006; 103: 5799-5804
Non-patent Document 23: Nemoto *et al.*, Pathobiology 1997; 65: 195-203
Non-patent Document 24: Craven *et al.*, Int J Cancer 1995; 60: 791-797
Non-patent Document 25: Neubauer *et al.*, Blood 1994; 84: 1931-1941
Non-patent Document 26: Holland *et al.*, Cancer Res 2005; 65: 9294-9303
Non-patent Document 27: Pavlaki *et al.*, Cancer Metastasis Rev. 2003; 22: 177-203
Non-patent Document 28: Vaisitti *et al.*, J Biol Regul Homeost Agents. 2005; 19: 105-12
Non-patent Document 29: Pardridge *et al.*, J Pharmacol Exp Ther. 1998; 286: 548-54

Summary of the Invention

[Problems to be Solved by the Invention]

An objective of the present invention is to provide anti-anexelekto (AXL) antibodies, and anticancer agents comprising the antibodies as an active ingredient. More specifically, an objective of the present invention is to decrease immunogenicity in humans by humanizing the mouse-derived anti-AXL antibodies.

[Means for Solving the Problems]

The present inventors discovered that certain types of antibodies that bind to AXL have actions of decreasing AXL expression level *in vitro*, and antitumor activity *in vivo*. Furthermore, antibodies that bind to FND1 domain of AXL were found to have stronger antitumor activity than antibodies that bind to other AXL domains.

Furthermore, the present inventors succeeded in obtaining humanized anti-AXL antibodies by humanizing the obtained anti-AXL antibodies described above.

These humanized antibodies are expected to have decreased immunogenicity in humans than mouse-derived anti-AXL antibodies.

More specifically, the present invention relates to the following:

[1] an antibody that recognizes FND1 domain of AXL, which is an antibody of any one of (1) to (6) below:

(1) an antibody comprising a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 33 to 37, a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 38 to 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(2) an antibody comprising a heavy chain variable region of SEQ ID NO: 2 (H0);

(3) an antibody comprising a light chain variable region comprising a CDR1 comprising the

amino acid sequence of any one of SEQ ID NOs: 84 to 89, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 91;

(4) an antibody comprising a light chain variable region of SEQ ID NO: 65 (L0);

(5) an antibody comprising the heavy chain variable region of (1) and the light chain variable region of (3); and

(6) an antibody having one or more amino acid substitutions, deletions, additions, and/or insertions in the antibody of any one of (1) to (5), which has equivalent activity as the antibody of any one of (1) to (5);

[2] a humanized antibody that recognizes FND1 domain of AXL, which is an antibody of any one of (1) to (6) below:

(1) an antibody comprising a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 33 to 37, a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 38 to 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49, as well as an FR1 comprising the amino acid sequence of SEQ ID NO: 51, an FR2 comprising the amino acid sequence of SEQ ID NO: 53, an FR3 comprising the amino acid sequence of SEQ ID NO: 109 or 58, and an FR4 comprising the amino acid sequence of SEQ ID NO: 61;

(2) an antibody comprising the heavy chain variable region of SEQ ID NO: 2 (H0);

(3) an antibody comprising a light chain variable region comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 84 to 89, a CDR2 comprising the amino acid sequence SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 91, as well as an FR1 comprising the amino acid sequence of SEQ ID NO: 93, an FR2 comprising the amino acid sequence of SEQ ID NO: 96, an FR3 comprising the amino acid sequence of SEQ ID NO: 101, and an FR4 comprising the amino acid sequence of SEQ ID NO: 103;

(4) an antibody comprising the light chain variable region of SEQ ID NO: 65 (L0);

(5) an antibody comprising the heavy chain variable region of (1) and the light chain variable region of (3); and

(6) an antibody having one or more amino acid substitutions, deletions, additions, and/or insertions in the antibody of any one of (1) to (5), which has equivalent activity as the antibody of any one of (1) to (5);

[3] the antibody of [1] or [2], wherein the amino acid residue at position 94 by Kabat numbering in the heavy chain variable region is glycine;

[4] the antibody of any one of [1] to [3], wherein the amino acid sequence of the heavy chain variable region comprises at least one of the following amino acid residues:

(1) the amino acid residue at position 31 by Kabat numbering in the heavy chain variable region

is aspartic acid, glutamic acid, lysine, or arginine;
(2) the amino acid residue at position 40 by Kabat numbering in the heavy chain variable region is proline;
(3) the amino acid residue at position 41 by Kabat numbering in the heavy chain variable region is arginine;
(4) the amino acid residue at position 43 by Kabat numbering in the heavy chain variable region is glutamine or glutamic acid;
(5) the amino acid residue at position 44 by Kabat numbering in the heavy chain variable region is arginine;
(6) the amino acid residue at position 48 by Kabat numbering in the heavy chain variable region is isoleucine;
(7) the amino acid residue at position 61 by Kabat numbering in the heavy chain variable region is glutamic acid, lysine, or arginine;
(8) the amino acid residue at position 62 by Kabat numbering in the heavy chain variable region is glutamic acid;
(9) the amino acid residue at position 64 by Kabat numbering in the heavy chain variable region is glutamine;
(10) the amino acid residue at position 65 by Kabat numbering in the heavy chain variable region is aspartic acid;
(11) the amino acid residue at position 73 by Kabat numbering in the heavy chain variable region is asparagine; and
(12) the amino acid residue at position 105 by Kabat numbering in the heavy chain variable region is glutamic acid or arginine;
[5] the antibody of any one of [1] to [3], wherein the amino acid sequence of the heavy chain variable region comprises at least one of the following amino acid residues:
(1) the amino acid residue at position 41 by Kabat numbering in the heavy chain variable region is arginine;
(2) the amino acid residue at position 43 by Kabat numbering in the heavy chain variable region is glutamine;
(3) the amino acid residue at position 44 by Kabat numbering in the heavy chain variable region is arginine;
(4) the amino acid residue at position 61 by Kabat numbering in the heavy chain variable region is arginine; and
(5) the amino acid residue at position 73 by Kabat numbering in the heavy chain variable region is asparagine;
[6] the antibody of any one of [1] to [5], wherein the amino acid sequence of the light chain

variable region comprises at least one of the following amino acid residues:

(1) the amino acid residue at position 17 by Kabat numbering in the light chain variable region is arginine;

(2) the amino acid residue at position 24 by Kabat numbering in the light chain variable region is glutamine;

(3) the amino acid residue at position 27 by Kabat numbering in the light chain variable region is glutamic acid or arginine;

(4) the amino acid residue at position 29 by Kabat numbering in the light chain variable region is alanine;

(5) the amino acid residue at position 42 by Kabat numbering in the light chain variable region is glutamic acid or glutamine;

(6) the amino acid residue at position 45 by Kabat numbering in the light chain variable region is lysine;

(7) the amino acid residue at position 100 by Kabat numbering in the light chain variable region is arginine;

(8) the amino acid residue at position 104 by Kabat numbering in the light chain variable region is valine; and

(9) the amino acid residue at position 107 by Kabat numbering in the light chain variable region is glutamic acid;

[7] the antibody of any one of [1] to [5], wherein the amino acid sequence of the light chain variable region comprises at least one of the following amino acid residues:

(1) the amino acid residue at position 17 by Kabat numbering in the light chain variable region is arginine;

(2) the amino acid residue at position 24 by Kabat numbering in the light chain variable region is glutamine;

(3) the amino acid residue at position 27 by Kabat numbering in the light chain variable region is arginine;

(4) the amino acid residue at position 29 by Kabat numbering in the light chain variable region is alanine;

(5) the amino acid residue at position 45 by Kabat numbering in the light chain variable region is lysine;

(6) the amino acid residue at position 100 by Kabat numbering in the light chain variable region is arginine;

(7) the amino acid residue at position 104 by Kabat numbering in the light chain variable region is valine; and

(8) the amino acid residue at position 107 by Kabat numbering in the light chain variable region

is glutamic acid;

[8] the antibody of any one of [1] to [5], comprising at least any one of the following heavy chain variable regions:

(1) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(2) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 34, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(3) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 35, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(4) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 39, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(5) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 40, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(6) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(7) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(8) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 43, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(9) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 44, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(10) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(11) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 46, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(12) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 36, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(13) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(14) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49; and

(15) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

[9] the antibody of any one of [1] to [7], which is selected from the group consisting of (1) to (25) below:

(1) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(2) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 85, 90, and 91, respectively;

(3) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 86, 90, and 91, respectively;

(4) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 87, 90, and 91, respectively;

(5) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 88, 90, and 91, respectively;

(6) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 34, 38, and 49, respectively, and a light

chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(7) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 35, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(8) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 39, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(9) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 40, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(10) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3comprising the amino acid sequences of SEQ ID NOs: 33, 41, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(11) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 42, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(12) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 43, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(13) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 44, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(14) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 44, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 85, 90, and 91, respectively;

(15) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 45, and 49, respectively, and a light

chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(16) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 46, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(17) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 44, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 89, 90, and 91, respectively;

(18) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 36, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(19) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 37, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(20) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 47, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(21) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 48, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(22) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 48, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 85, 90, and 91, respectively;

(23) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 48, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 87, 90, and 91, respectively;

(24) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 47, and 49, respectively, and a light

chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 85, 90, and 91, respectively; and

(25) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 47, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 87, 90, and 91, respectively;

[10] the antibody of [1] or [2] comprising the heavy chain variable region of any one of SEQ ID NOs: 2 to 32, and the light chain variable region of any one of SEQ ID NOs: 65 to 83;

[11] a chimeric antibody comprising the heavy chain variable region of SEQ ID NO: 1, the light chain variable region of SEQ ID NO: 64, and human antibody-derived constant regions, or the antibody of [1] having one or more amino acid substitutions, deletions, additions, and/or insertions in the chimeric antibody, which has equivalent activity as the chimeric antibody;

[12] the antibody of any one of [1] to [11], wherein the amino acid residue at position 42 by Kabat numbering in the amino acid sequence of the light chain variable region is lysine;

[13] a pharmaceutical composition comprising the antibody of any one of [1] to [12] as an active ingredient;

[14] an anticancer agent comprising the antibody of any one of [1] to [12] as an active ingredient;

[15] the anticancer agent of [14] wherein the cancer is pancreatic cancer, gastric cancer, lung cancer, osteosarcoma, colon cancer, prostate cancer, melanoma, endometrial cancer, ovarian cancer, uterine leiomyoma, thyroid cancer, cancer stem cell, breast cancer, bladder cancer, renal cancer, glioma, neuroblastoma, or esophageal cancer;

[16] the anticancer agent of [14], wherein the cancer is glioma, gastric cancer, endometrial cancer, non-small-cell lung cancer, pancreatic adenocarcinoma, or breast cancer; and

[17] the anticancer agent of [14], wherein the cancer is pancreatic adenocarcinoma or breast cancer.

The present invention further provides the following:

[18] a method of treating cancer, comprising a step of administering the antibody of any one of [1] to [12] to a subject (for example, a mammal such as a human); and

[19] use of the antibody of any one of [1] to [12] in the manufacture of an anticancer agent.

The hybridoma (Accession No. FERM BP-10854) selected by the present inventors was deposited at the International Patent Organism Depositary of the National Institute of Advanced Industrial Science and Technology. The following section provides a description of the contents, specifying the deposition.

(a) Name and Address of the Depositary Institution

Name: International Patent Organism Depositary, National Institute of Advanced

Industrial Science and Technology

Address: Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan 305-8566

(b) Acceptance Date (Deposition Date): July 5, 2007

(c) Accession No.

AXL No. 225 #070402 (Ax225) (Accession No. FERM BP-10854)

The amino acid sequences of the heavy chain variable region and the light chain variable region of the antibody produced by the hybridoma are shown in SEQ ID NO: 1 and SEQ ID NO: 64, respectively. The amino acid sequences of the heavy chain variable region CDR1, CDR2, CDR3, FR1, FR2, FR3, and FR4 are shown in SEQ ID NO: 33, SEQ ID NO: 38, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 57, and SEQ ID NO: 60, respectively. The amino acid sequences of the light chain variable region CDR1, CDR2, CDR3, FR1, FR2, FR3, and FR4 are shown in SEQ ID NO: 84, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 95, SEQ ID NO: 100, and SEQ ID NO: 102, respectively.

Preferred antibody heavy chain variable regions and light chain variable regions of the present invention as well as the amino acid sequences of their CDR1, CDR2, CDR3, FR1, FR2, FR3, and FR4, and their corresponding SEQ ID NOs are shown in Table 1 and Table 2.

[Table 1]

HEAVY CHAIN	FULL LENGTH OF VARIABLE REGION	CDR1	CDR2	CDR3	FR1	FR2	FR3	FR4
chH	1	33	38	49	50	52	57	60
H0	2	33	38	49	51	53	58	61
H9	3	33	38	49	51	53	59	61
H17	4	34	38	49	51	53	59	61
H18	5	35	38	49	51	53	59	61
H19	6	33	38	49	51	54	59	61
H20	7	33	38	49	51	55	59	61
H21	8	33	39	49	51	53	59	61
H22	9	33	40	49	51	53	59	61
H23	10	33	41	49	51	53	59	61
H24	11	33	42	49	51	53	59	61
H25	12	33	43	49	51	53	59	61
H26	13	33	44	49	51	53	59	61
H26	13	33	44	49	51	53	59	61
H26	13	33	44	49	51	53	59	61
H26	13	33	44	49	51	53	59	61
H26	13	33	44	49	51	53	59	61
H27	14	33	45	49	51	53	59	61
H28	15	33	46	49	51	53	59	61
H30	16	33	38	49	51	53	59	62
H31	17	33	44	49	51	54	59	61
H32	18	33	44	49	51	53	59	62
H33	19	33	44	49	51	54	59	62
H34	20	36	38	49	51	53	59	61
H35	21	37	38	49	51	53	59	61
H36	22	33	38	49	51	56	59	61
H37	23	33	47	49	51	53	59	61
H38	24	33	48	49	51	53	59	61
H39	25	33	38	49	51	53	59	63
H40	26	36	38	49	51	56	59	61
H41	27	37	38	49	51	56	59	61
H46	28	33	48	49	51	56	59	61
H47	29	33	47	49	51	56	59	61
H48	30	33	38	49	51	56	59	63
H49	31	33	48	49	51	56	59	63
H50	32	33	47	49	51	56	59	63

[Table 2]

LIGHT CHAIN	FULL LENGTH OF VARIABLE REGION	CDR1	CDR2	CDR3	FR1	FR2	FR3	FR4
chL	64	84	90	91	92	95	100	102
L0	65	84	90	91	93	96	101	103
L1	66	85	90	91	93	96	101	103
L3	67	86	90	91	93	96	101	103
L4	68	84	90	91	93	97	101	103
L5	69	84	90	91	93	98	101	103
L10	70	84	90	91	93	96	101	104
L11	71	85	90	91	93	96	101	104
L12	72	85	90	91	93	97	101	104
L13	73	85	90	91	93	98	101	104
L21	83	89	90	91	93	96	101	104
L25	74	84	90	91	94	96	101	103
L27	75	87	90	91	93	96	101	103
L28	76	84	90	91	93	99	101	103
L29	77	84	90	91	93	96	101	105
L31	78	87	90	91	93	99	101	103
L34	79	87	90	91	94	99	101	103
L35	80	87	90	91	93	99	101	105
L36	81	88	90	91	93	96	101	103
L37	82	87	90	91	94	99	101	105

An antibody having one or more amino acid substitutions, deletions, additions, and/or insertions in the amino acid sequence of any of the antibodies mentioned above, and having equivalent activity to that antibody is also within the scope of the present invention.

In the present invention "an antibody having equivalent activity" means, for example, "an antibody having at least equivalent binding activity or *in vivo* activity". Specifically, "an antibody having one or more amino acid substitutions, deletions, additions, and/or insertions in the amino acid sequence of H0 and/or L0, and having antigen binding activity or *in vivo* activity that is at least equivalent compared to the H0/L0 antibody" is also within the scope of the present invention. Herein, *in vivo* activity includes antitumor activity and such in *in vivo* examinations. Specifically, the examinations include mouse xenograft model examinations employed in Example 6 of the present application.

Preferably, antibodies of the present invention are humanized antibodies that bind to the FND1 domain of AXL. That is, in another perspective, the present invention provides

humanized antibodies that bind to AXL.

Furthermore, in another perspective, the present invention provides pharmaceutical compositions comprising any of the above-mentioned antibodies of the present invention. Preferably, the pharmaceutical compositions are anticancer agents.

Moreover, in another perspective, the present invention provides agents that decrease the level of AXL expression and diagnostic agents comprising any of the above-mentioned antibodies of the present invention.

[Effects of the Invention]

In comparison to mouse-derived anti-AXL antibodies, these humanized antibodies are expected to have decreased immunogenicity in humans.

Brief Description of the Drawings

Fig. 1 shows the antitumor activities and binding domains of anti-AXL antibodies. -: TGI (%) < 30, +: TGI (%) < 60, ++: 60 =< TGI (%).

Fig. 2 shows photographs depicting the results of an experiment that evaluates the activity of Ax225 antibody to induce downmodulation of AXL in cancer cells. This antibody was shown to induce downmodulation of AXL protein.

Fig.3 depicts a graph showing the antitumor effect of anti-AXL antibody on human pancreatic adenocarcinoma-xenografted mouse model.

Fig. 4 depicts a graph showing the expression levels of humanized Ax225 antibodies.

Fig. 5 depicts a graph showing the expression levels of humanized Ax225 antibodies.

Fig. 6 depicts a graph showing the tumor growth inhibiting effects of the humanized Ax225 antibody (H9/L0). The asterisk (*) indicates that $p < 0.05$.

Fig. 7 depicts a graph showing the antitumor effects of humanized anti-AXL antibodies (H47/L0, H47/L11, H50/L0, and H50/L11) on human pancreatic adenocarcinoma-xenografted mouse model. The asterisk (*) indicates that $p < 0.05$.

Mode for Carrying Out the Invention

Humanized Antibodies

An example of preferred embodiments of the antibodies of the present invention is a humanized antibody that binds to AXL. Humanized antibodies can be produced using known methods. Humanized antibodies are also called reshaped human antibodies.

In the present invention, a humanized antibody is composed of complementarity determining regions (CDRs) of a non-human animal-derived antibody, human antibody-derived framework regions (FRs), and human antibody-derived constant regions. Preferably, the

non-human animal-derived antibody is Ax225 antibody (Application No. PCT/JP2008/070739) produced by the hybridoma deposited under Accession No. FERM BP-10854 (National Institute of Advanced Industrial Science and Technology, International Patent Organism Depository, Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan, acceptance date (deposition date): July 5, 2007). The method for obtaining Ax225 antibody is described in Referential Example 1. Binding of Ax225 antibody to the FND1 domain of the AXL is described in Referential Example 2. Referential Example 3 describes that Ax225 antibody has the activity of downmodulation of AXL.

Common genetic engineering techniques for producing humanized antibodies are also known (see EP Patent Application Publication No. EP 125023 and WO 96/02576). A humanized antibody is obtained by linking the obtained DNA to a DNA encoding a human antibody constant region or a modified human antibody constant region, then incorporating this into an expression vector, and transfecting the vector into a host to produce antibodies (see EP Patent Application Publication No. EP 239400 and WO 96/02576).

The humanized Ax225 antibody, a conjugate of the CDR regions of Ax225 antibody and the framework regions (FRs) of a human antibody, can be produced as follows. First, respective variable region sequences of the heavy chain (H chain) and the light chain (L chain) of humanized Ax225 antibody are designed, and several synthetic oligo-DNA fragments encoding these regions are designed. These oligo-DNA fragments are fused by assembly PCR to produce a gene encoding the full length of the variable regions (see the method described in WO 98/13388).

For the human antibody framework regions which will be fused with CDRs, those that will allow the CDRs to form favorable antigen binding sites will be selected. When necessary, amino acid substitutions, deletions, additions, and/or insertions can be carried out on the framework regions in the antibody variable regions.

Furthermore, the above-mentioned CDR sequences may have one or more amino acid substitutions, deletions, additions, and/or insertions. A CDR sequence that has undergone one or more amino acid substitutions, deletions, additions, and/or insertions preferably has equivalent or better properties as compared to the CDR sequence before modification in terms of binding activity, neutralizing activity, stability, immunogenicity, and/or pharmacokinetics. The number of amino acids that are substituted, deleted, added, and/or inserted is not particularly limited, but is preferably three amino acids or less per CDR, more preferably two amino acids or less, and more preferably one amino acid.

Amino acid substitutions, deletions, additions, and/or insertions can be carried out by the above-described methods.

A constant region used in the antibodies of the present invention is not particularly

limited, and any constant region may be used. Preferred examples of the constant region to be used in the antibodies of the present invention include human antibody-derived constant regions (for example, Cγ1, Cγ2, Cγ3, Cγ4, Cμ, Cδ, Cα1, Cα2, and Cε for the H chain, and Cκ, Cλ, and such for the L chain). Particularly preferred examples of natural human antibody constant regions include constant regions derived from IgG1, IgG2, or IgG4.

When the antibody constant region of the present invention originates from IgG1, the antibody antitumor effect of the present invention which will be mentioned later is expected to be enhanced by its ADCC and CDC.

When the antibody constant region of the present invention originates from IgG4, the side effects of the antibody of the present invention are expected to be reduced.

Furthermore, a human antibody-derived constant region may have one or more amino acid substitutions, deletions, additions, and/or insertions for the purpose of decreasing heterogeneity, enhancing ADCC, prolonging half-life in plasma, and such. Herein, one or more amino acids are for example, 30 amino acids or less, preferably 15 amino acids or less, more preferably 10 amino acids or less, and particularly preferably two amino acids or less.

The antibodies of the present invention include not only bivalent antibodies represented by IgG, but also monovalent antibodies, or polyvalent antibodies represented by IgM, as long as they have binding activity and/or neutralizing activity against the AXL (preferably the FND1 domain). The polyvalent antibodies of the present invention include polyvalent antibodies having antigen binding sites which are all the same, and polyvalent antibodies having antigen binding sites which are partly or all different. The antibodies of the present invention are not limited to whole antibody molecules, and may be minibodies (low-molecular-weight antibodies) or their variants as long as they bind to the AXL.

The minibodies are antibodies that include an antibody fragment wherein a part of a whole antibody (for example, whole IgG) is missing, and they are not particularly limited as long as they have binding activity and/or neutralizing activity against AXL. The minibodies of the present invention are not particularly limited so long as they include a part of a whole antibody, but preferably include either VH or VL, and particularly preferably include both VH and VL. Another preferred example of the minibodies of the present invention includes minibodies comprising antibody CDRs. The CDRs included in the minibodies may be all six CDRs of the antibody or some of the CDRs.

The minibodies of the present invention preferably have a smaller molecular weight compared to the whole antibody; however, they may form multimers such as a dimer, trimer, or tetramer, and their molecular weight may become larger than the whole antibody.

Specific examples of antibody fragments include, for example, Fab, Fab', F(ab')2, and Fv. Specific examples of minibodies (low molecular weight antibodies) include Fab, Fab',

F(ab')2, Fv, scFv (single-chain Fv), diabody, sc(Fv)2 (single-chain (Fv)2), etc. Polymers (such as dimers, trimers, tetramers, or polymers) of these antibodies are also included in the minibodies of the present invention.

Antibody fragments can be obtained by producing an antibody fragment by treating the antibody with an enzyme. Known examples of enzymes used to produce antibody fragments include papain, pepsin, plasmin, etc. Alternatively, genes encoding these antibody fragments can be constructed, introduced into an expression vector, and then expressed in suitable host cells (see, for example, Co, M.S. *et al.*, J. Immunol. (1994) 152, 2968-2976; Better, M. and Horwitz, A.H., Methods in Enzymology (1989) 178, 476-496; Plueckthun, A. and Skerra, A., Methods in Enzymology (1989) 178, 476-496; Lamoyi, E., Methods in Enzymology (1989) 121, 652-663; Rousseaux, J. *et al.*, Methods in Enzymology (1989) 121, 663-669; and Bird, R.E. *et al.*, TIBTECH (1991) 9, 132-137).

Digestive enzymes cleave a specific position of an antibody fragment to yield an antibody fragment with a specific structure, as indicated below. An arbitrary portion of an antibody can be deleted by applying genetic engineering techniques to an antibody fragment enzymatically obtained in this manner.

Antibody fragments obtainable by using the digestive enzymes mentioned above are as follows:

Papain digestion: F(ab)2 or Fab

Pepsin digestion: F(ab')2 or Fab'

Plasmin digestion: Facb

As long as they have an AXL binding activity and/or neutralizing activity, minibodies of the present invention can include antibody fragments having a deletion of an arbitrary region.

"Diabody" refers to bivalent minibodies constructed by gene fusion (see Holliger, P. *et al.*, Proc. Natl. Acad. Sci. U. S. A. (1993) 90, 6444-6448; EP 404,097; WO 93/11161, etc.). Diabodies are dimers composed of two polypeptide chains. Normally, VL and VH within the same chain of the polypeptide chains that forms a dimer are both bound by linkers. The linkers of the polypeptides in a diabody are typically too short to allow the VL and VH to bind to each other. Specifically, the number of amino acid residues that constitute a linker is preferably 2 to 12 residues, more preferably 3 to 10 residues, and particularly about five residues. Thus, the VL and VH encoded in the same polypeptide chain cannot form a single-chain variable region fragment, but instead form a dimer with a different single-chain variable region fragment. As a result, a diabody has two antigen-binding sites.

An scFv is an antibody of a single-chain polypeptide obtained by linking VH and VL through a linker or such (Huston, J.S. *et al.*, Proc. Natl. Acad. Sci. U. S. A. (1988) 85, 5879-5883; and Plickthun, "The Pharmacology of Monoclonal Antibodies" Vol.113, Ed

Resenburg and Moore, Springer Verlag, New York, pp.269-315, (1994)). The H chain V region and L chain V region in an scFv may be derived from any antibody described herein. There is no particular limitation on the peptide linkers that link the V regions. For example, any arbitrary single-chain peptide comprising about three to 25 residues can be used as a linker. Specifically, for example, peptide linkers which are mentioned below may be used.

The V regions of both chains can be linked by, for example, the PCR method described above. To link the V regions using the PCR method, a DNA encoding the entire or desired partial amino acid sequence of the DNAs below are used as templates.

a DNA sequence encoding the H chain or the H chain V region of the antibody, and

a DNA sequence encoding the L chain or the L chain V region of the above antibody

Each DNA encoding the V regions of the H chain or L chain is amplified by the PCR method using pairs of primers with sequences corresponding to the sequences at both ends of the DNA to be amplified. Next, a DNA encoding the peptide linker portion is prepared. The DNA encoding the peptide linker can also be synthesized by PCR. Nucleotide sequences that can link the amplification products of each separately synthesized V region are added to the 5′ side of the primers used at this time. Next, a PCR reaction is carried out using the "H chain V region DNA", the "peptide linker DNA", and the "L chain V region DNA" together with the primers for the assembly PCR.

The primers for the assembly PCR consist of a combination of a primer that anneals to the 5′ side of the "H chain V region DNA" and a primer that anneals to the 3′ side of the "L chain V region DNA". Therefore, the primers for the assembly PCR consist of a primer set that can amplify the DNA encoding the entire sequence of the scFv to be synthesized. Conversely, nucleotide sequences that can link to each V region DNA are added to the "peptide linker DNA". As a result, these DNAs are linked together and the full length of scFv is finally produced as an amplification product of the primers used for the assembly PCR. Once a DNA encoding an scFv is prepared, an expression vector comprising the DNA and recombinant cells transformed with the expression vector can be acquired with ordinary methods. The scFv can also be acquired by expressing the DNA encoding the scFv in cultures of the resulting recombinant cells.

The order of VHs and VLs to be linked is not particularly limited, and they may be arranged in any order. Examples of arrangements are listed below:

[VH] linker [VL]

[VL] linker [VH]

An sc(Fv)2 is a minibody in which two VHs and two VLs are linked by a linker or such to form a single chain (Hudson, *et al.*, J. Immunol. Methods (1999) 231: 177-189). An sc(Fv)2 can be prepared, for example, by connecting scFvs with a linker.

The sc(Fv)2 of the present invention include antibodies preferably in which two VHs

and two VLs are arranged in the order of: VH, VL, VH, and VL ([VH] linker [VL] linker [VH] linker [VL]), beginning from the N terminus of a single-chain polypeptide; however the order of the two VHs and two VLs is not limited to the above arrangement, and they may be arranged in any order. Examples of arrangements are listed below:

[VL] linker [VH] linker [VH] linker [VL]
[VH] linker [VL] linker [VL] linker [VH]
[VH] linker [VH] linker [VL] linker [VL]
[VL] linker [VL] linker [VH] linker [VH]
[VL] linker [VH] linker [VL] linker [VH]

The amino acid sequence of the VH or VL in the minibodies may include substitutions, deletions, additions, and/or insertions. Additionally, a part may be deleted or another polypeptide may be added as long as there is antigen binding activity when VH and VL are associated. Moreover, the variable region may be chimerized or humanized.

Any arbitrary peptide linker that can be introduced by genetic engineering, a synthetic compound linker (for example, those disclosed in Protein Engineering, (1996) 9(3), 299-305) or such, can be used as the linker to link antibody variable regions in the present invention.

Peptide linkers are preferred in the present invention. There is no particular limitation on the length of the peptide linkers, and the length can be suitably selected by those skilled in the art according to the purpose of use. Normally, they are one to 100 amino acids, preferably from three to 50 amino acids, more preferably from five to 30 amino acids, and particularly preferably from 12 to 18 amino acids (for example, 15 amino acids).

For example, such peptide linkers include:

Ser
Gly Ser
Gly Gly Ser
Ser Gly Gly
Gly Gly Gly Ser (SEQ ID NO: 110)
Ser Gly Gly Gly (SEQ ID NO: 111)
Gly Gly Gly Gly Ser (SEQ ID NO: 112)
Ser Gly Gly Gly Gly (SEQ ID NO: 113)
Gly Gly Gly Gly Gly Ser (SEQ ID NO: 114)
Ser Gly Gly Gly Gly Gly (SEQ ID NO: 115)
Gly Gly Gly Gly Gly Gly Ser (SEQ ID NO: 116)
Ser Gly Gly Gly Gly Gly Gly (SEQ ID NO: 117)
(Gly Gly Gly Gly Ser (SEQ ID NO: 112))n
(Ser Gly Gly Gly Gly (SEQ ID NO: 113))n

wherein n is an integer of one or more.

The amino acid sequence of the peptide linker can be suitably selected by those skilled in the art according to the objective. For example, n which determines the length of the peptide linker is ordinarily one to five, preferably one to three, and more preferably one or two.

A synthetic compound linker (chemical cross-linking agent) is cross-linking agents ordinarily used to cross-link peptides and such. Examples of cross-linking agents that are available on the market include N-hydroxysuccinimide (NHS), disuccinimidylsuberate (DSS), bis(sulfosuccinimidyl)suberate (BS3), dithiobis(succinimidylpropionate) (DSP), dithiobis(sulfosuccinimidylpropionate) (DTSSP), ethyleneglycol bis(succinimidylsuccinate) (EGS), ethyleneglycol bis(sulfosuccinimidylsuccinate) (sulfo-EGS), disuccinimidyl tartrate (DST), disulfosuccinimidyl tartrate (sulfo-DST), bis[2-(succinimidooxycarbonyloxy)ethyl]sulfone (BSOCOES), and bis[2-(sulfosuccinimidooxycarbonyloxy)ethyl]sulfone (sulfo-BSOCOES).

Normally, three linkers are required when four antibody variable regions are linked. The multiple linkers used may be identical or different.

The antibodies of the present invention include antibodies with one or more amino acid residue additions in the amino acid sequence of the antibodies of the present invention. Furthermore, fusion proteins produced by fusing these antibodies with other peptides or proteins are also included. The fusion proteins can be produced by a method of fusing a polynucleotide encoding an antibody of the present invention and a polynucleotide encoding another peptide or polypeptide in-frame and inserting this into an expression vector, then expressing this in a host, and methods known to those skilled in the art may be used. Known peptides, for example, FLAG (Hopp, T. P. *et al.*, BioTechnology (1988) 6: 1204-1210), 6 x His containing six His (histidine) residues, 10 x His, influenza agglutinin (HA), human c-myc fragment, VSV-GP fragment, p18HIV fragment, T7-tag, HSV-tag, E-tag, SV40T antigen fragment, lck tag, α-tubulin fragment, B-tag, Protein C fragment, and such can be used as other peptides or polypeptides that are fused to the antibody of the present invention. Examples of other polypeptides that are fused to an antibody of the present invention are glutathione-S-transferase (GST), influenza agglutinin (HA), immunoglobulin constant region, β-galactosidase, maltose-binding protein (MBP), and such. Fusion polypeptides can be prepared by fusing a commercially available polynucleotide which encodes such a peptide or polypeptide with a polynucleotide encoding an antibody of the present invention, and by expressing the resulting fusion polynucleotide.

The antibodies of the present invention may be conjugated antibodies which are bound to various molecules including macromolecular substances such as polyethylene glycol (PEG) or hyaluronic acid, radioactive materials, fluorescent substances, luminescent substances, enzymes, and toxins. Such conjugated antibodies can be obtained by performing chemical modifications

on obtained antibodies. Methods for modifying antibodies are already established in this field (for example, US 5057313 and US 5156840). The term "antibody" in the present invention also includes such conjugated antibodies.

The antibodies of the present invention also include antibodies with modified sugar chains. It is known that cytotoxic activity of antibodies can be enhanced by modifying the sugar chains of an antibody. Known examples of antibodies with modified sugar chains are the following:

glycosylated antibodies (for example, WO 99/54342);

antibodies deficient in fucose attached to sugar chains (for example, WO 00/61739 and WO 02/31140);

antibodies having a sugar chain with bisecting GlcNAc (for example, WO 02/79255), and such.

Furthermore, antibodies used in the present invention may be bispecific antibodies. Bispecific antibodies refer to antibodies that comprise within the same antibody molecule, variable regions recognizing different epitopes. In the present invention, the bispecific antibodies may be those recognizing different epitopes on the AXL molecule, or alternatively, those in which one of the antigen binding sites recognizes AXL, and the other antigen binding site recognizes another substance. Furthermore, from a different perspective, they may be bispecific antibodies in which one of the antigen binding sites recognizes AXL, and the other antigen binding site recognizes an antigen on human effector cells. Examples of antigens bound by the other antigen binding site of bispecific, AXL-recognizing antibody of the present invention include CD2, CD3, CD16, CD19, CD20, CD25, CD28, CD33, CD30, CD44, CD44v6, CD52, VEGF, VEGFR, EGF, EGFR, EGFRvIII, HER-2 neu, HER-3, HER-4, cMET, EpCAM, IGF-1R, TRAIL-R2, Tie-1, PDGFR-alpha, NKG2D, CCR5, Gas6, Mer, Tyro3, NCAM, Transferin receptor, Folate binding protein, IL-15, IL-15R, CEA, CA125, MUC-1, ganglioside GD3, Glypican-3, GM2, and Sonic Hedgehog (Shh).

Examples of the different epitopes on the AXL molecule bound by the other antigen binding site of bispecific, AXL-recognizing antibody of the present invention include IgD1, IgD2, and FND2.

Methods for producing bispecific antibodies are known. For example, a bispecific antibody can be produced by linking two types of antibodies that recognize different antigens. Each of the linked antibodies may be a half molecule, with the H and L chains, or a quarter molecule comprising only the H chain. Alternatively, fused cells that produce bispecific antibodies can be prepared by fusing hybridomas producing different monoclonal antibodies. Bispecific antibodies can also be prepared with genetic engineering techniques.

Antibodies of the present invention may differ in their amino acid sequences, molecular weights, isoelectric points, or the presence/absence or form of sugar chains depending on the

later-described antibody-producing cells or hosts, or methods of purification. However, as long as the obtained antibodies have functions equivalent to the antibodies of the present invention, they are included in the present invention. For example, in some cases the amino acids included in the amino acid sequences described in the present invention are subjected to modification (for example, modification of the N-terminal glutamine to pyroglutamic acid by pyroglutamylation is a modification well known to those skilled in the art) after translation, but even when amino acids undergo post-translational modification in this manner, they are, as a matter of course, included in the amino acid sequences of the present invention. Furthermore, when an antibody of the present invention is expressed in prokaryotic cells such as *Escherichia coli*, a methionine residue is added to the N terminus of the amino acid sequence of the original antibody. Antibodies of the present invention also include such antibodies.

Polypeptides such as the anti-AXL antibodies of the present invention can be produced by methods known to those skilled in the art.

Anti-AXL antibodies can be prepared by a gene recombinant technique known to those skilled in the art based on a sequence of an anti-AXL antibody obtained, for example. Specifically, a polynucleotide encoding an antibody is constructed based on the sequence of an antibody that recognizes AXL, introduced into an expression vector, and then expressed in suitable host cells (see, for example, Co, M.S. *et al.*, J. Immunol. (1994) 152, 2698-2976; Better, M. and Horwitz, A.H., Methods Enzymol. (1989) 178, 476-496; Plueckthun, A. and Skerra, A., Methods Enzymol. (1989) 178, 497-515; Lamoyi, E., Methods Enzymol. (1986) 121, 652-663; Rousseaux, J. *et al.*, Methods Enzymol. (1986) 121, 663-669; and Bird, R.E. and Walker, B.W., Trends Biotechnol. (1991) 9, 132-137).

The vectors include, for example, M13-series vectors, pUC-series vectors, pBR322, pBluescript, and pCR-Script. When the purpose is subcloning or excision of cDNAs, examples of vectors include pGEM-T, pDIRECT, and pT7 in addition to the above-mentioned vectors. When using vectors for the purpose of producing the antibodies of this invention, expression vectors are particularly useful. When the objective is to express the vector in *E. coli*, for example, the vector should have characteristics that will lead to amplification in *E. coli*. Additionally, when *E. coli* such as JM109, DH5α, HB101, or XL1-Blue is used as a host, the vector must have a promoter, for example, a lacZ promoter (Ward *et al.* Nature (1989) 341, 544-546; FASEB J. (1992) 6, 2422-2427), araB promoter (Better *et al.* Science (1988) 240, 1041-1043), or T7 promoter that can allow efficient expression in *E. coli*. Other examples of such vectors include pGEX-5X-1 (manufactured by Pharmacia), "QIAexpress system" (manufactured by QIAGEN), pEGFP, and pET (in this case, the host is preferably BL21 expressing T7 RNA polymerase).

Furthermore, the expression plasmid vector may comprise a signal sequence for

antibody secretion. For production into the periplasm of *E. coli*, the pelB signal sequence (Lei, S. P. *et al.* J. Bacteriol. (1987) 169, 4379) may be used as the signal sequence for antibody secretion. For example, calcium chloride methods or electroporation methods may be used to introduce the vector into a host cell.

In addition to *E. coli*, a vector for producing an antibody of the present invention may be, for example, expression vectors derived from mammals (e.g., pcDNA3 (manufactured by Invitrogen), pEF-BOS (Nucleic Acids Res. 1990, 18(17), p5322), pEF, pCDM8), expression vectors derived from insect cells (e.g., "Bac-to-BAC baculovirus expression system" (manufactured by GIBCO-BRL), pBacPAK8), expression vectors derived from plants (e.g., pMH1, pMH2), expression vectors derived from animal viruses (e.g., pHSV, pMV, pAdexLcw), expression vectors derived from retroviruses (e.g., pZIPneo), expression vectors derived from yeasts (e.g., "Pichia Expression Kit" (manufactured by Invitrogen), pNV11, SP-Q01), and expression vectors derived from *Bacillus subtilis* (e.g., pPL608, pKTH50).

When an objective is expression in animal cells such as CHO, COS, and NIH3T3 cells, the expression plasmid vector must have a promoter necessary for expression in the cells, for example, an SV40 promoter (Mulligan *et al.* Nature (1979) 277, 108), MMLV-LTR promoter, EF1α promoter (Mizushima *et al.* Nucleic Acids Res. (1990) 18, 5322), CMV promoter, etc. It is even more preferable that the vector also carries a gene for selecting transformants (for example, a drug-resistance gene enabling selection by a drug (neomycin, G418, or such)). Examples of vectors with such characteristics include pMAM, pDR2, pBK-RSV, pBK-CMV, pOPRSV, pOP13, and such.

In addition, when an objective is to stably express a gene and amplify the gene copy number in cells, a method can be adopted in which CHO cells with a defective nucleic acid synthesis pathway is introduced with a vector containing a DHFR gene (for example, pSV2-dhfr ("Molecular Cloning 2nd edition" Cold Spring Harbor Laboratory Press, (1989))) which complements the defect, and methotrexate (MTX) is used for amplification. Alternatively, when an objective is transient gene expression, a method can be used in which a COS cell, which carries an SV40 T antigen-expressing gene on its chromosome, is transformed with a vector containing the SV40 replication origin (for example, pcD). The replication origin used may be those derived from polyoma viruses, adenoviruses, bovine papilloma viruses (BPV), and such. Furthermore, to increase the gene copy number in host cell systems, the expression vector may contain, as a selection marker, an aminoglycoside transferase (APH) gene, thymidine kinase (TK) gene, *E. coli* xanthine guanine phosphoribosyl transferase (Ecogpt) gene, dihydrofolate reductase (dhfr) gene, and such.

The resulting antibodies of the present invention may be isolated from the inside or outside (such as medium) of host cells, and purified as substantially pure and homogenous

antibodies. Methods for isolation and purification commonly used for antibody purification may be used for the isolation and purification of antibodies, and they are not limited to any method. Antibodies may be isolated and purified by appropriately selecting and combining, for example, column chromatographies, filtration, ultrafiltration, salting out, solvent precipitation, solvent extraction, distillation, immunoprecipitation, SDS-polyacrylamide gel electrophoresis, isoelectric focusing, dialysis, and recrystallization.

Chromatography includes, for example, affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel filtration, reverse-phase chromatography, and adsorption chromatography (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed Daniel R. Marshak *et al.*, Cold Spring Harbor Laboratory Press, 1996). These chromatographies can be carried out using liquid phase chromatographies such as HPLC and FPLC. Columns used for affinity chromatography include protein A column and protein G column. Examples of columns using protein A column include Hyper D, POROS, and Sepharose FF (GE Healthcare Biosciences). The present invention also includes antibodies that are highly purified using these purification methods.

The AXL-binding activity of the obtained antibodies can be measured using methods known to those skilled in the art. For example, Biacore, ELISA (enzyme linked immunosorbent assay), EIA (enzyme immunoassay), RIA (radioimmunoassay), or fluoroimmunoassay may be used as the method for measuring antigen binding activity of antibodies. For example, when using enzyme immunoassay, a sample containing an antibody, for example, a culture supernatant of antibody-producing cells or a purified antibody is added to an antigen-coated plate. A secondary antibody labeled with an enzyme such as alkaline phosphatase is added, the plate is incubated, and after washing, an enzyme substrate such as *p*-nitrophenylphosphate is added, and the absorbance is measured to evaluate the antigen binding activity.

Agents for lowering the AXL expression level

The present invention also provides agents that lower the AXL expression level comprising an anti-AXL antibody. The agent that lowers the AXL expression level reduces AXL expression level in cells expressing AXL. There is no particular limitation on the cells that express AXL. Examples of these cells include cancer cells (Calu-1, MDA-MB-231, DU-145, etc.).

The reduction in the expression level of AXL may be a reduction in the amount of AXL already present by the degradation of AXL, or such, or may be a reduction in the amount of newly expressed AXL by suppressing the expression of AXL.

The agents that lower the AXL expression level comprising the anti-AXL antibody of the present invention can be expressed as methods for lowering the expression level of AXL

using an anti-AXL antibody. Moreover, the agents that lower the AXL expression level comprising the anti-AXL antibody of the present invention can be expressed as a use of an anti-AXL antibody for producing an agent for lowering the AXL expression level.

The anti-AXL antibodies of the present invention are expected to exhibit angiogenesis inhibitory activity, tumor-growth-suppressive effect, and such by lowering AXL expression levels.

Pharmaceutical compositions

The cell-growth suppressants or agents that lower the AXL expression level of the present invention can be administered by either oral administration methods or parenteral administration methods. Parenteral administration methods are particularly preferred. Specific examples of such administration methods include administration by injection, transnasal administration, transpulmonary administration, and transcutaneous administration. The pharmaceutical compositions of the present invention can be administered systemically or locally by injection, for example, by intravenous injection, intramuscular injection, intraperitoneal injection, subcutaneous injection, or such. Suitable methods of administration can also be selected according to the age and symptoms of the patient. The dosage can be selected, for example, within the range of 0.0001 mg to 1,000 mg per kilogram body weight per administration. Alternatively, the dosage can be selected, for example, within the range of 0.001 to 100,000 mg/body per patient. However, the dosage of the pharmaceutical compositions of the present invention is not limited thereto.

The cell-growth suppressants or agents for lowering the AXL expression level of the present invention can be formulated according to ordinary methods (for example, Remington's Pharmaceutical Science, latest edition, Mark Publishing Company, Easton, USA), and may comprise pharmaceutically acceptable carriers or additives. Examples of the carriers and additives include, but are not limited to, surfactants, vehicles, colorants, fragrances, preservatives, stabilizers, buffers, suspension agents, isotonic agents, binders, disintegration agents, lubricants, fluidity promoters, and flavoring agents. Other commonly used carriers can be used appropriately. Specific examples of such carriers include light silicic anhydride, lactose, crystalline cellulose, mannitol, starch, carmellose calcium, carmellose sodium, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, polyvinylacetal diethylaminoacetate, polyvinylpyrrolidone, gelatin, medium-chain fatty-acid triglycerides, polyoxyethylene hydrogenated castor oil 60, saccharose, carboxymethyl cellulose, cornstarch, inorganic salts, etc.

All prior art reference cited herein are incorporated by reference in their entirety.

Examples

Although the present invention will be explained in more detail by the following Examples, the present invention is not limited by these Examples.

[Referential Example 1] Method for obtaining Ax225 Antibody

1-1. Preparation of antigen

Hamster ovary cells (CHO ($dhfr^-$) cells) were transfected with the expression vector for a fusion protein (hAXL-ECD-mIgG2aFc), in which the extracellular domain of human AXL and an Fc domain of mouse IgG2a were fused, and CHO cell lines that produce hAXL-ECD-mIgG2aFc protein were cloned with G418 selection. The culture supernatant of the hAXL-ECD-mIgG2aFc protein-producing CHO cell lines collected using serum-free medium (CHO-S-SFM II; Gibco) was added to a Protein G Column (HiTrap Protein G HP, GE Healthcare) equilibrated with a binding buffer (20 mM phosphate buffer, pH 7.0). After the unbound proteins were washed with the binding buffer, fractions of hAXL-ECD-mIgG2aFc protein were collected with an elution buffer (100 mM glycine-HCl, pH 2.7) into tubes containing neutralizing buffer (1 M Tris-HCl, pH 9.0). Then the buffer of the purified protein was replaced with phosphate-buffered physiological saline (pH 7.35–7.65; Takara Bio) and the purified protein was concentrated using an ultrafiltration kit for a molecular weight fraction of 10 kDa (Centricon (registered trademark), Millipore). The concentration of the purified protein was calculated from the absorbance at 280 nm using a molar absorption coefficient calculated according to the calculation formula of Pace *et al.* (Prof. Sci. (1995) 4: 2411-2423).

1-2. Preparation of anti-AXL-antibody-producing hybridoma deposited under Accession No. FERM BP-10854

Four BALB/c mice (male, six weeks old at the start of immunization, Charles River Laboratories Japan) and two MRL/lpr mice (male, six weeks old at the start of immunization, Charles River Laboratories Japan) were immunized as described below with the antigen prepared in the previous section (hAXL-ECD-mIgG2aFc protein). Antigen emulsified with Freund's complete adjuvant (FCA) (H37 Ra, Difco Laboratories) was administered subcutaneously at 40 μg/head as the initial immunization. Two weeks later, antigen emulsified with Freund's incomplete adjuvant (FIA) (Difco Laboratories) was administered subcutaneously at 40 μg/head. The animals were subsequently immunized three times more at one week intervals. Increases in the serum antibody titer in response to the antigen were confirmed by ELISA (Enzyme linked immunosorbent assay) as indicated in the following section, followed by a final immunization of

intravenous administration of antigen diluted with phosphate-buffered physiological saline (phosphate-buffered saline without calcium ions or magnesium ions, PBS(–); Nissui Pharmaceutical) at 10 μg/head. Three days after the final immunization, mouse spleen cells and mouse myeloma cells P3X63Ag8U.1 (referred to as P3U1, ATCC CRL-1597) were fused according to ordinary methods using PEG 1500 (Roche Diagnostics). The fused cells were cultured in RPMI1640 medium (Invitrogen) containing 10% FBS (Invitrogen) (hereafter referred to as 10% FBS/RPMI1640). On the day after fusion, the fused cells were suspended in semifluid medium (StemCells) followed by the selective culture and colonization of the hybridomas. Hybridoma colonies were picked from the medium on the ninth or tenth day after fusion and seeded into a 96-well plate containing HAT selective medium (10% FBS/RPMI1640, 2 vol% HAT 50x concentrate [Dainippon Pharmaceutical] and 5 vol% BM-Condimed H1 [Roche Diagnostics]) at one colony per well. After culture for three to four days, the supernatant was collected from each well and the hybridomas with binding activity to the extracellular domain of human AXL were selected by measuring their binding activity to the aforementioned antigen and to a control protein fused with the Fc domain of mouse IgG2a by ELISA, as indicated in the following section.

The binding activities of the supernatants of the selected hybridomas are shown in Table 3.

[Table 3]

AXL	2ndSC Abs	2ndSC Abs	2ndSC Abs	2ndSC Abs	IgG
Clone No.	AXL-mFc	FGFR2-mFc	Abs Δ	AXL-His	Binding
225	0. 629	0. 055	0. 574	0. 642	0. 859

The hybridoma selected by the present inventors was deposited at the International Patent Organism Depositary of the National Institute of Advanced Industrial Science and Technology. The following section provides a description of the contents, specifying the deposition.

(a) Name and Address of the Depositary Institution

Name: International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology

Address: Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan 305-8566

(b) Acceptance Date (Deposition Date): July 5, 2007

(c) Accession No.

AXL No. 225 #070402 (Ax225) (Accession No. FERM BP-10854)

1-3. Binding activity to human AXL

Antigen (hAXL-ECD-mIgG2aFc protein) diluted to 1 μg/mL with coating buffer (100 mM sodium bicarbonate [pH 9.6], 0.02% sodium azide) or control protein fused with the Fc domain of mouse IgG2a was dispensed into a 96-well plate (Nunc-Immuno™ 96 MicroWell™ MaxiSorp™ plate; Nalge Nunc International) at 80 μL/well, followed by incubation overnight or longer at 4°C. After it was washed three times with phosphate-buffered physiological saline containing 0.05 vol% Tween (registered trademark) 20 (tPBS(–)), the plate was blocked overnight or longer at 4°C with diluent buffer (1/5 dilution of BlockingOne; Nacalai Tesque). Then the buffer was removed and mouse antiserum or hybridoma culture supernatant diluted with diluent buffer was added to the plate at 80 μL/well, followed by incubation for one hour at room temperature. The plate was then washed three times with tPBS(–), and HRP-labeled anti-mouse IgG antibody (Stressgen), diluted 1/5000 with diluent buffer, was added at 80 μL/well, followed by incubation for one hour at room temperature. After the plate had been washed five times with tPBS(–), a chromogenic substrate, Peroxidase Substrate (Kirkegaad & Perry Laboratories), was added at 80 μL/well, followed by incubation for 20 minutes at room temperature. Following the addition of Peroxidase Stop Solution (Kirkegaad & Perry Laboratories) at 80 μL/well, the absorbance at 405 nm was measured with a Microplate Reader Model 3550 (Bio-Rad Laboratories).

1-4. Purification of antibody from hybridoma culture supernatant

The resulting hybridomas described above were cultured in HAT selective medium using low-IgG FBS (Invitrogen) as the FBS. Protein G beads (Pharmacia), in which the solvent was replaced with wash buffer (20 mM sodium acetate buffer, pH 5.0), were added to 20–50 mL of the culture supernatant at 50 μL per 10 mL of culture supernatant, followed by mixing by inversion overnight at 4°C. After the Protein G beads had been recovered and washed with wash buffer, the antibody was eluted with elution buffer (50 mM sodium acetate buffer, pH 3.3), followed immediately by neutralization with neutralizing buffer (Tris-HCl buffer, pH 7.8). The buffer was replaced with phosphate-buffered physiological saline (pH 7.35–7.65; Nissui Pharmaceutical) and the purified antibody was concentrated using an ultrafiltration kit for a molecular weight fraction of 10 kDa (Amicon (registered trademark), Millipore), followed by sterilization with a 0.22 μm sterilization filter (Millipore GV, Millipore).

[Referential Example 2] Binding activity to human AXL-FND1 and human AXL-IgD2

2-1. Binding activity to human AXL-FND1 and human AXL-IgD2

The binding abilities of anti-AXL monoclonal antibody to AXL-fibronectin type 3 domain 1 (AXL-FND1) and AXL immunoglobulin family domain 2 (AXL-IgD2) were tested.

2-2. Preparation of human recombinant AXL-FND1 and human recombinant AXL-IgD2 expression vectors

Human recombinant AXL-FND1 was prepared by amplifying by PCR a region equivalent to the 225th to 331st amino acids from full-length human AXL cDNA (O'Bryan, *et al.*, Mol. Cell. Biol. (1991) 11: 5016-5031) (GenBank No. NM_021913), cloning the amplified products to pET-41a(+) (Novagen) to express fusion proteins with GST-tag, and constructing pET-AXL-FND1. Other domains were prepared by amplifying by PCR a region AXL-IgD2 equivalent to the 137th to 224th amino acids, and cloning the amplified products to pET-41a(+) to express fusion proteins with GST-tag.

Each of the prepared vectors (5 μl) was transformed to DH5α (Toyobo Co., Ltd., Cat. No. DNA-903) by a heat shock method and then cultured in SOC medium. Colonies were selected after culturing overnight at 37°C on an LB plate containing kanamycin.

2-3. Purification of human recombinant AXL-FND1 and human recombinant AXL-IgD2

Each of the produced colonies were precultured overnight at 37°C in 20 mL of LB medium containing kanamycin and then transferred to 500 mL of medium. The each colony was cultured to an A_{600} of 0.5 ± 0.05 and IPTG was added to be a concentration of 0.5 mM. After culturing for one hour at 37°C, the bacterial cells were collected and suspended in Buffer A (50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.5 mM PMSF, and 1 mM DTT). Freezing and thawing was repeated twice using liquid nitrogen. NP-40 was then added to 0.5% and the cells were homogenized with an ultrasonic homogenizer (30 seconds x 5) and centrifuged for 30 minutes at 244,000 x G, and then the supernatant was recovered.

Human recombinant AXL-FND1 was purified in the manner described below using the resulting supernatant. Solubilized *E. coli* supernatant was mixed with Glutathione Sepharose™ 4B Fast Flow (GE Healthcare) and stirred for one hour at 4°C with a rotator. After centrifugation for five minutes at 500 x G, the supernatant was discarded and the Glutathione Sepharose™ 4B was washed by adding Buffer A. This washing procedure was repeated three times. After transferring the human recombinant AXL-FND1 from the washed Glutathione Sepharose™ 4 Fast Flow to a mini-column, it was separated and eluted from the Glutathione Sepharose™ 4 Fast Flow with 50 mM Tris-HCl (pH 7.5) and 25 mM glutathione. Each of other AXL domains was expressed, separated, and eluted in the same manner.

2-4. Evaluation of binding activity of anti-AXL antibody to AXL-FND1 by western blotting

The human recombinant AXL-FND1 separated and eluted from the Glutathione Sepharose™ 4 Fast Flow, as well as AXL–IgD1, AXL–IgD2, AXL–FND2, AXL–IgD1+IgD2, AXL–IgD2+FND1, and AXL–FND1+FND2 were quantified using the BIO-RAD Dc Protein Assay. 1 μg each was mixed with NuPAGE (registered trademark) Sample Buffer (Invitrogen), and electrophoresed with NuPAGE (registered trademark) 10% Bis-Tris Gel. The electrophoresed gel was transferred to an Immobilon™-FL (Millipore) PVDF membrane. The PVDF membrane containing the transferred protein was blocked with Odyssey (registered trademark) Blocking Buffer (LI-COR) and immersed in a primary antibody solution in which anti-AXL antibody was diluted to 5 μg/mL, and incubated overnight at 4°C. The PVDF membrane containing the transferred protein and immersed in the primary antibody solution was washed four times for five minutes each with 0.1% TBS-T (TBS (Tris-Buffered Saline (Takara)) containing 0.1% Tween-20). The PVDF membrane immersed in anti-AXL antibody was immersed in Alexa Fluor (registered trademark) 680 Goat Anti-mouse IgG (H+L) (Invitrogen) secondary antibody solution diluted to 80 ng/mL and incubated for one hour at room temperature. After washing the PVDF membrane immersed in the secondary antibody solution three times for five minutes each with 0.1% TBS-T, the membrane was washed for five minutes with TBS-T containing 0.01% SDS and then washed for five minutes with TBS. The binding of the washed PVDF membrane was then evaluated by scanning with the Odyssey (registered trademark) far infrared imaging system.

2-5. Results

The evaluation results are shown in Fig. 1.

Anti-AXL antibody produced by a hybridoma deposited under Accession No. FERM BP-10854 (Ax225) was demonstrated to recognize FND1 of AXL (Fig. 1). Anti-AXL antibody produced by a hybridoma deposited under Accession No. FERM BP-10857 (Ax284) was considered to recognize FND1 and IgD2 of AXL (Fig. 1). Anti-AXL antibody produced by a hybridoma deposited under Accession No. FERM BP-10850 (Ax7) and anti-AXL antibody produced by a hybridoma deposited under Accession No. FERM BP-10851 (Ax51) were demonstrated to recognize IgD2 of AXL (Fig. 1).

[Referential Example 3] Assay of the induction of AXL protein downmodulation by the antibody Ax225

The ability of the anti-AXL monoclonal antibody to induce the downmodulation of AXL within cancer cells was tested. Human non-small-cell lung cancer cell line Calu-1 was seeded into six-well plates at a density of 4×10^5 cells/well and 24 hours later, the medium was replaced

with medium from which the serum had been removed (serum-starved medium) and then the cells were cultured overnight. Next, the anti-AXL monoclonal antibody prepared as described above was added at 2 μg/mL, and recombinant GAS6 (R&D) was added at 200 ng/mL to act as the positive control, followed by incubation for 24 hours at 37°C. Next, the cells were washed with PBS(–) and the protein was extracted from the cells with the previously described cell lysis buffer. The cell lysis products, immunoprecipitated with a commercially available anti-AXL antibody (Santa Cruz™), were separated on 7% NuPAGE (Invitrogen), immunoblotted by western blotting, and tyrosine phosphorylation assay, as previously described.

25 μg of each protein solution was suspended in NuPAGE-LDS sample buffer (Invitrogen), heated for 10 minutes at 70°C, and electrophoresed for one hour at 150 V on 7% NuPAGE (Invitrogen). The gels separated by electrophoresis were electrophoretically transferred to a 0.45 μm polyvinylidene difluoride filter (Immobilon-FL, Millipore) over the course of one hour at 30 mA in NuPAGE transfer buffer (Invitrogen) and the buffer containing 20 vol% methanol. The filter was washed with TBS (50 mM Tris-HCl [pH 7.6], 150 mM NaCl) and then blocked by incubation overnight in Odyssey blocking buffer (Li-COR). The filter was washed four times for five minutes each with TBST and then incubated for two hours at room temperature with anti-AXL antibody (diluted 1:15,000 with TBST; Santa Cruz) and anti-actin antibody (diluted 1:5,000 with TBST). After the filter had been washed four times for five minutes each with TBST, it was incubated for one hour with Alexa 680-labeled anti-rabbit secondary antibody (Invitrogen) diluted 1:10,000 with TBST and IRDye 800-labeled anti-goat secondary antibody (Rockland) diluted 1:10,000 with TBST. After it had been washed three times for five minutes each with TBST, the filter was washed again once for five minutes with TBS, and then scanned with the Odyssey infrared imaging system (Li-COR).

The AXL blots were observed to weaken following exposure to the Ax225 antibody (Fig. 2). Therefore, the Ax225 antibody can induce the downmodulation of AXL protein.

[Referential Example 4] Measurement of antitumor effects of the anti-AXL antibody in a mouse xenograft model with human pancreatic adenocarcinoma

1. Preparation of a mouse xenograft model with human pancreatic adenocarcinoma

The human pancreatic adenocarcinoma cell line PANC-1, purchased from Dainippon Pharmaceutical (currently Dainippon Sumitomo Pharma), was prepared at 5 x 10^7 cells/mL with HBSS. 200 μL of the cell suspension (1 x 10^7 cells/mouse) was subcutaneously grafted into the inguinal region of a CAnN.Cg-Foxn1<nu>/CrlCrlj nu/nu (BALB-nu/nu) mouse purchased from Charles River Laboratories, Japan. The mouse was subjected to the experiment when the mean tumor volume had reached about 210 mm^3.

2. Antibody preparation and administration

The Ax225 antibody was prepared at 2 mg/mL with PBS and administered twice a week for two weeks at 20 mg/kg into the peritoneal cavity of the mouse xenografted with human pancreatic adenocarcinoma. As the negative control, PBS was administered in the same manner. Gemzar (Eli Lilly Japan) was prepared at 12 mg/mL with physiological saline as the positive control and administered intraperitoneally at 120 mg/kg twice a week for two weeks.

3. Evaluation of antitumor effects

The antitumor effects in a mouse xenograft model with human pancreatic adenocarcinoma were calculated as tumor-growth-suppressive effects by comparing the tumor growth in the antibody-treated group with the tumor growth in the negative control group four days after the final administration (Fig. 3).

Tumor-growth-suppressive effect (%) = (1 – amount of tumor growth in the antibody-treated group/amount of tumor growth in the control group) x 100

4. Statistical processing

Tumor volume was expressed as the mean ± standard deviation. Statistical analysis consisted of a comparison between the control group and the treated group by the LSD method using the SAS Preclinical Package Ver. 5.0. Reliability of 95% (*: $p<0.05$) was determined to constitute significance.

5. Results

The Ax225 antibody inhibited tumor growth and demonstrated antitumor effects (Fig. 3).

[Referential Example 5] Measurement of antitumor effects of anti-AXL antibody on mouse xenograft model with human pancreatic adenocarcinoma (2)

1. Preparation of mouse xenograft model with human pancreatic adenocarcinoma

Human pancreatic adenocarcinoma cell line PANC-1 purchased from Dainippon Pharmaceutical (currently Dainippon Sumitomo Pharma) was prepared to 5×10^7 cells/mL with HBSS. 200 μL of the cell suspension (1×10^7 cells/mouse) were subcutaneously grafted to the inguinal regions of CAnN.Cg-Foxn1<nu>/CrlCrlj nu/nu (BALB-nu/nu) mice purchased from Charles River Laboratories, Japan. The mice were used in the experiment when the mean tumor volume reached about 270 mm^3.

2. Antibody preparation and administration

The Ax225 antibody and anti-AXL antibodies obtained similarly as Ax225 antibody but have a different epitope were prepared to 2 mg/mL with PBS and administered into the peritoneal cavity of the mice xenografted with human pancreatic adenocarcinoma twice a week for two weeks at 20 mg/kg. PBS was administered in the same manner for use as a negative control. Gemzar (Eli Lilly Japan) was prepared to 12 mg/mL with physiological saline for use as a positive control and administered intraperitoneally twice a week for two weeks at 120 mg/kg.

3. Evaluation of antitumor effects

Antitumor effects in a mouse xenograft model with human pancreatic adenocarcinoma were calculated as tumor-growth-suppressive effects by comparing with the amount of tumor growth of a negative control group four days after final administration.

Tumor growth suppressive effect (%) = (1 – amount of tumor growth of the antibody-treated group/amount of tumor growth of the control group) x 100

4. Results

The results for suppression of tumor growth are shown in Fig. 1. A tumor-growth-suppressive effect (%) of lower than 30% is indicated as "–", that of 30% or more is indicated as "+", and that of 60% or more is indicated as "++".

Antibodies that bind to FND-1 demonstrated 60% or more of TGI activity even if administration was begun at the time when mean tumor volumes had reached about 270 mm^3. This finding that anti-AXL antibodies that bind to FND-1 have such significant antitumor effects *in vivo* was determined for the first time in this study and was completely unexpected.

[Example 1] Production of chimeric antibodies

Production of chimeric antibody expression vectors

To produce chimeric Ax225 antibodies, in which the human IgG1 constant region and Ax225 antibody variable region are fused, two synthetic oligo-DNAs, a sense strand and an antisense strand, were designed for each of the H and L chains such that the 5' end of the cDNA encoding the human IgG1 constant region (H chain: human γ1; L chain: human κ) and the 3' end of the cDNA encoding Ax225 antibody variable region were fused. Hereinafter, they will be referred to as H-chain sense fusion primer (A), H chain antisense fusion primer (B), L chain sense fusion primer (C), and L chain antisense fusion primer (D). Each of the synthetic

oligo-DNAs was admixed, and a gene encoding the chimeric Ax225 antibody was produced by assembly PCR. The first step of expressing the chimeric antibody by assembly PCR involved performing PCR according to the following conditions using four combinations: a sense strand produced by adding a Kozak sequence and a restriction enzyme site to the H chain 5' end of Ax225 cDNA (E) and the above-mentioned (B); an antisense strand produced by adding a restriction enzyme site to the H chain 3' end region of Ax225 cDNA (F) and the above-mentioned (A); a sense strand produced by adding a restriction enzyme site and a Kozak sequence to the L chain 5' end region of Ax225 cDNA (G) and the above-mentioned (D); and an antisense strand produced by adding a restriction enzyme site to the L chain 3' end region of Ax225 cDNA (H) and the above-mentioned (C). A reaction mixture comprising the attached PCR Buffer, dNTPs, PrimeSTAR, a cDNA encoding the Ax225 H chain or L chain, and one synthetic oligo-DNA was heated at 98°C for one minute, and then subjected to PCR reaction consisting of 30 cycles of 98°C for 10 seconds, 55°C for 10 seconds, and 72°C for one minute. The second PCR was performed using the gene fragments amplified in the first PCR reaction as the template. For the H chain, mixture of the fragments amplified using the (E) (B) and (F) (A) combinations were used, and for the L chain, mixture of the fragments amplified using the (G) (D) and (H) (C) combinations were used. As primers, (E) and (F) were used for the H chain and (G) and (H) were used for the L chain. After heating at 98°C for one minute, PCR reaction consisting of 30 cycles of 98°C for 10 seconds, 55°C for 10 seconds, and 72°C for one minute thirty seconds was carried out.

The obtained amplified fragments were cloned into an animal cell expression vector. The nucleotide sequence of each DNA fragment was determined using BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) on an ABI PRISM 3730xL DNA Sequencer or an ABI PRISM 3700 DNA Sequencer (Applied Biosystems) according to the method described in the attached manual.

Expression and purification of chimeric antibodies

Human embryonic renal cancer cell-derived FreeStyle™ 293-F cells (Invitrogen) were suspended in FreeStyle™ 293 Expression Medium (Invitrogen), and were seeded at 30 mL per 125-mL flask (CORNING) at a cell density of 1×10^6 cells/mL. Opti-MEM I Reduced Serum Medium (Invitrogen) was added to the prepared plasmid DNA mixture solution (a total of 30 μg) to make the volume 1 mL. Furthermore, Opti-MEM I Reduced Serum Medium (Invitrogen) was added to 60 μL of 293 fectin (Invitrogen) to make the volume 1 mL, and this was mixed with the plasmid DNA mixture solution. The mixture solution was incubated at room temperature for 20 minutes, and was added to the cell suspension. This was incubated for three to six days in a CO_2 incubator (37°C, 8% CO_2). After the culture supernatant was collected, the

cells were removed by centrifugation (approximately 2,000 g for five minutes at room temperature), and this was also passed through a 0.22 μm MILLEX®-GV filter (Millipore). Each sample was stored at 4°C until use. Antibodies were purified from this supernatant using Protein A Sepharose (GE Healthcare). The absorbance at 280 nm was measured on an ND-1000 Spectrophotometer (NanoDrop), and the concentration was calculated by the method of Pace *et al.* (Protein Science (1995) 4: 2411-2423).

[Example 2] Humanization of Ax225 antibody

Affinity measurements using Biacore

To screen for humanized Ax225 antibodies, assessment of binding activity to AXL-FND1 was carried out by the following method using Biacore.

2-1. Preparation of expression vectors for human recombinant AXL-FND1

To prepare human recombinant AXL-FND1, the region corresponding to the amino acid positions 225 to 331 in the full-length human AXL cDNA (O'Bryan *et al.*, Mol. Cell. Biol. 1991; 11: 5016-5031) (GenBank # NM_021913) was amplified by PCR, and this was cloned into pET-41b(+) (Novagen) for expression of the fusion protein with GST-tag, and pET-AXL-FND1 was constructed.

Each of the produced vectors (5 μL) was transfected into BL21-CodonPlus (DE3) RIPL (Stratagene, Cat # 230280) by the heat shock method, cells were cultured in an SOC medium and then cultured overnight on a kanamycin-containing LB plate at 37°C, and then colonies were selected.

2-2. Purification of human recombinant AXL-FND1

Each of the produced colonies was pre-cultured in 3 mL of kanamycin-containing MagicMedia (Invitrogen) for three hours at 37°C, transferred to 500 mL of medium, and cultured overnight at 25°C. Bacterial cells were then collected and suspended in Buffer A (20 mM Tris-HCl (pH 8),10 mM EDTA,30 mM NaCl, Protease inhibitor mixture (complete Mini, EDTA-free (Roche))), and then lysozyme solution was added at a final concentration of 2 mg/mL. This was incubated on ice for one hour, then upon addition of Triton-X100 at a final concentration of 0.5% and NaCl at a final concentration of 100 mM, this was incubated on ice for ten minutes. After the cells were disrupted (30 seconds x 5) using an ultrasonic homogenizer, they were centrifuged at 244,000 x G for 20 minutes, and the supernatant was collected.

Using the obtained supernatant, human recombinant AXL-FND1 was purified as

follows. Glutathione Sepharose™ 4B Fast Flow (GE Healthcare) was washed with PBS, then mixed with a solubilized *E. coli* supernatant, and left for binding overnight at 4°C. Glutathione Sepharose™ 4B Fast Flow was collected and washing operation with 30 mL of washing buffer (20 mM Tris-HCl (pH 8), 500 mM NaCl, 1% Triton-X100, protease inhibitor mixture) was repeated four times. Glutathione Sepharose™ 4B Fast Flow was transferred to a column, and further washing operation was carried out using 5 mL of the washing buffer. Separation and elution from Glutathione Sepharose™ 4B Fast Flow to obtain the human recombinant AXL-FND1 involved three repetitions of an elution operation using 5 mL of elution buffer (100 mM Tris-HCl (pH 8), 20 mM glutathione, 120 mM NaCl).

2-3. Affinity analysis with Biacore using Protein A

Kinetic analyses of antigen-antibody reactions of humanized AXL antibodies were carried out using Biacore T100 (BIACORE). rec-Protein A (Zymed) (hereinafter, Protein A) was immobilized onto a sensor chip, various antibodies were bound to it, then an antigen as the analyte was flushed to measure the antibody-antigen interaction. Human recombinant AXL-FND1 (prepared in 2-2; hereinafter referred to as GST-FND1) prepared at various concentrations was used as the antigen. From the sensorgrams obtained through the measurements, kinetic parameters, namely binding rate constant k_a (1/Ms) and dissociation rate constant k_d (1/s), were calculated, and K_d (M) was calculated based on these values. Biacore T100 Evaluation Software (BIACORE) was used to calculate each of the parameters.

The sensor chips were produced by immobilizing approximately 3000 RU of Protein A onto CM5 (BIACORE) by amine coupling. Using the produced sensor chip, kinetic analyses were carried out on the interaction between the Protein A-bound antibodies and GST-FND1. HBS-EP+ was used as the running buffer, and the flow rate was set to 30 μL/min. Each antibody was prepared at 1 μg/mL using the running buffer, and was subjected to binding with Protein A for two minutes. GST-FND1 as the analyte was prepared at 0.5 and 2.0 μg/mL using HBS-EP+. Measurements were carried out by first binding the various humanized AXL antibodies, chimeric AXL antibodies, or mouse AXL antibodies of interest to Protein A, then applying an analyte solution to them for a two-minute interaction, and then switching to HBS-EP+ (BIACORE) for a two-minute measurement of the dissociation phase. After completion of measurement of the dissociation phase, 30 μL of 10 mM glycine-HCl (pH 2.0) was used for washing to regenerate the sensor chip. Such binding, dissociation, and regeneration were defined as one cycle of analysis. All experiments were performed at 25°C.

Selection of each framework sequence

To humanize the Ax225 antibody, the variable region sequences of the Ax225 antibody

and the human germline sequences were compared. Of these, FR sequences which will serve as a template for humanization are summarized in Table 4. As for the humanized variable region H chain, the sequence comprising FR1, FR2, FR3(2), and FR4 described in Table 4 was defined as H0 (SEQ ID NO: 2). As for the L chain, the sequence comprising FR1, FR2, FR3, and FR4 was defined as L0 (SEQ ID NO: 65). CDRs and FRs were determined according to Kabat numbering.

In the sequence of H chain FR3, the residue at position 94 by Kabat numbering has been reported to have great influence on the three-dimensional structure of CDR3 (Morea *et al.*, J. Mol. Biol. 1998, 275: 269-294). This residue is glycine (G) in Ax225 (SEQ ID NO: 57), but as indicated for FR3(1) (SEQ ID NO: 109) in Table 4, the corresponding residue in the germline sequence selected for humanization was arginine (R). Therefore, substitution of arginine would take place as a result of humanization, and decrease in activity was expected. Accordingly, the residue of Ax225 at position 94 in the H chain was kept and the sequence of FR3(2) in which this residue is glycine (SEQ ID NO: 58) was used.

[Table 4]

H0	Germline	SEQUENCE
FR1	Germline: hVH_2_26	QVTLKESGPVLVKPTETLTLTCTVSGFSLS (SEQ ID NO: 51)
FR2	Germline: hVH_1_24	WVRQAPGKGLEWMG (SEQ ID NO: 53)
FR3(1)	Germline: hVH_2_26	RLTISKDTSKSQVVLTMTNMDPVDTATYYCAR (SEQ ID NO: 109)
FR3(2)	Germline: hVH_2_26 (G is substituted at position 94)	RLTISKDTSKSQVVLTMTNMDPVDTATYYCAG (SEQ ID NO: 58)
FR4	Germline: hJH1	WGQGTLVTVSS (SEQ ID NO: 61)

L0	Germline	
FR1	Germline: hVK_2_30	DVVMTQSPLSLPVTLGQPASISC (SEQ ID NO: 93)
FR2	Germline: hVK_1D_8	WYQQKPGKAPELLIY (SEQ ID NO: 96)
FR3	Germline: hVK_2_40	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC (SEQ ID NO: 101)
FR4	Germline: hJK2	FGQGTKLEIK (SEQ ID NO: 103)

Production of humanized Ax225 variable regions H0 and L0

Synthetic oligo-DNAs were designed for the H chain and L chain to produce variable

regions of humanized Ax225 antibody in which the CDR regions of Ax225 antibody are grafted into the FR regions of the humanized template sequences. Each of the synthetic oligo-DNAs was admixed, and a gene encoding the variable regions of humanized Ax225 was produced by assembly PCR. The H chain was named H0 and the L chain was named L0. Assembly PCR was carried out using KOD-Plus (TOYOBO), and by PCR method according to the following conditions. Amplified fragments were obtained by subjecting a reaction mixture comprising the attached PCR Buffer, dNTPs, $MgSO_4$, KOD-Plus, and 10 pmol of synthetic oligo-DNA to heating at 94°C for five minutes, and then performing two PCR reaction cycles consisting of 94°C for two minutes, 55°C for two minutes, and 68°C for two minutes, and then adding a primer including a restriction enzyme site and a Kozak sequence added to the 5' end of the variable region and a primer including a restriction enzyme site added to the 3' end of the variable region at 10 pmols each, and then performing 35 cycles of PCR reaction consisting of 94°C for 30 seconds, 55°C for 30 seconds, and 68°C for one minute. The obtained amplified fragments were cloned using an animal cell expression vector, and fused to the constant region.

Here, amidation of the C-terminal amino group due to deletion of the C-terminal amino acid lysine residue and deletion of two C-terminal amino acids glycine and lysine is reported as heterogeneity derived from the H-chain C-terminal sequence of IgG antibody (Anal. Biochem. 2007 Jan 1; 360 (1): 75-83). A known method for decreasing such heterogeneity is to delete two H-chain C-terminal amino acids, namely to delete glycine at position 446 and lysine at position 447 by EU numbering (Patent Document 4: WO 2009/041613). Since absence of heterogeneity derived from the H-chain C-terminal sequence is desirable for the humanized Ax225 antibody as well, the IgG1 sequence in which the glycine at position 446 and lysine at position 447 by EU numbering in human IgG1 are deleted (SEQ ID NO: 106) was used as the constant region sequence. On the other hand, for the L chain, natural-type human κ chain (SEQ ID NO: 107) was used as the constant region sequence.

Discovery of amino acid residues essential to maintenance of activity

As described earlier, glycine in the Ax225 H chain at position 94 by Kabat numbering was predicted to play an important role in the binding with AXL.

Therefore, to evaluate the effect of this residue on activity, a modified form (H6; SEQ ID NO: 108) was produced, wherein the residue of the chimeric antibody H chain (chH; SEQ ID NO: 1) at position 94 by Kabat numbering was substituted with the residue for human germline sequence which is arginine. The mutant was produced by performing assembly PCR which utilizes PCR. Specifically, sense-strand and antisense-strand oligo-DNAs that were designed based on the amino acid sequence containing the modified site were synthesized. The sense strand oligo-DNA containing the modified site and the antisense oligo-DNA which binds to the

vector into which the gene to be modified is inserted, and the antisense strand oligo-DNA containing the modified site and the sense strand oligo-DNA that binds to the vector into which the gene to be modified is inserted were combined respectively, and by performing PCR using PrimeSTAR (TAKARA), two fragments, 5'-end and 3'-end fragments containing the modified site were produced. Each mutant was produced by linking the two fragments by assembly PCR. The produced mutants were inserted into an expression vector that enables expression of the inserted gene in animal cells, and the nucleotide sequence of the obtained expression vector was determined by a method known to those skilled in the art.

Expression and purification of chH/L0 (H-chain chH/SEQ ID NO: 1; L chain L0/SEQ ID NO: 65) and H6/L0 (H chain H6/SEQ ID NO: 108; L chain L0/SEQ ID NO: 65) were carried out according to the method of Example 1. The sequences for chH and H6 are completely the same except for position 94 by Kabat numbering, so the effect of this residue alone can be evaluated.

The results of affinity evaluation of chH/L0 and H6/L0 by Biacore are shown in Table 5. A significant decrease in affinity was confirmed for H6/L0 in comparison to chH/L0. This showed that for binding of the Ax225 antibody and humanized Ax225 antibody to AXL-FND1, glycine is preferred as the residue at position 94 by Kabat numbering in the H chain, and arginine is inappropriate.

[Table 5]

H	L	KD(M)
chH	L0	1.59E-09
H6	L0	1.23E-08

Production of H9, an H-chain variable region with improved affinity

The amino acid residue at position 73 by Kabat numbering of H0 (SEQ ID NO: 2), which is the H chain of H0/L0, is threonine (T), but upon consideration based on a three-dimensional structural model, substitution of this residue with asparagine (N) as in the sequence of the Ax225 antibody was expected to increase in affinity.

Therefore, the threonine (T) at position 73 by Kabat numbering in FR3 of H0 (SEQ ID NO: 2) was replaced with asparagine (N) to produce H9 (SEQ ID NO: 3). The mutant was produced by performing assembly PCR which utilizes PCR. Specifically, first, sense-strand and antisense-strand oligo-DNAs that were designed based on the amino acid sequence containing the modified site were synthesized. The sense strand oligo-DNA containing the modified site and the antisense oligo-DNA which binds to the vector into which the gene to be

modified is inserted, and the antisense strand oligo-DNA containing the modified site and the sense strand oligo-DNA that binds to the vector into which the gene to be modified is inserted were combined respectively, and by performing PCR using PrimeSTAR (TAKARA), two fragments, 5'-end and 3'-end fragments containing the modified site were produced. Each mutant was produced by linking the two fragments by assembly PCR. The produced mutants were inserted into an expression vector that enables expression of the inserted gene in animal cells, and the nucleotide sequence of the obtained expression vector was determined by a method known to those skilled in the art. Antibodies were produced and purified according to the method of Example 1.

Affinity measurement of H9/L0 was carried out by the method of Example 2, and the result is shown in Table 6. Increase in affinity was confirmed for H9/L0 in comparison to H0/L0.

[Table 6]

	KD(M)
H0/L0	4.44E-09
H9/L0	3.58E-09

[Example 3] Identification of mutation sites which will change the isoelectric point

Identification of mutation sites

As a method for regulating the plasma half-life of an antibody, a method of controlling the surface charge of an antibody molecule by modifying amino acid residues exposed to the surface of an antibody molecule is known (Patent Documents 2 and 3). Specifically, it is known that decreasing the isoelectric point (pI) value of an antibody enables prolongation of the plasma half-life of the antibody. Conversely, it is known that increasing the isoelectric point of an antibody shortens its plasma half-life, and improves its tissue distribution properties (Non-patent Documents 28 and 29).

From the above, a humanized Ax225 antibody with a modified isoelectric point is expected to have a stronger antitumor activity due to prolonged plasma half-life or improved tissue distribution properties. Therefore, amino acid residues that allow regulation of pharmacokinetics of the humanized Ax225 antibody by adjusting the surface charge on the antibody molecule without having effects on the antibody's binding activity against antigens and three-dimensional structure were identified. Specifically, mutation sites that can change the isoelectric point without greatly reducing affinity as measured by Biacore were searched for in the variable region of H9/L0 (H chain H9/SEQ ID NO: 3; L chain L0/SEQ ID NO: 65).

As a result of using a three-dimensional structural model of the humanized Ax225 antibody to screen for mutation sites that can change the isoelectric point of the variable region without greatly decreasing the binding to AXL, several mutation sites were found. Modifications in the H chain and L chain that decrease the isoelectric point are shown in Table 7 and Table 8 (modification sites in the H chain for decreasing the isoelectric point), and Table 9 (modification sites in the L chain for decreasing the isoelectric point); and modifications in the H chain and L chain that increase the isoelectric point are shown in Table 10 and Table 11 (modification sites in the H chain for increasing the isoelectric point), and Table 12 (modification sites in the L chain for increasing the isoelectric point). Each of the variants were produced and purified by the method of Example 1.

Affinity evaluation of each of the variants by Biacore was carried out by the method of Example 2. As shown in Tables 13 to 15, the affinity of each of the variants did not show a large decrease compared to that of H9/L0. The SEQ ID NOs of the H chain and L chain of each of the variants are also shown in Tables 13 to 15.

[Table 7]

NAME	CLASSIFICATION	H9 SEQUENCE	MUTATED POSITION (kabat No.)	AMINO ACID BEFORE MUTATION(H9)	AMINO ACID AFTER MUTATION	SEQUENCE AFTER MUTATION
H17	CDR1	SFGVD (SEQ ID NO:33)	31	S	D	DFGVD (SEQ ID NO:34)
H18	CDR1	SFGVD (SEQ ID NO:33)	31	S	E	EFGVD (SEQ ID NO:35)
H19	FR2	WVRQAPGKGLEWMG (SEQ ID NO:53)	43	K	Q	WVRQAPGQGLEWMG (SEQ ID NO:54)
H20	FR2	WVRQAPGKGLEWMG (SEQ ID NO:53)	40 43 48	A K M	P E I	WVRQPPGEGLEWIG (SEQ ID NO:55)
H21	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	61	S	E	VIWGGGSTNYNEALKS (SEQ ID NO:39)
H22	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	62	A	E	VIWGGGSTNYNSELKS (SEQ ID NO:40)
H23	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	64	K	Q	VIWGGGSTNYNSALQS (SEQ ID NO:41)
H24	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	65	S	D	VIWGGGSTNYNSALKD (SEQ ID NO:42)
H25	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	64 65	K S	Q D	VIWGGGSTNYNSALQD (SEQ ID NO:43)
H26	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	61 64 65	S K S	E Q D	VIWGGGSTNYNEALQD (SEQ ID NO:44)

[Table 8]

NAME	CLASSIFICATION	H9 SEQUENCE	MUTATED POSITION (kabat No.)	AMINO ACID BEFORE MUTATION (H9)	AMINO ACID AFTER MUTATION	SEQUENCE AFTER MUTATION
H27	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	62	A	E	VIWGGGSTNYNSELQD (SEQ ID NO:45)
			64	K	Q	
			65	S	D	
H28	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	61	S	E	VIWGGGSTNYNEELQD (SEQ ID NO:46)
			62	A	E	
			64	K	Q	
			65	S	D	
H30	FR4	WGQGTLVTVSS (SEQ ID NO:61)	105	Q	E	WGEGTLVTVSS (SEQ ID NO:62)
H31	FR2	WVRQAPGKGLEWMG (SEQ ID NO:53)	43	K	Q	WVRQAPGQGLEWMG (SEQ ID NO:54)
	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	61	S	E	VIWGGGSTNYNEALQD (SEQ ID NO:44)
			64	K	Q	
			65	S	D	
	FR4	WGQGTLVTVSS (SEQ ID NO:61)	105	Q	E	WGEGTLVTVSS (SEQ ID NO:62)
H32	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	61	S	E	VIWGGGSTNYNEALQD (SEQ ID NO:44)
			64	K	Q	
			65	S	D	
	FR4	WGQGTLVTVSS (SEQ ID NO:61)	105	Q	E	WGEGTLVTVSS (SEQ ID NO:62)
H33	FR2	WVRQAPGKGLEWMG (SEQ ID NO:53)	43	K	Q	WVRQAPGQGLEWMG (SEQ ID NO:54)
	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	61	S	E	VIWGGGSTNYNEALQD (SEQ ID NO:44)
			64	K	Q	
			65	S	D	
	FR4	WGQGTLVTVSS (SEQ ID NO:61)	105	Q	E	WGEGTLVTVSS (SEQ ID NO:62)

Table 8 is a continuation of Table 7.

[Table 9]

NAME	CLASSIFICATION	LO SEQUENCE	MUTATED POSITION (kabat No.)	AMINO ACID BEFORE MUTATION (LO)	AMINO ACID AFTER MUTATION	SEQUENCE AFTER MUTATION
L1	CDR1	RSSQNIVHTNGNTYLE (SEQ ID NO:84)	24	R	Q	QSSQNIVHTNGNTYLE (SEQ ID NO:85)
L3	CDR1	RSSQNIVHTNGNTYLE (SEQ ID NO:84)	24	R	Q	QSSENIVHTNGNTYLE (SEQ ID NO:86)
			27	Q	E	
L4	FR2	WYQQKPGKAPELLIY (SEQ ID NO:96)	42	K	E	WYQQKPGEAPELLIY (SEQ ID NO:97)
L5	FR2	WYQQKPGKAPELLIY (SEQ ID NO:96)	42	K	Q	WYQQKPGQAPELLIY (SEQ ID NO:98)
L10	FR4	FGQGTKLEIK (SEQ ID NO:103)	104	L	V	FGQGTKVEIE (SEQ ID NO:104)
			107	K	E	
L11	CDR1	RSSQNIVHTNGNTYLE (SEQ ID NO:84)	24	R	Q	QSSQNIVHTNGNTYLE (SEQ ID NO:85)
	FR4	FGQGTKLEIK (SEQ ID NO:103)	104	L	V	FGQGTKVEIE (SEQ ID NO:104)
			107	K	E	
L12	CDR1	RSSQNIVHTNGNTYLE (SEQ ID NO:84)	24	R	Q	QSSQNIVHTNGNTYLE (SEQ ID NO:85)
	FR2	WYQQKPGKAPELLIY (SEQ ID NO:96)	42	K	E	WYQQKPGEAPELLIY (SEQ ID NO:97)
	FR4	FGQGTKLEIK (SEQ ID NO:103)	104	L	V	FGQGTKVEIE (SEQ ID NO:104)
			107	K	E	
L13	CDR1	RSSQNIVHTNGNTYLE (SEQ ID NO:84)	24	R	Q	QSSQNIVHTNGNTYLE (SEQ ID NO:85)
	FR2	WYQQKPGKAPELLIY (SEQ ID NO:96)	42	K	Q	WYQQKPGQAPELLIY (SEQ ID NO:98)
	FR4	FGQGTKLEIK (SEQ ID NO:103)	104	L	V	FGQGTKVEIE (SEQ ID NO:104)
			107	K	E	

[Table 10]

NAME	CLASSIFICATION	H9 SEQUENCE	MUTATED POSITION (kabat No.)	AMINO ACID BEFORE MUTATION (H9)	AMINO ACID AFTER MUTATION	SEQUENCE AFTER MUTATION
H34	CDR1	SFGVD (SEQ ID NO:33)	31	S	K	KFGVD (SEQ ID NO:36)
H35	CDR1	SFGVD (SEQ ID NO:33)	31	S	R	RFGVD (SEQ ID NO:37)
H36	FR2	WVRQAPGKGLEWMG (SEQ ID NO:53)	41	P	R	WVRQARGQRLEWMG (SEQ ID NO:56)
			43	K	Q	
			44	G	R	
H37	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	61	S	R	VIWGGGSTNYNRALKS (SEQ ID NO:47)
H38	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	61	S	K	VIWGGGSTNYNKALKS (SEQ ID NO:48)
H39	FR4	WGQGTLVTVSS (SEQ ID NO:61)	105	Q	R	WGRGTLVTVSS (SEQ ID NO:63)
H40	CDR1	SFGVD (SEQ ID NO:33)	31	S	K	KFGVD (SEQ ID NO:36)
	FR2	WVRQAPGKGLEWMG (SEQ ID NO:53)	41	P	R	WVRQARGQRLEWMG (SEQ ID NO:56)
			43	K	Q	
			44	G	R	
H41	CDR1	SFGVD (SEQ ID NO:33)	31	S	R	RFGVD (SEQ ID NO:37)
	FR2	WVRQAPGKGLEWMG (SEQ ID NO:53)	41	P	R	WVRQARGQRLEWMG (SEQ ID NO:56)
			43	K	Q	
			44	G	R	
H46	FR2	WVRQAPGKGLEWMG (SEQ ID NO:53)	41	P	R	WVRQARGQRLEWMG (SEQ ID NO:56)
			43	K	Q	
			44	G	R	
	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	61	S	K	VIWGGGSTNYNKALKS (SEQ ID NO:48)

[Table 11]

NAME	CLASSIFICATION	H9 SEQUENCE	MUTATED POSITION (kabat No.)	AMINO ACID BEFORE MUTATION (H9)	AMINO ACID AFTER MUTATION	SEQUENCE AFTER MUTATION
H47	FR2	WVRQAPGKGLEWMG (SEQ ID NO:53)	41	P	R	WVRQARGQRLEWMG (SEQ ID NO:56)
			43	K	Q	
			44	G	R	
	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	61	S	R	VIWGGGSTNYNRALKS (SEQ ID NO:47)
H48	FR2	WVRQAPGKGLEWMG (SEQ ID NO:53)	41	P	R	WVRQARGQRLEWMG (SEQ ID NO:56)
			43	K	Q	
			44	G	R	
	FR4	WGQGTLVTVSS (SEQ ID NO:61)	105	Q	R	WGRGTLVTVSS (SEQ ID NO:63)
H49	FR2	WVRQAPGKGLEWMG (SEQ ID NO:53)	41	P	R	WVRQARGQRLEWMG (SEQ ID NO:56)
			43	K	Q	
			44	G	R	
	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	61	S	K	VIWGGGSTNYNKALKS (SEQ ID NO:48)
	FR4	WGQGTLVTVSS (SEQ ID NO:61)	105	Q	R	WGRGTLVTVSS (SEQ ID NO:63)
H50	FR2	WVRQAPGKGLEWMG (SEQ ID NO:53)	41	P	R	WVRQARGQRLEWMG (SEQ ID NO:56)
			43	K	Q	
			44	G	R	
	CDR2	VIWGGGSTNYNSALKS (SEQ ID NO:38)	61	S	R	VIWGGGSTNYNRALKS (SEQ ID NO:47)
	FR4	WGQGTLVTVSS (SEQ ID NO:61)	105	Q	R	WGRGTLVTVSS (SEQ ID NO:63)

Table 11 is a continuation of Table 10.

[Table 12]

NAME	CLASSIFICATION	LO SEQUENCE	MUTATED POSITION (kabat No.)	AMINO ACID BEFORE MUTATION (LO)	AMINO ACID AFTER MUTATION	SEQUENCE AFTER MUTATION
L25	FR1	DVVMTQSPLSLPVTLGQPASISC (SEQ ID NO:93)	17	Q	R	DVVMTQSPLSLPVTLGRPASISC (SEQ ID NO:94)
L27	CDR1	RSSQNIVHTNGNTYLE (SEQ ID NO:84)	27	Q	R	RSSRNIVHTNGNTYLE (SEQ ID NO:87)
L28	FR2	WYQQKPGKAPELLIY (SEQ ID NO:96)	45	E	K	WYQQKPGKAPKLLIY (SEQ ID NO:99)
L29	FR4	FGQGTKLEIK (SEQ ID NO:103)	100	Q	R	FGRGTKLEIK (SEQ ID NO:105)
L31	CDR1	RSSQNIVHTNGNTYLE (SEQ ID NO:84)	27	Q	R	RSSRNIVHTNGNTYLE (SEQ ID NO:87)
	FR2	WYQQKPGKAPELLIY (SEQ ID NO:96)	45	E	K	WYQQKPGKAPKLLIY (SEQ ID NO:99)
L34	FR1	DVVMTQSPLSLPVTLGQPASISC (SEQ ID NO:93)	17	Q	R	DVVMTQSPLSLPVTLGRPASISC (SEQ ID NO:94)
	CDR1	RSSQNIVHTNGNTYLE (SEQ ID NO:84)	27	Q	R	RSSRNIVHTNGNTYLE (SEQ ID NO:87)
	FR2	WYQQKPGKAPELLIY (SEQ ID NO:96)	45	E	K	WYQQKPGKAPKLLIY (SEQ ID NO:99)
L35	CDR1	RSSQNIVHTNGNTYLE (SEQ ID NO:84)	27	Q	R	RSSRNIVHTNGNTYLE (SEQ ID NO:87)
	FR2	WYQQKPGKAPELLIY (SEQ ID NO:96)	45	E	K	WYQQKPGKAPKLLIY (SEQ ID NO:99)
	FR4	FGQGTKLEIK (SEQ ID NO:103)	100	Q	R	FGRGTKLEIK (SEQ ID NO:105)
L37	FR1	DVVMTQSPLSLPVTLGQPASISC (SEQ ID NO:93)	17	Q	R	DVVMTQSPLSLPVTLGRPASISC (SEQ ID NO:94)
	CDR1	RSSQNIVHTNGNTYLE (SEQ ID NO:84)	27	Q	R	RSSRNIVHTNGNTYLE (SEQ ID NO:87)
	FR2	WYQQKPGKAPELLIY (SEQ ID NO:96)	45	E	K	WYQQKPGKAPKLLIY (SEQ ID NO:99)
	FR4	FGQGTKLEIK (SEQ ID NO:103)	100	Q	R	FGRGTKLEIK (SEQ ID NO:105)

[Table 13]

HEAVY CHAIN	LIGHT CHAIN	SEQ ID NO OF HEAVY CHAIN	SEQ ID NO OF LIGHT CHAIN	KD(M)
H9	L0	3	65	3.58E-09
H9	L1	3	66	2.49E-09
H9	L3	3	67	2.97E-09
H9	L4	3	68	2.64E-09
H9	L5	3	69	2.53E-09
H9	L10	3	70	2.92E-09
H9	L11	3	71	2.40E-09
H9	L12	3	72	2.55E-09
H9	L13	3	73	2.45E-09
H17	L0	4	65	3.46E-09
H18	L0	5	65	4.31E-09
H19	L0	6	65	2.63E-09
H20	L0	7	65	3.21E-09
H21	L0	8	65	2.35E-09
H22	L0	9	65	3.14E-09
H23	L0	10	65	2.39E-09
H24	L0	11	65	2.56E-09
H25	L0	12	65	2.50E-09
H26	L0	13	65	2.50E-09
H26	L10	13	70	2.38E-09
H26	L11	13	71	2.71E-09
H26	L12	13	72	2.79E-09
H26	L13	13	73	2.50E-09
H27	L0	14	65	3.03E-09
H28	L0	15	65	2.94E-09
H30	L0	16	65	2.70E-09
H31	L0	17	65	2.54E-09
H31	L10	17	70	3.03E-09
H31	L11	17	71	3.05E-09
H31	L12	17	72	3.14E-09
H32	L0	18	65	2.82E-09
H32	L10	18	70	2.80E-09
H32	L11	18	71	2.74E-09
H32	L12	18	72	2.64E-09
H32	L13	18	73	2.74E-09
H33	L0	19	65	3.04E-09
H33	L11	19	71	3.06E-09
H33	L12	19	72	3.06E-09
H33	L13	19	73	3.02E-09

[Table 14]

HEAVY CHAIN	LIGHT CHAIN	SEQ ID NO OF HEAVY CHAIN	SEQ ID NO OF LIGHT CHAIN	KD(M)
H9	L25	3	74	3.54E-09
H9	L27	3	75	3.07E-09
H9	L28	3	76	3.06E-09
H9	L29	3	77	3.49E-09
H9	L31	3	78	2.46E-09
H9	L34	3	79	2.80E-09
H9	L35	3	80	2.37E-09
H9	L37	3	82	2.69E-09
H34	L0	20	65	5.94E-09
H35	L0	21	65	3.65E-09
H36	L0	22	65	1.96E-09
H36	L1	22	66	2.03E-09
H36	L10	22	70	1.87E-09
H36	L11	22	71	2.08E-09
H36	L27	22	75	1.94E-09
H36	L28	22	76	1.95E-09
H36	L31	22	78	1.67E-09
H36	L34	22	79	2.01E-09
H36	L35	22	80	1.94E-09
H36	L37	22	82	2.20E-09
H37	L0	23	65	2.92E-09
H38	L0	24	65	2.92E-09
H39	L0	25	65	3.43E-09
H40	L0	26	65	3.51E-09
H41	L0	27	65	2.35E-09
H46	L0	28	65	1.87E-09
H46	L1	28	66	1.84E-09
H46	L10	28	70	1.88E-09
H46	L11	28	71	1.80E-09
H46	L27	28	75	1.90E-09
H46	L28	28	76	1.86E-09
H46	L31	28	78	1.90E-09
H46	L34	28	79	2.22E-09
H46	L35	28	80	2.04E-09
H46	L37	28	82	2.30E-09

[Table 15]

HEAVY CHAIN	LIGHT CHAIN	SEQ ID NO OF HEAVY CHAIN	SEQ ID NO OF LIGHT CHAIN	KD(M)
H47	L0	29	65	1.84E-09
H47	L1	29	66	1.89E-09
H47	L10	29	70	1.89E-09
H47	L11	29	71	1.83E-09
H47	L27	29	75	1.60E-09
H47	L28	29	76	1.52E-09
H47	L31	29	78	1.83E-09
H47	L34	29	79	2.20E-09
H47	L35	29	80	1.68E-09
H47	L37	29	82	2.38E-09
H48	L0	30	65	2.09E-09
H48	L1	30	66	2.09E-09
H48	L10	30	70	2.06E-09
H48	L11	30	71	2.00E-09
H48	L27	30	75	1.99E-09
H48	L28	30	76	2.07E-09
H48	L31	30	78	1.98E-09
H48	L34	30	79	2.27E-09
H48	L35	30	80	2.07E-09
H48	L37	30	82	2.19E-09
H49	L0	31	65	2.06E-09
H49	L1	31	66	1.91E-09
H49	L10	31	70	2.05E-09
H49	L11	31	71	1.92E-09
H49	L27	31	75	2.08E-09
H49	L28	31	76	2.11E-09
H49	L31	31	78	1.94E-09
H49	L34	31	79	2.21E-09
H49	L35	31	80	2.08E-09
H49	L37	31	82	2.43E-09
H50	L0	32	65	1.89E-09
H59	L1	32	66	1.86E-09
H50	L10	32	70	1.90E-09
H50	L11	32	71	1.82E-09
H50	L27	32	75	1.95E-09
H50	L28	32	76	1.97E-09
H50	L31	32	78	1.93E-09
H50	L34	32	79	2.36E-09
H50	L35	32	80	2.24E-09
H50	L37	32	82	2.72E-09

[Example 4] Introduction of mutations that suppress deamidation reactions

Antibodies used for pharmaceuticals have heterogeneity even though they are monoclonal antibodies obtained from a clone derived from a single antibody-producing cell. Such antibody heterogeneity occurs due to modifications such as oxidation and deamidation, and is known to increase during long term storage or when subjected to stress conditions such as heat stress or light stress (Reference Document: Heterogeneity of Monoclonal Antibodies: Journal of Pharmaceutical Sciences, vol. 97, No. 7, 2426-2447). However, when developing an antibody as a pharmaceutical, the physicochemical properties of that protein, particularly homogeneity and stability, are extremely important, and reducing the heterogeneity of the substance of interest and, if possible, being a single substance is desired.

Deamidation reaction takes place non-enzymatically in the asparagine (N) and glutamine (Q) side chains, and is a reaction in which the amides present in the asparagine and glutamine side chains are changed to carboxylic acids. Deamidation reaction which takes place during storage causes the above-mentioned heterogeneity; therefore, it is desirably suppressed as much as possible. Furthermore, it is reported that the deamidation reaction readily occurs particularly at a site where asparagine (N) and glycine (G) are next to each other (···NG···) (Geiger *et al.*, J. Biol. Chem. 1987; 262: 785-794). Since a sequence in which asparagine (N) and glycine (G) are next to each other exists in CDR1 of L0 (SEQ ID NO: 65), amino acid substitution at this site was considered to enable suppression of the deamidation reaction.

Specifically, suppression of deamidation reaction by amino acid substitution was carried out as follows. Substitution of alanine (A) for glycine (G) at position 29 by Kabat numbering in L0 (SEQ ID NO: 65) was considered to enable suppression of the deamidation reaction. Therefore, L36 (SEQ ID NO: 81) in which glycine (G) is replaced with alanine (A) at position 29 by Kabat numbering in L0 (SEQ ID NO: 65) was produced. Similarly, substitution of alanine (A) for glycine (G) at position 29 by Kabat numbering in L11 (SEQ ID NO: 71) was considered to enable suppression of the deamidation reaction. Therefore, L21 (SEQ ID NO: 83) in which glycine (G) is replaced with alanine (A) at position 29 by Kabat numbering in L11 (SEQ ID NO: 71) was produced. These were used to produce H9/L36 (H chain: H9/SEQ ID NO: 3; L chain: L36/SEQ ID NO: 81), H36/L36 (H chain: H36/SEQ ID NO: 22; L chain: L36/SEQ ID NO: 81), and H32/L21 (H chain: H32/SEQ ID NO: 18; L chain: L21/SEQ ID NO: 83). These variants were produced and purified by the method of Example 1.

Affinity measurements on the produced variants were carried out by the method of Example 2. The results are shown in Table 16. All variants had affinity that was not remarkably decreased compared to that of H9/L0, and suppression of the deamidation reaction was considered to be possible.

[Table 16]

H	L	KD(M)
H9	L0	3.58E-09
H9	L36	3.60E-09
H36	L36	2.59E-09
H32	L21	3.81E-09

[Example 5] Discovery of mutation sites for increasing the antibody expression level

As a method for producing antibody pharmaceuticals, a method that uses mammalian cells to construct a stable transfectant that produces the antibody of interest is generally used. Herein, since the level of antibody expression by the stable transfectant is an important factor linked to production cost of antibody pharmaceuticals, it is desirable that the antibody expression level is sufficiently high.

To evaluate the effects of Ax225 antibody humanization on the antibody expression level, four types of antibodies, chimeric chH/chL antibody (H chain chH/SEQ ID NO: 1; L chain chL/SEQ ID NO: 64); H0/chL wherein the H chain alone is humanized (H chain H0/SEQ ID NO: 2; L chain chL/SEQ ID NO: 64); chH/L0 wherein the L chain alone is humanized (H chain chH/SEQ ID NO: 1; L chain L0/SEQ ID NO: 65); and H0/L0 wherein both chains are humanized (H chain H0/SEQ ID NO: 2; L chain L0/SEQ ID NO: 65), were expressed according to the method of Example 1.

Quantification of antibody concentration in the culture supernatant by Biacore-Q

Quantification of antibody concentration in the culture supernatant using Biacore-Q (BIACORE) was carried out by the following method.

The sensor chips were produced by immobilizing approximately 5,000 RU of recombinant Protein A onto CM5 (GE Healthcare) by amine coupling. HBS-EP was used as the running buffer, 10 mM glycine-HCl (pH 1.5) was used as the regeneration buffer, and the flow rate was set to 5 μL/min. Furthermore, to produce a calibration curve, the chimeric antibody or the humanized antibody expressed and purified by the method of Example 1 were prepared at concentrations of 2,000, 1,000, 500, 250, 125, and 62.5 ng/mL.

The collected culture supernatant was suitably diluted using HBS-EP to produce antibody concentrations that correctly lie on the calibration curve. The prepared culture supernatant and the samples for calibration curve production were subjected to Biacore-Q, and

by measuring and analyzing using BIACORE Q Control Software on COM1, antibody concentration in the culture supernatant was calculated.

As a result, it was found that humanizing the L chain increased the level of Ax225 antibody expression by two- to three-folds (Fig. 4).

Identification of mutation sites that increase the antibody expression level

From the three-dimensional structural model, the residue at position 42 by Kabat numbering in the L chain FR2 was expected to contribute greatly to the expression level. Specifically, substituting lysine (K) for the residue at position 42 by Kabat numbering was expected to enable enhancement of the expression level. This speculation does not contradict the fact that the residue at position 42 by Kabat numbering in the chimeric antibody L chain (chL/SEQ ID NO: 64) is glutamine (Q), whereas this residue in the humanized antibody L chain (L0/SEQ ID NO: 65) is lysine (K).

To evaluate the effect of the residue at position 42 by Kabat numbering in the L chain on the expression level, H32/L11 (H chain H32/SEQ ID NO: 18; L chain L11/SEQ ID NO: 71) and H32/L12 (H chain H32/SEQ ID NO: 18; L chain L12/SEQ ID NO: 72) were expressed by the method of Example 1, and the antibody concentration in the culture supernatant was measured. The difference between the two types of L chains, L11 and L12, used herein is that the residue at position 42 by Kabat numbering in the L11 is lysine (K) whereas that in L12 is replaced with glutamic acid (E). Since the sequences of the two are completely identical except for position 42 by Kabat numbering, the influence of this residue alone can be evaluated.

The results are shown in Fig. 5. H32/L11 whose position 42 by Kabat numbering is lysine showed an approximately three-fold enhancement of the expression level as compared to H32/L12 in which this residue is glutamic acid. Therefore, substituting lysine for the residue at position 42 by Kabat numbering was shown to enable great enhancement of the expression level (Fig. 5).

[Example 6] Measurement of antitumor effect of humanized Ax225 antibody on mouse xenograft model with human pancreatic adenocarcinoma

6-1. Production of mouse xenograft model with human pancreatic adenocarcinoma

Human pancreatic adenocarcinoma cell line PANC-1 obtained from Dainippon Pharma Co., Ltd. (currently Dainippon Sumitomo Pharma Co., Ltd.) was prepared using HBSS to provide 2.5×10^7 cells/mL. Two-hundred microliters of the cell suspension solution (5×10^6 cells/mouse) was inoculated subcutaneously to the inguinal region of CAnN.Cg-Foxnl<nu>/CrlCrlj nu/nu (BALB-nu/nu) mice purchased from Japan Charles River

Co. Ltd. When the tumor volume reached approximately 240 mm^3, the mice were subjected to the experiment.

6-2. Antibody preparation and administration

The antibody was prepared at 1 mg/mL in PBS, and was administered intraperitoneally to human pancreatic adenocarcinoma-xenografted mice at 10 mg/kg once a week for two weeks. As a negative control, PBS was administered similarly.

6-3. Evaluation of antitumor effects

The antitumor effect in the human pancreatic adenocarcinoma-xenografted mouse model was calculated as a tumor growth inhibiting effect by comparison with the amount of tumor growth in the negative control group seven days after the final administration.

[tumor growth inhibiting effect (%)] = (1 - [amount of tumor growth in the antibody-treated group] / [amount of tumor growth in the control group]) x 100

6-4. Statistical treatment

The tumor volume was expressed by the mean ± standard deviation. Statistical analyses were carried out by comparing the control group and the treated group by the LSD method using SAS Preclinical Package Version 5.0. A 95% confidence level (*; $p < 0.05$) was used to indicate significance.

6-5. Results

As shown in Fig. 6, similarly to the mouse antibody and the chimeric antibody, the humanized Ax225 antibody (H9/L0) showed significant tumor growth inhibiting effects as compared to the PBS-administered group.

[Example 7] Measurement of antitumor effects of humanized anti-AXL antibodies on mouse xenograft model with human pancreatic adenocarcinoma (2)

7-1. Production of mouse xenograft model with human pancreatic adenocarcinoma

Human pancreatic adenocarcinoma cell line PANC-1 obtained from Dainippon Pharma Co., Ltd. (currently Dainippon Sumitomo Pharma Co., Ltd.) was prepared using HBSS to provide 2.5 x 10^7 cells/mL. Two-hundred microliters of the cell suspension solution (5 x 10^6 cells/mouse) was inoculated subcutaneously to the inguinal region of CAnN.Cg-Foxnl<nu>/CrlCrlj nu/nu (BALB-nu/nu) mice purchased from Japan Charles River Co. Ltd. When the tumor volume reached approximately 200 mm^3, the mice were subjected to

the experiment.

7-2. Antibody preparation and administration

Each antibody was prepared at 1 mg/mL in histidine buffer solution (20 mM Histidine-HCl, 150 mM NaCl, pH 6.0), and was administered into caudal vein of human pancreatic adenocarcinoma-xenografted mice at 10 mg/kg once a week for two weeks. As a negative control, histidine buffer solution was administered similarly.

7-3. Evaluation of antitumor effects

The antitumor effects in the human pancreatic adenocarcinoma-xenografted mouse model were calculated as a tumor growth inhibiting effect by comparing the amount of tumor in each antibody-administered group and the amount of tumor in the negative control group seven days after the final administration.

7-4. Statistical treatment

The tumor volume was expressed by the mean ± standard deviation. Statistical analyses were carried out by comparing the control group and the treated group by the LSD method using SAS Preclinical Package Version 5.0. A 95% confidence level (*; $p < 0.05$) was used to indicate significance.

7-5. Results

As shown in Fig. 7, the antibody-administered groups showed significant tumor growth inhibiting effects as compared to the histidine buffer solution-administered group.

Industrial Applicability

The present inventors succeeded in obtaining humanized anti-AXL antibodies. The anti-AXL antibodies of the present invention have high antitumor activity, and are useful as antitumor agents and diagnostic agents for cancer.

CLAIMS

1. An antibody that recognizes FND1 domain of AXL, which is an antibody of any one of (1) to (6) below:

(1) an antibody comprising a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 33 to 37, a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 38 to 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(2) an antibody comprising a heavy chain variable region of SEQ ID NO: 2 (H0);

(3) an antibody comprising a light chain variable region comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 84 to 89, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 91;

(4) an antibody comprising a light chain variable region of SEQ ID NO: 65 (L0);

(5) an antibody comprising the heavy chain variable region of (1) and the light chain variable region of (3); and

(6) an antibody having one or more amino acid substitutions, deletions, additions, and/or insertions in the antibody of any one of (1) to (5), which has equivalent activity as the antibody of any one of (1) to (5).

2. A humanized antibody that recognizes FND1 domain of AXL, which is an antibody of any one of (1) to (6) below:

(1) an antibody comprising a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 33 to 37, a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 38 to 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49, as well as an FR1 comprising the amino acid sequence of SEQ ID NO: 51, an FR2 comprising the amino acid sequence of SEQ ID NO: 53, an FR3 comprising the amino acid sequence of SEQ ID NO: 109 or 58, and an FR4 comprising the amino acid sequence of SEQ ID NO: 61;

(2) an antibody comprising the heavy chain variable region of SEQ ID NO: 2 (H0);

(3) an antibody comprising a light chain variable region comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 84 to 89, a CDR2 comprising the amino acid sequence SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 91, as well as an FR1 comprising the amino acid sequence of SEQ ID NO: 93, an FR2 comprising the amino acid sequence of SEQ ID NO: 96, an FR3 comprising the amino acid sequence of

SEQ ID NO: 101, and an FR4 comprising the amino acid sequence of SEQ ID NO: 103;
(4) an antibody comprising the light chain variable region of SEQ ID NO: 65 (L0);
(5) an antibody comprising the heavy chain variable region of (1) and the light chain variable region of (3); and
(6) an antibody having one or more amino acid substitutions, deletions, additions, and/or insertions in the antibody of any one of (1) to (5), which has equivalent activity as the antibody of any one of (1) to (5).

3. The antibody of claim 1 or 2, wherein the amino acid residue at position 94 by Kabat numbering in the heavy chain variable region is glycine.

4. The antibody of any one of claims 1 to 3, wherein the amino acid sequence of the heavy chain variable region comprises at least one of the following amino acid residues:
(1) the amino acid residue at position 31 by Kabat numbering in the heavy chain variable region is aspartic acid, glutamic acid, lysine, or arginine;
(2) the amino acid residue at position 40 by Kabat numbering in the heavy chain variable region is proline;
(3) the amino acid residue at position 41 by Kabat numbering in the heavy chain variable region is arginine;
(4) the amino acid residue at position 43 by Kabat numbering in the heavy chain variable region is glutamine or glutamic acid;
(5) the amino acid residue at position 44 by Kabat numbering in the heavy chain variable region is arginine;
(6) the amino acid residue at position 48 by Kabat numbering in the heavy chain variable region is isoleucine;
(7) the amino acid residue at position 61 by Kabat numbering in the heavy chain variable region is glutamic acid, lysine, or arginine;
(8) the amino acid residue at position 62 by Kabat numbering in the heavy chain variable region is glutamic acid;
(9) the amino acid residue at position 64 by Kabat numbering in the heavy chain variable region is glutamine;
(10) the amino acid residue at position 65 by Kabat numbering in the heavy chain variable region is aspartic acid;
(11) the amino acid residue at position 73 by Kabat numbering in the heavy chain variable region is asparagine; and
(12) the amino acid residue at position 105 by Kabat numbering in the heavy chain variable

region is glutamic acid or arginine.

5. The antibody of any one of claims 1 to 3, wherein the amino acid sequence of the heavy chain variable region comprises at least one of the following amino acid residues:
(1) the amino acid residue at position 41 by Kabat numbering in the heavy chain variable region is arginine;
(2) the amino acid residue at position 43 by Kabat numbering in the heavy chain variable region is glutamine;
(3) the amino acid residue at position 44 by Kabat numbering in the heavy chain variable region is arginine;
(4) the amino acid residue at position 61 by Kabat numbering in the heavy chain variable region is arginine; and
(5) the amino acid residue at position 73 by Kabat numbering in the heavy chain variable region is asparagine.

6. The antibody of any one of claims 1 to 5, wherein the amino acid sequence of the light chain variable region comprises at least one of the following amino acid residues:
(1) the amino acid residue at position 17 by Kabat numbering in the light chain variable region is arginine;
(2) the amino acid residue at position 24 by Kabat numbering in the light chain variable region is glutamine;
(3) the amino acid residue at position 27 by Kabat numbering in the light chain variable region is glutamic acid or arginine;
(4) the amino acid residue at position 29 by Kabat numbering in the light chain variable region is alanine;
(5) the amino acid residue at position 42 by Kabat numbering in the light chain variable region is glutamic acid or glutamine;
(6) the amino acid residue at position 45 by Kabat numbering in the light chain variable region is lysine;
(7) the amino acid residue at position 100 by Kabat numbering in the light chain variable region is arginine;
(8) the amino acid residue at position 104 by Kabat numbering in the light chain variable region is valine; and
(9) the amino acid residue at position 107 by Kabat numbering in the light chain variable region is glutamic acid.

7. The antibody of any one of claims 1 to 5, wherein the amino acid sequence of the light chain variable region comprises at least one of the following amino acid residues:
(1) the amino acid residue at position 17 by Kabat numbering in the light chain variable region is arginine;
(2) the amino acid residue at position 24 by Kabat numbering in the light chain variable region is glutamine;
(3) the amino acid residue at position 27 by Kabat numbering in the light chain variable region is arginine;
(4) the amino acid residue at position 29 by Kabat numbering in the light chain variable region is alanine;
(5) the amino acid residue at position 45 by Kabat numbering in the light chain variable region is lysine;
(6) the amino acid residue at position 100 by Kabat numbering in the light chain variable region is arginine;
(7) the amino acid residue at position 104 by Kabat numbering in the light chain variable region is valine; and
(8) the amino acid residue at position 107 by Kabat numbering in the light chain variable region is glutamic acid.

8. The antibody of any one of claims 1 to 5, comprising at least any one of the following heavy chain variable regions:
(1) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;
(2) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 34, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;
(3) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 35, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;
(4) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 39, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;
(5) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 40, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;

(6) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;
(7) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;
(8) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 43, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;
(9) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 44, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;
(10) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;
(11) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 46, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;
(12) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 36, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;
(13) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49;
(14) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49; and
(15) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 33, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 49.

9. The antibody of any one of claims 1 to 7, which is selected from the group consisting of (1) to (25) below:
(1) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ

ID NOs: 84, 90, and 91, respectively;

(2) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 85, 90, and 91, respectively;

(3) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 86, 90, and 91, respectively;

(4) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 87, 90, and 91, respectively;

(5) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 88, 90, and 91, respectively;

(6) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 34, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(7) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 35, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(8) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 39, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(9) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 40, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;

(10) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3comprising the amino acid sequences of SEQ ID NOs: 33, 41, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ

ID NOs: 84, 90, and 91, respectively;
(11) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 42, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;
(12) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 43, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;
(13) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 44, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;
(14) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 44, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 85, 90, and 91, respectively;
(15) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 45, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;
(16) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 46, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;
(17) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 44, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 89, 90, and 91, respectively;
(18) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 36, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;
(19) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 37, 38, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ

ID NOs: 84, 90, and 91, respectively;
(20) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 47, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;
(21) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 48, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 84, 90, and 91, respectively;
(22) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 48, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 85, 90, and 91, respectively;
(23) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 48, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 87, 90, and 91, respectively;
(24) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 47, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 85, 90, and 91, respectively; and
(25) an antibody comprising a heavy chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 33, 47, and 49, respectively, and a light chain variable region comprising CDRs 1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 87, 90, and 91, respectively.

10. The antibody of claim 1 or 2 comprising the heavy chain variable region of any one of SEQ ID NOs: 2 to 32, and the light chain variable region of any one of SEQ ID NOs: 65 to 83.

11. A chimeric antibody comprising the heavy chain variable region of SEQ ID NO: 1, the light chain variable region of SEQ ID NO: 64, and human antibody-derived constant regions, or the antibody of claim 1 having one or more amino acid substitutions, deletions, additions, and/or insertions in the chimeric antibody, which has equivalent activity as the chimeric antibody.

12. The antibody of any one of claims 1 to 11, wherein the amino acid residue at position 42 by Kabat numbering in the amino acid sequence of the light chain variable region is lysine.

13. A pharmaceutical composition comprising the antibody of any one of claims 1 to 12 as an active ingredient.

14. An anticancer agent comprising the antibody of any one of claims 1 to 12 as an active ingredient.

15. The anticancer agent of claim 14, wherein the cancer is pancreatic cancer, gastric cancer, lung cancer, osteosarcoma, colon cancer, prostate cancer, melanoma, endometrial cancer, ovarian cancer, uterine leiomyoma, thyroid cancer, cancer stem cell, breast cancer, bladder cancer, renal cancer, glioma, neuroblastoma, or esophageal cancer.

16. The anticancer agent of claim 14, wherein the cancer is glioma, gastric cancer, endometrial cancer, non-small-cell lung cancer, pancreatic adenocarcinoma, or breast cancer.

17. The anticancer agent of claim 14, wherein the cancer is pancreatic adenocarcinoma or breast cancer.

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<130> C1-A0906P

<150> JP 2009-118725
<151> 2009-05-15

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65 70 75 80

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala
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Thr Leu Val Thr Val Ser Ala
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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
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100 105 110

Thr Leu Val Thr Val Ser Ser
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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
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Thr Leu Val Thr Val Ser Ser

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
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Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
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Thr Leu Val Thr Val Ser Ser
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20 25 30

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu

65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
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Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

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20 25 30

Gly Val Asp Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp Ile
35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
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Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ser Phe

20 25 30

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Glu Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

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20 25 30

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Glu Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Gln
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
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Thr Leu Val Thr Val Ser Ser

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Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ser Phe
20 25 30

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Asp Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

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Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ser Phe
20 25 30

Gly Val Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Gln
50 55 60

Asp Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu

65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
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20 25 30

Gly Val Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Glu Ala Leu Gln
50 55 60

Asp Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
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100 105 110

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20 25 30

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Glu Leu Gln
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Asp Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

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Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ser Phe
20 25 30

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Glu Glu Leu Gln
50 55 60

Asp Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
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Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
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Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ser Phe
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        35                  40                  45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
    50                  55                  60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65                  70                  75                  80

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Glu Ala Leu Gln
50 55 60

Asp Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
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20 25 30

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Glu Ala Leu Gln
50 55 60

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65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Glu Ala Leu Gln
50 55 60

Asp Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
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20 25 30

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
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20 25 30

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Arg Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

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20 25 30

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Lys Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu

65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

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20 25 30

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35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Arg Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
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Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Lys Phe

20 25 30

Gly Val Asp Trp Val Arg Gln Ala Arg Gly Gln Arg Leu Glu Trp Met
35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

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<400> 27

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Arg Phe
20 25 30

Gly Val Asp Trp Val Arg Gln Ala Arg Gly Gln Arg Leu Glu Trp Met
35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

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<400> 28

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Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1               5                  10                  15

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ser Phe
            20                  25                  30

Gly Val Asp Trp Val Arg Gln Ala Arg Gly Gln Arg Leu Glu Trp Met
        35                  40                  45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Lys Ala Leu Lys
    50                  55                  60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65                  70                  75                  80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
                85                  90                  95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser
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115

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<400> 29

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ser Phe
20 25 30

Gly Val Asp Trp Val Arg Gln Ala Arg Gly Gln Arg Leu Glu Trp Met
35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Arg Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

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Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ser Phe
20 25 30

Gly Val Asp Trp Val Arg Gln Ala Arg Gly Gln Arg Leu Glu Trp Met
35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu

65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Arg Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

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<400> 31

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ser Phe
20 25 30

Gly Val Asp Trp Val Arg Gln Ala Arg Gly Gln Arg Leu Glu Trp Met
35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Lys Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Arg Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

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<400> 32

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ser Phe

20 25 30

Gly Val Asp Trp Val Arg Gln Ala Arg Gly Gln Arg Leu Glu Trp Met
35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Arg Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Arg Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
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<400> 33

Ser Phe Gly Val Asp
1 5

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<400> 34

Asp Phe Gly Val Asp
1 5

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Glu Phe Gly Val Asp
1 5

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Lys Phe Gly Val Asp
1 5

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<400> 37

Arg Phe Gly Val Asp
1 5

<210> 38
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<400> 38

Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys Ser

1 5 10 15

<210> 39
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<400> 39

Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Glu Ala Leu Lys Ser
1 5 10 15

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<400> 40

Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Glu Leu Lys Ser
1 5 10 15

<210> 41
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<400> 41

Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Gln Ser
1 5 10 15

<210> 42
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<400> 42

Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys Asp
1 5 10 15

<210> 43
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<400> 43

Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Gln Asp
1 5 10 15

<210> 44
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<400> 44

Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Glu Ala Leu Gln Asp
1 5 10 15

<210> 45
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<400> 45

Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Glu Leu Gln Asp
1 5 10 15

<210> 46
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<400> 46

Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Glu Glu Leu Gln Asp
1 5 10 15

<210> 47
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<400> 47

Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Arg Ala Leu Lys Ser
1 5 10 15

<210> 48
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<220>
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<400> 48

Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Lys Ala Leu Lys Ser
1 5 10 15

<210> 49

<211> 11
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<400> 49

Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr
1 5 10

<210> 50
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<400> 50

Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Leu Ser Leu Thr
20 25 30

<210> 51
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<220>
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<400> 51

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser
20 25 30

<210> 52
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<220>
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<400> 52

Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu Gly
1 5 10

<210> 53
<211> 14
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<400> 53

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met Gly
1 5 10

<210> 54
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<400> 54

Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
1 5 10

<210> 55
<211> 14
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<400> 55

Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp Ile Gly
1 5 10

<210> 56
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<400> 56

Trp Val Arg Gln Ala Arg Gly Gln Arg Leu Glu Trp Met Gly
1 5 10

<210> 57
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<400> 57

Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys
1 5 10 15

Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala Gly
20 25 30

<210> 58
<211> 32
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<400> 58

Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val Val Leu Thr
1 5 10 15

Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Gly
20 25 30

<210> 59
<211> 32
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<400> 59

Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Val Leu Thr
1 5 10 15

Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Gly
20 25 30

<210> 60
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<400> 60

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
1 5 10

<210> 61
<211> 11
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<220>
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<400> 61

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 62
<211> 11
<212> PRT
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<220>
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<400> 62

Trp Gly Glu Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 63

<211> 11
<212> PRT
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<400> 63

Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 64
<211> 112
<212> PRT
<213> Artificial

<220>
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<400> 64

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro

50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Thr Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 65
<211> 112
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<400> 65

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 66
<211> 112
<212> PRT
<213> Artificial

<220>
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<400> 66

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Gln Ser Ser Gln Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala

35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 67
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<212> PRT
<213> Artificial

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<400> 67

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Gln Ser Ser Glu Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 68
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<220>
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<400> 68

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Thr

20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Glu Ala
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 69
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<212> PRT
<213> Artificial

<220>
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<400> 69

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Gln Ala
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 70
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<400> 70

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly

1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
100 105 110

<210> 71
<211> 112
<212> PRT
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<220>
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<400> 71

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Gln Ser Ser Gln Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
100 105 110

<210> 72
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<400> 72

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Gln Ser Ser Gln Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Glu Ala
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
100 105 110

<210> 73
<211> 112
<212> PRT
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<220>
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<400> 73

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Gln Ser Ser Gln Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Gln Ala
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
100 105 110

<210> 74
<211> 112
<212> PRT

<213> Artificial

<220>
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<400> 74

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Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1               5                  10                  15

Arg Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Thr
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
        35                  40                  45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
                85                  90                  95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
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<210> 75

<211> 112
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<400> 75

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Arg Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 76
<211> 112
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<220>
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<400> 76

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys

100 105 110

<210> 77
<211> 112
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<220>
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<400> 77

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Arg Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 78
<211> 112
<212> PRT
<213> Artificial

<220>
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<400> 78

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Arg Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly

85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 79
<211> 112
<212> PRT
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<220>
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<400> 79

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Arg Pro Ala Ser Ile Ser Cys Arg Ser Ser Arg Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 80
<211> 112
<212> PRT
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<220>
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<400> 80

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Arg Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile

65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Arg Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 81
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<400> 81

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Thr
20 25 30

Asn Ala Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 82
<211> 112
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 82

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Arg Pro Ala Ser Ile Ser Cys Arg Ser Ser Arg Asn Ile Val His Thr
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro

50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Arg Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 83
<211> 112
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 83

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Gln Ser Ser Gln Asn Ile Val His Thr
20 25 30

Asn Ala Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Glu Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Ile Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
100 105 110

<210> 84
<211> 16
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 84

Arg Ser Ser Gln Asn Ile Val His Thr Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 85
<211> 16
<212> PRT
<213> Artificial

<220>
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<400> 85

Gln Ser Ser Gln Asn Ile Val His Thr Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 86
<211> 16
<212> PRT
<213> Artificial

<220>
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<400> 86

Gln Ser Ser Glu Asn Ile Val His Thr Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 87
<211> 16
<212> PRT
<213> Artificial

<220>
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<400> 87

Arg Ser Ser Arg Asn Ile Val His Thr Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 88
<211> 16
<212> PRT
<213> Artificial

<220>
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<400> 88

Arg Ser Ser Gln Asn Ile Val His Thr Asn Ala Asn Thr Tyr Leu Glu
1 5 10 15

<210> 89
<211> 16
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 89

Gln Ser Ser Gln Asn Ile Val His Thr Asn Ala Asn Thr Tyr Leu Glu
1 5 10 15

<210> 90
<211> 7
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 90

Lys Val Ser Asn Arg Phe Ser
1 5

<210> 91
<211> 9
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 91

Phe Gln Gly Ser His Ile Pro Phe Thr
1 5

<210> 92
<211> 23
<212> PRT
<213> Artificial

<220>
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<400> 92

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys

20

<210> 93
<211> 23
<212> PRT
<213> Artificial

<220>
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<400> 93

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys
20

<210> 94
<211> 23
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 94

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Arg Pro Ala Ser Ile Ser Cys

20

<210> 95
<211> 15
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 95

Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Glu Leu Leu Ile Tyr
1 5 10 15

<210> 96
<211> 15
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 96

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile Tyr
1 5 10 15

<210> 97
<211> 15
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 97

Trp Tyr Gln Gln Lys Pro Gly Glu Ala Pro Glu Leu Leu Ile Tyr
1 5 10 15

<210> 98
<211> 15
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 98

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Glu Leu Leu Ile Tyr
1 5 10 15

<210> 99
<211> 15
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 99

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 100
<211> 32
<212> PRT
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<220>
<223> an artificially synthesized sequence

<400> 100

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys
20 25 30

<210> 101
<211> 32
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 101

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
20 25 30

<210> 102
<211> 10
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 102

Phe Gly Thr Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 103
<211> 10
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 103

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 104
<211> 10
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 104

Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
1 5 10

<210> 105
<211> 10
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 105

Phe Gly Arg Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 106
<211> 328
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 106

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr

20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu

165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225 230 235 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr

305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro
325

<210> 107
<211> 107
<212> PRT
<213> Homo sapiens

<400> 107

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1 5 10 15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
20 25 30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
35 40 45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
50 55 60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65 70 75 80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
85 90 95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 108
<211> 119
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 108

Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Leu Ser Leu Thr Ser Phe
20 25 30

Gly Val Asp Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Gly Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65 70 75 80

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Arg Glu Gly Ser Lys Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ala
115

<210> 109
<211> 32
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 109

Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val Val Leu Thr
1 5 10 15

Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Arg
20 25 30

<210> 110
<211> 4
<212> PRT
<213> Artificial

<220>

<223> an artificially synthesized sequence

<400> 110

Gly Gly Gly Ser
1

<210> 111
<211> 4
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 111

Ser Gly Gly Gly
1

<210> 112
<211> 5
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 112

Gly Gly Gly Gly Ser
1 5

<210> 113
<211> 5
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 113

Ser Gly Gly Gly Gly
1 5

<210> 114
<211> 6
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 114

Gly Gly Gly Gly Gly Ser
1 5

<210> 115
<211> 6
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 115

Ser Gly Gly Gly Gly Gly
1 5

<210> 116
<211> 7
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 116

Gly Gly Gly Gly Gly Gly Ser
1 5

<210> 117
<211> 7
<212> PRT
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 117

Ser Gly Gly Gly Gly Gly Gly
1 5