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(54) **TUMOR-TARGETING MONOCLONAL
ANTIBODIES TO FZD10 AND USES
THEREOF**

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(57) **ABSTRACT**

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Related U.S. Application Data

(62) Division of application No. 12/308,095, filed on Dec. 8, 2008, now Pat. No. 8,221,751, filed as application No. PCT/JP2006/317155 on Aug. 24, 2006.
(60) Provisional application No. 60/815,257, filed on Jun. 21, 2006.

The present invention relates to an antibody or a fragment thereof which is capable of binding to a Frizzled homologue 10 (FZD10) protein, such as a mouse monoclonal antibody, a chimeric antibody and a humanized antibody. Also, the present invention relates to a method for treating and/or preventing FZD10-associated disease; a method for diagnosis or prognosis of FZD10-associated disease; and a method for in vivo imaging of FZD10 in a subject.

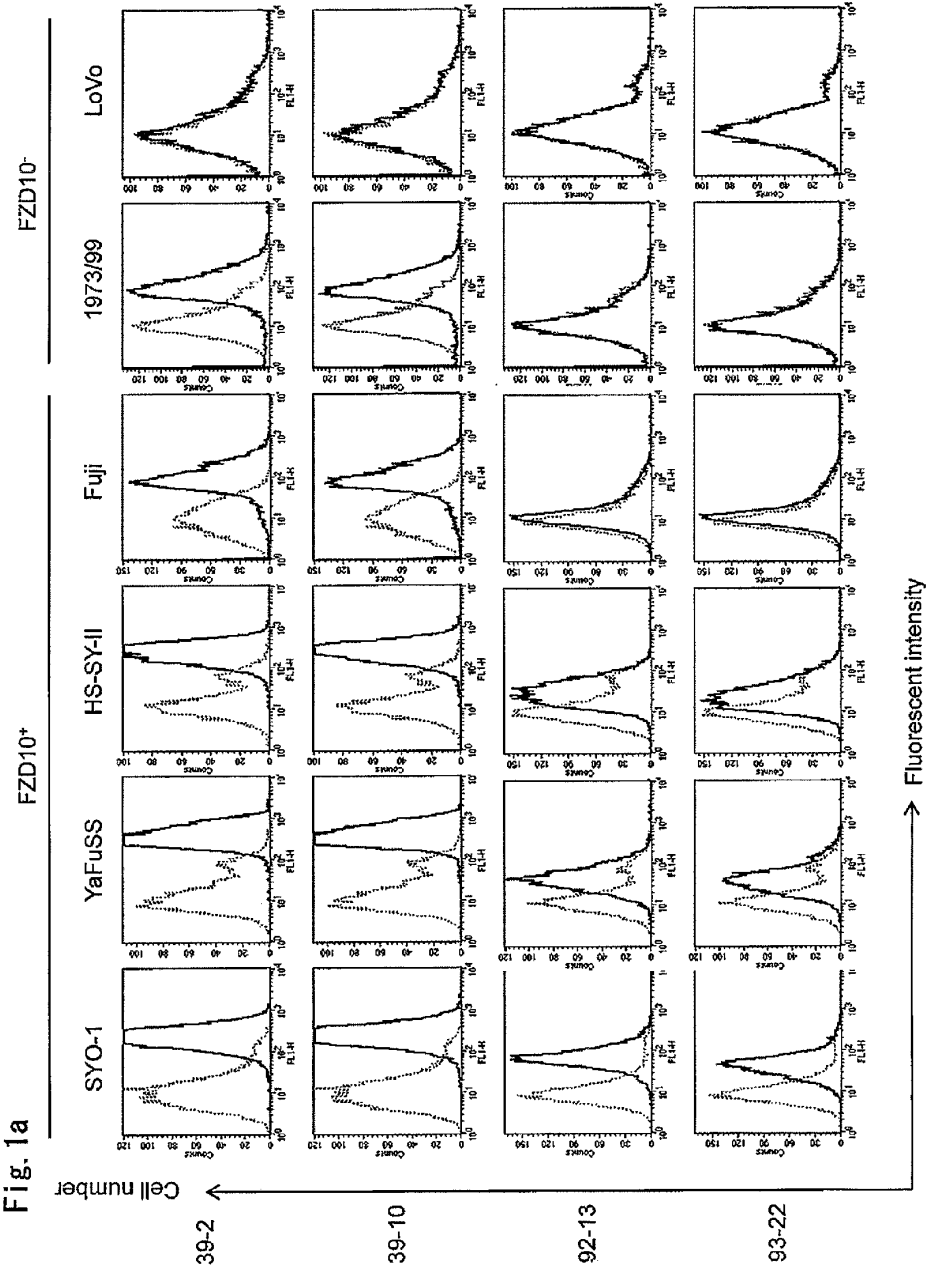


Fig. 1b

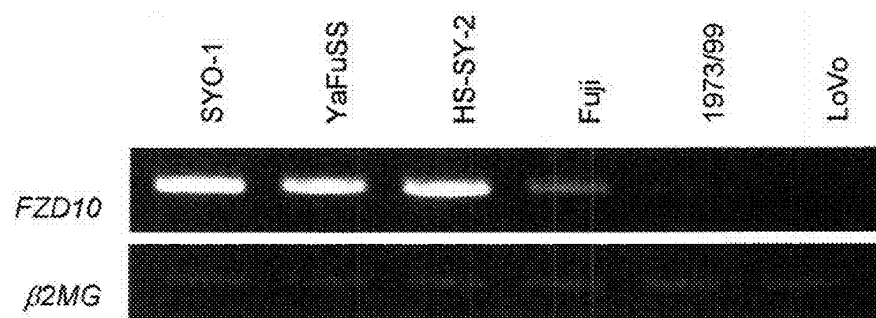


Fig. 1c

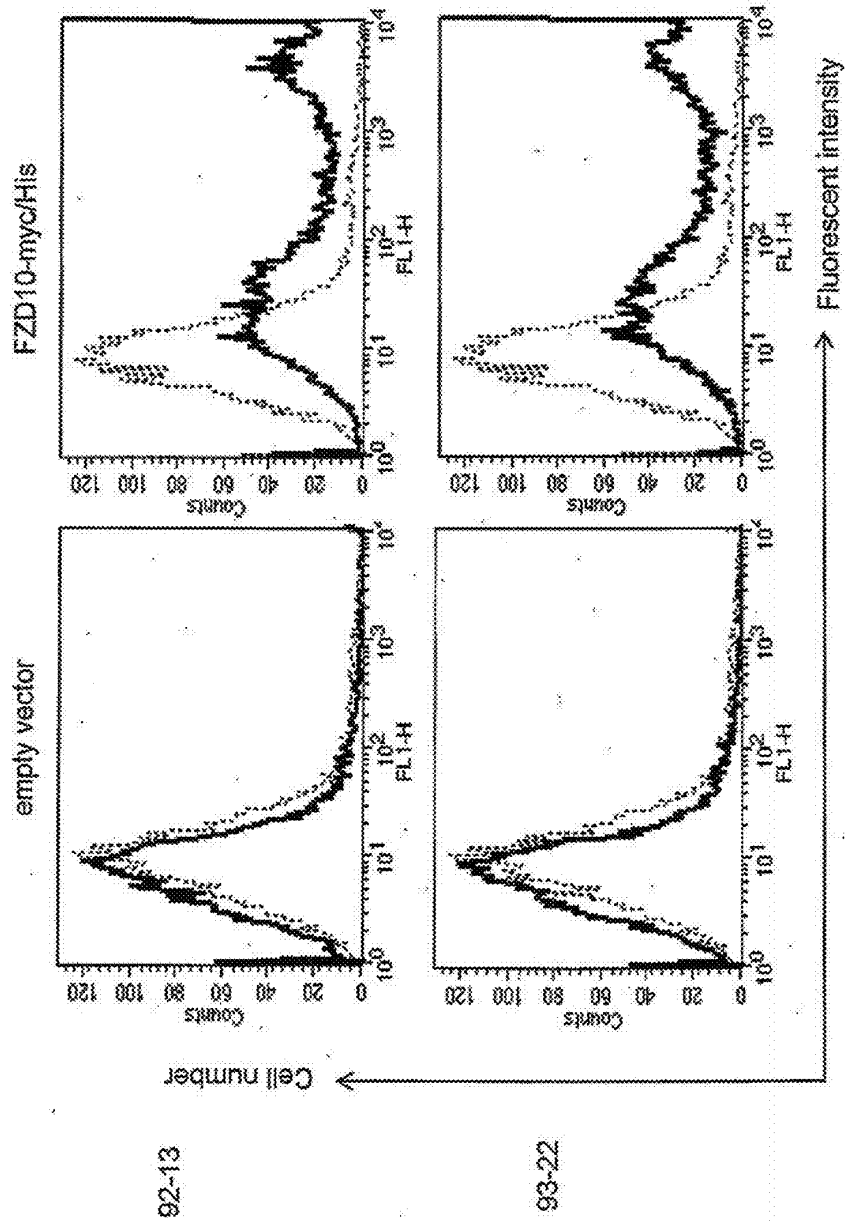


Fig. 1d

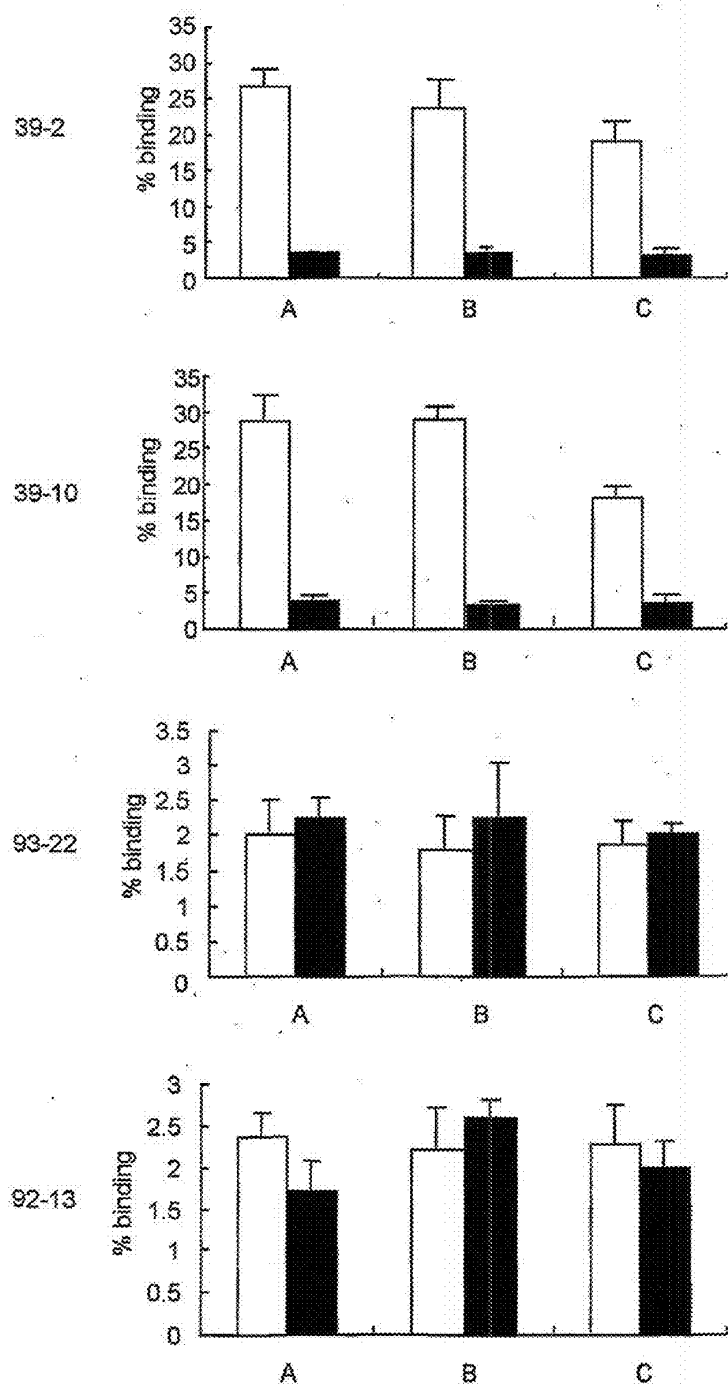


Fig. 1e

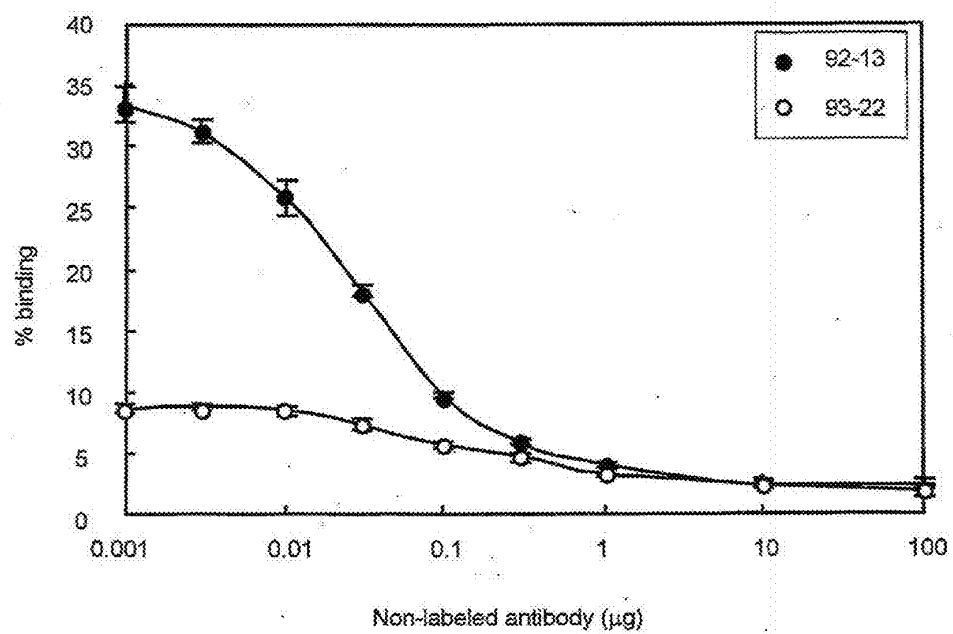


Fig. 1f

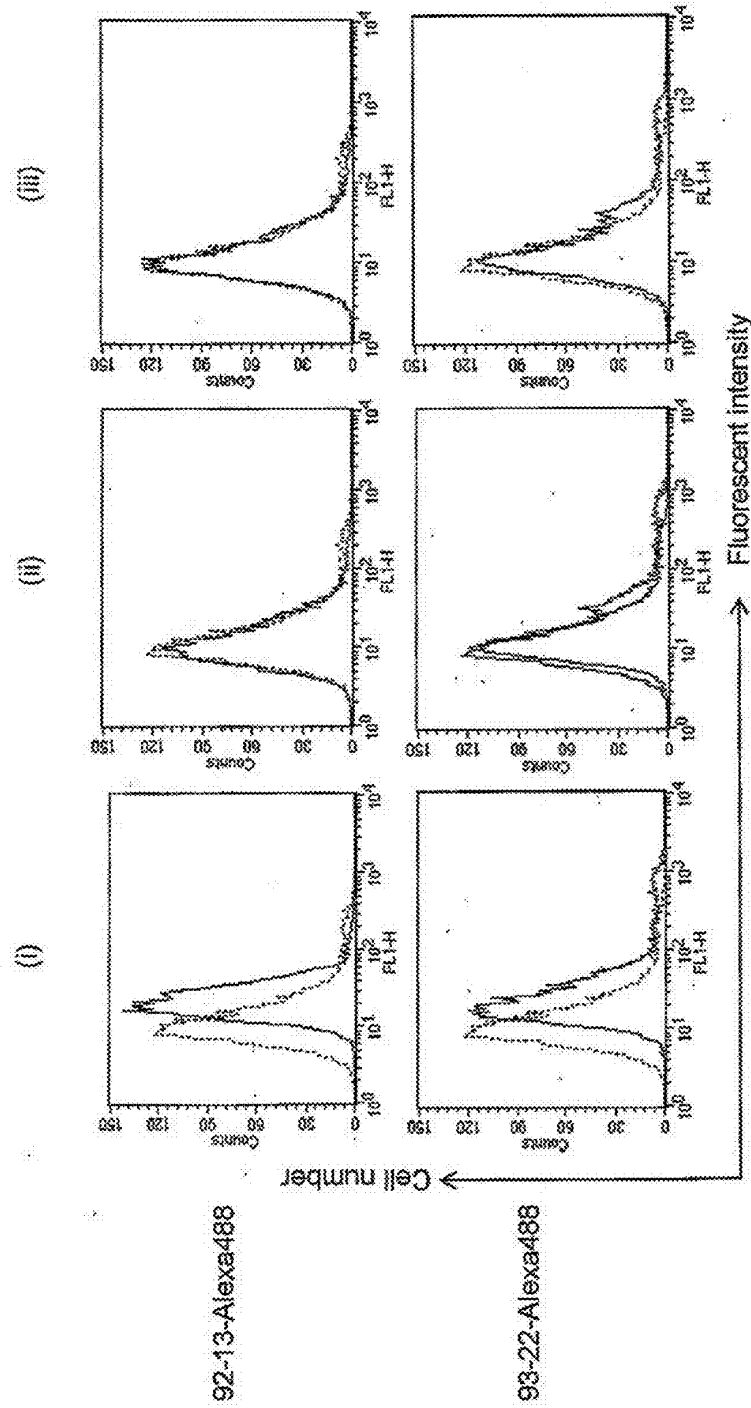


Fig. 2

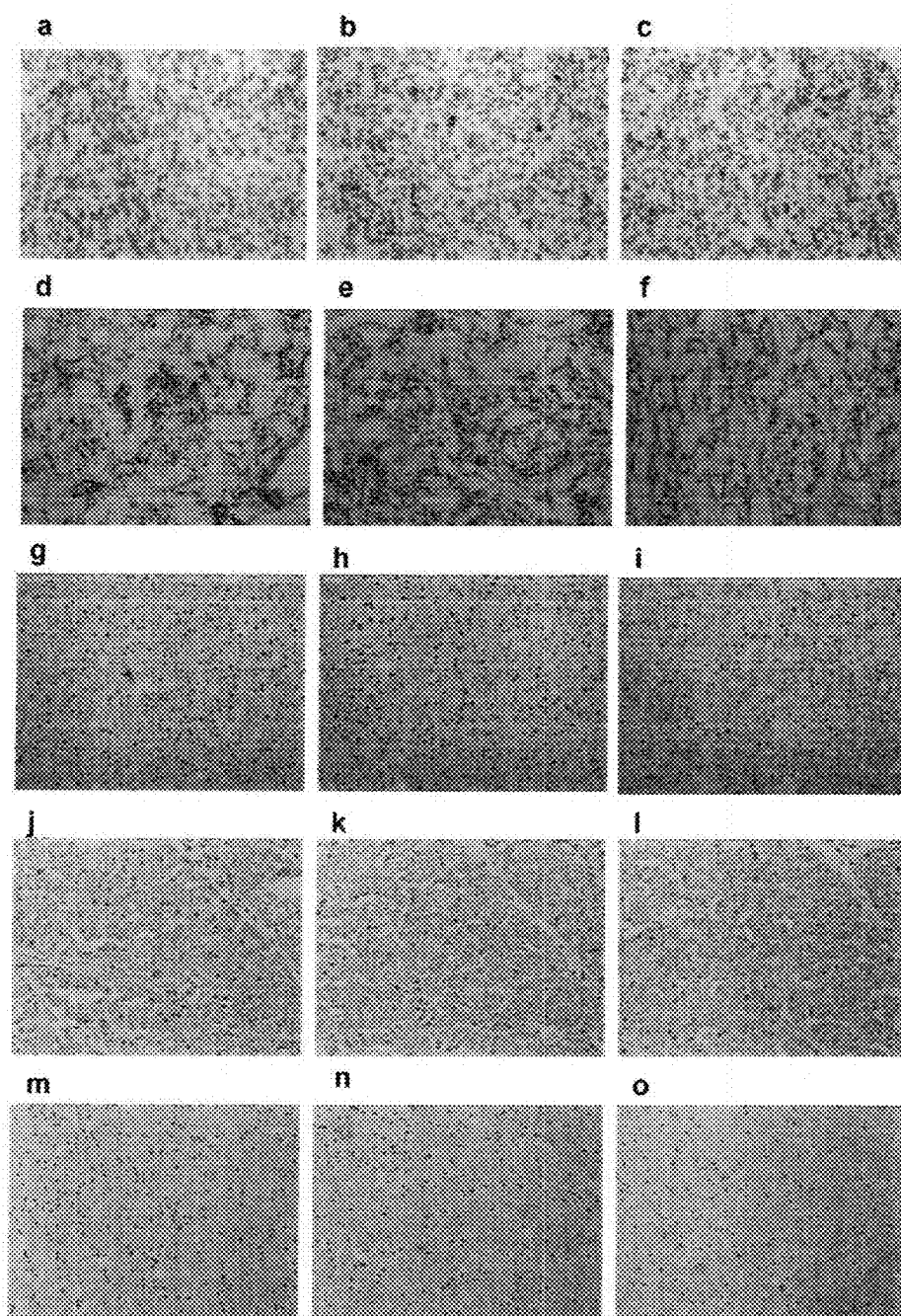


Fig. 3

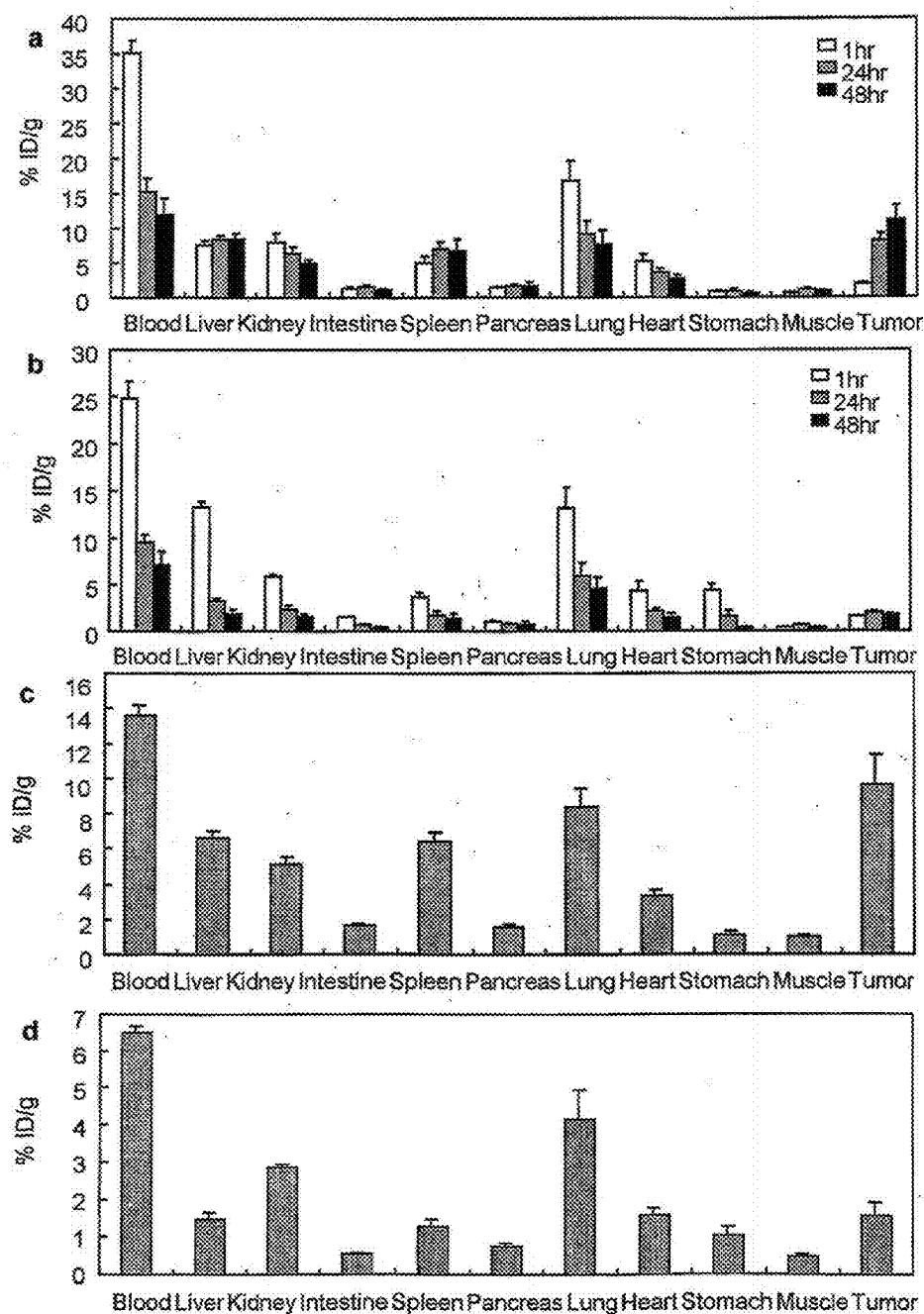


Fig. 4a

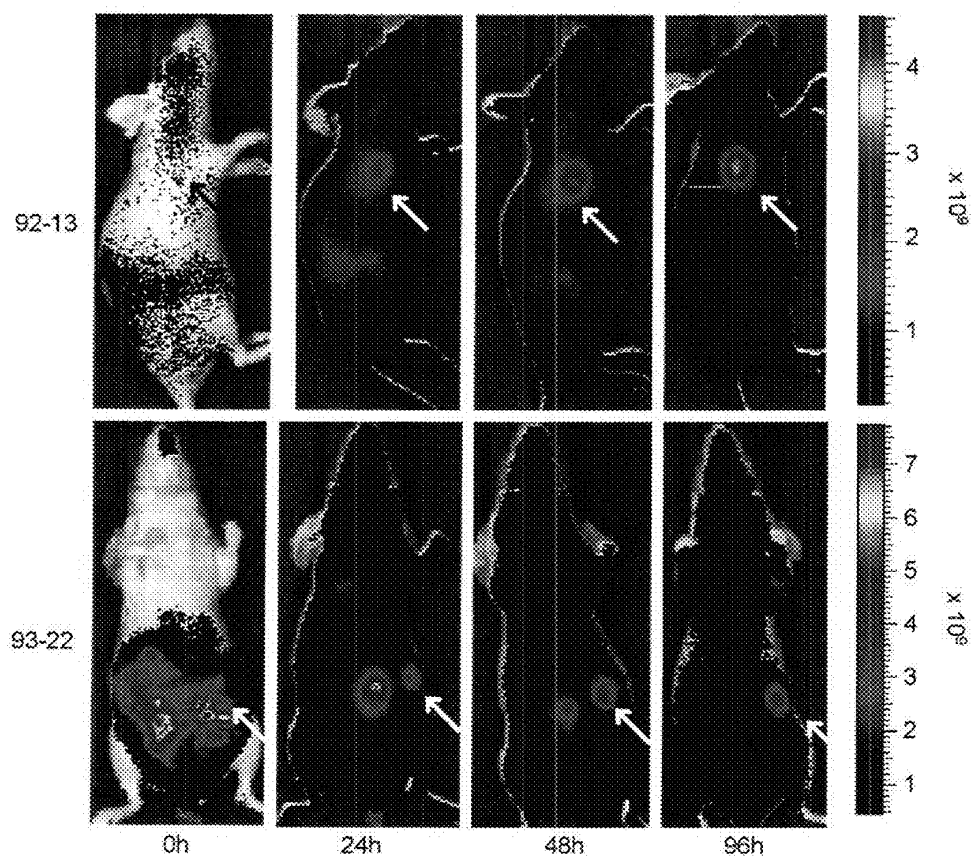


Fig. 4b

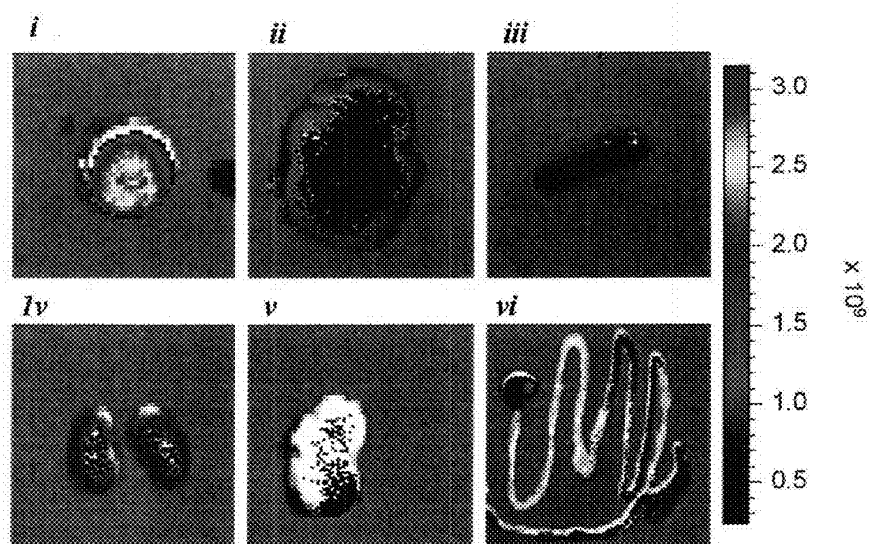


Fig. 4c

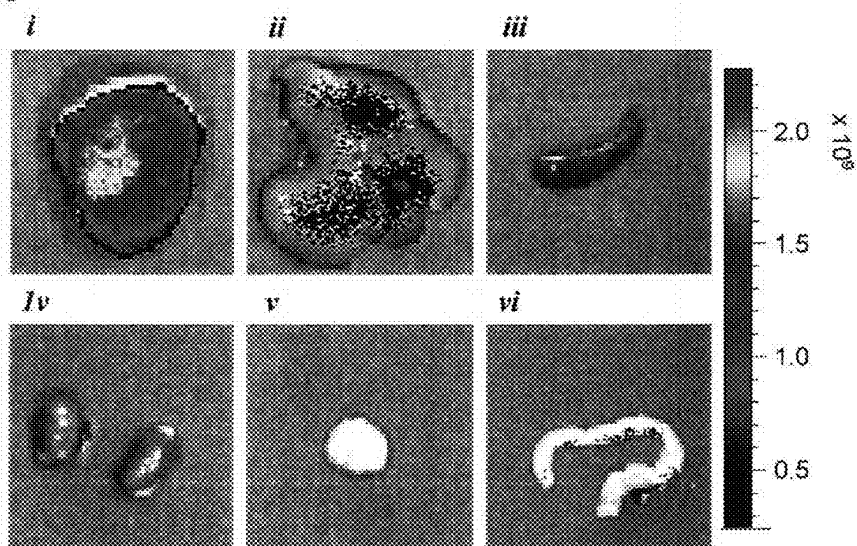


Fig. 5a

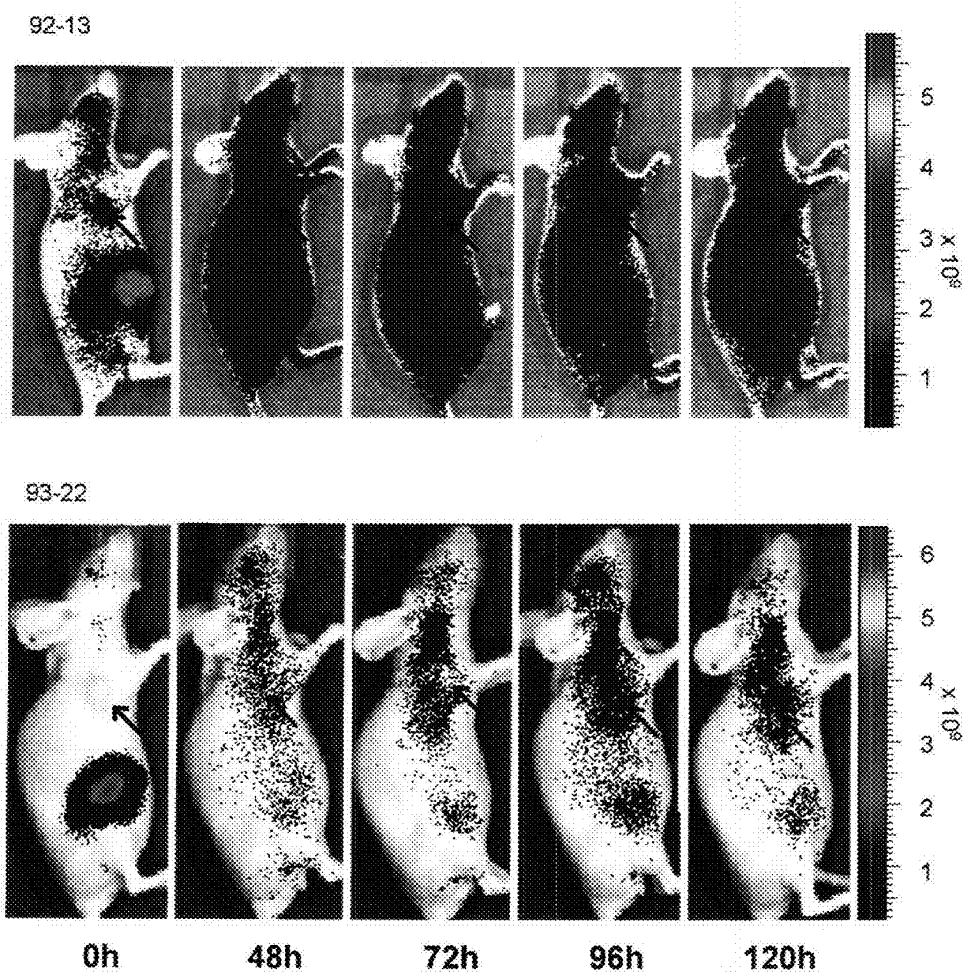


Fig. 5b

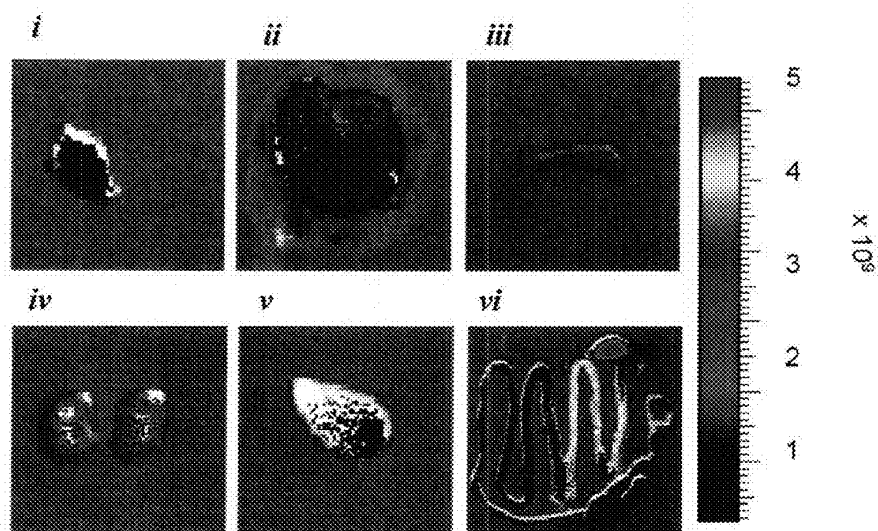


Fig. 5c

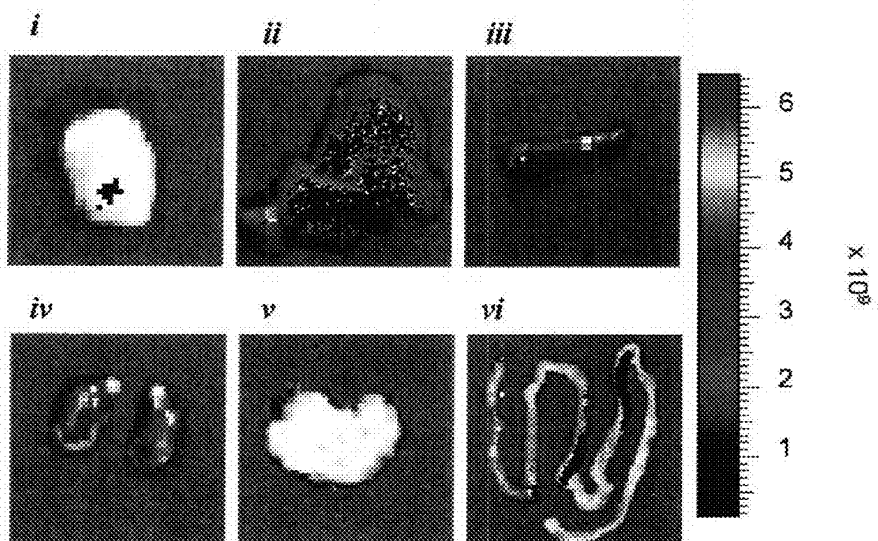


Fig. 6

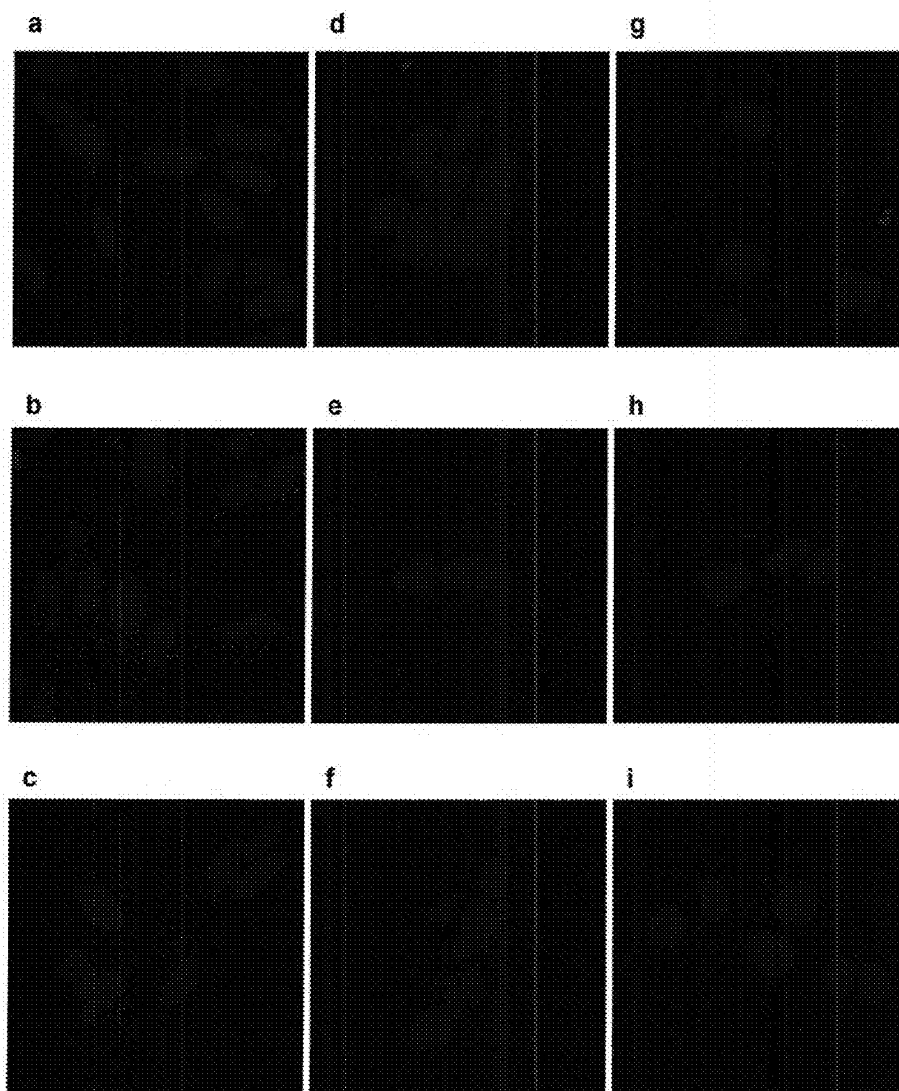


Fig. 7

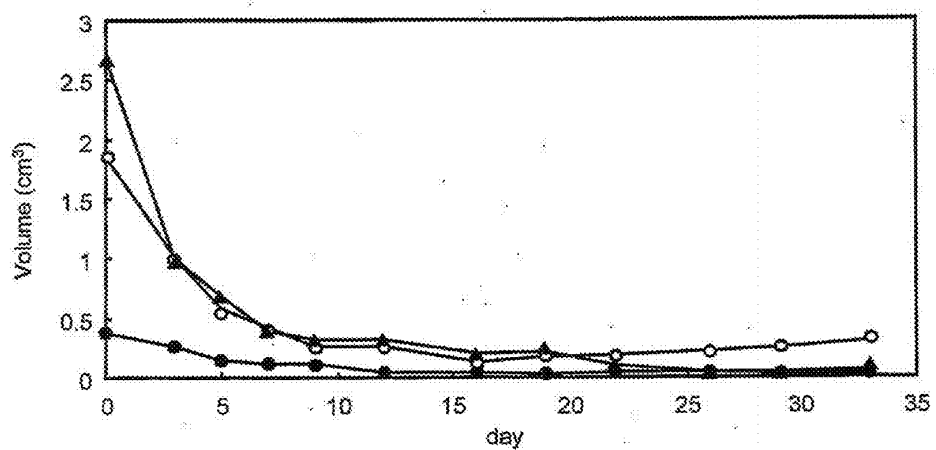
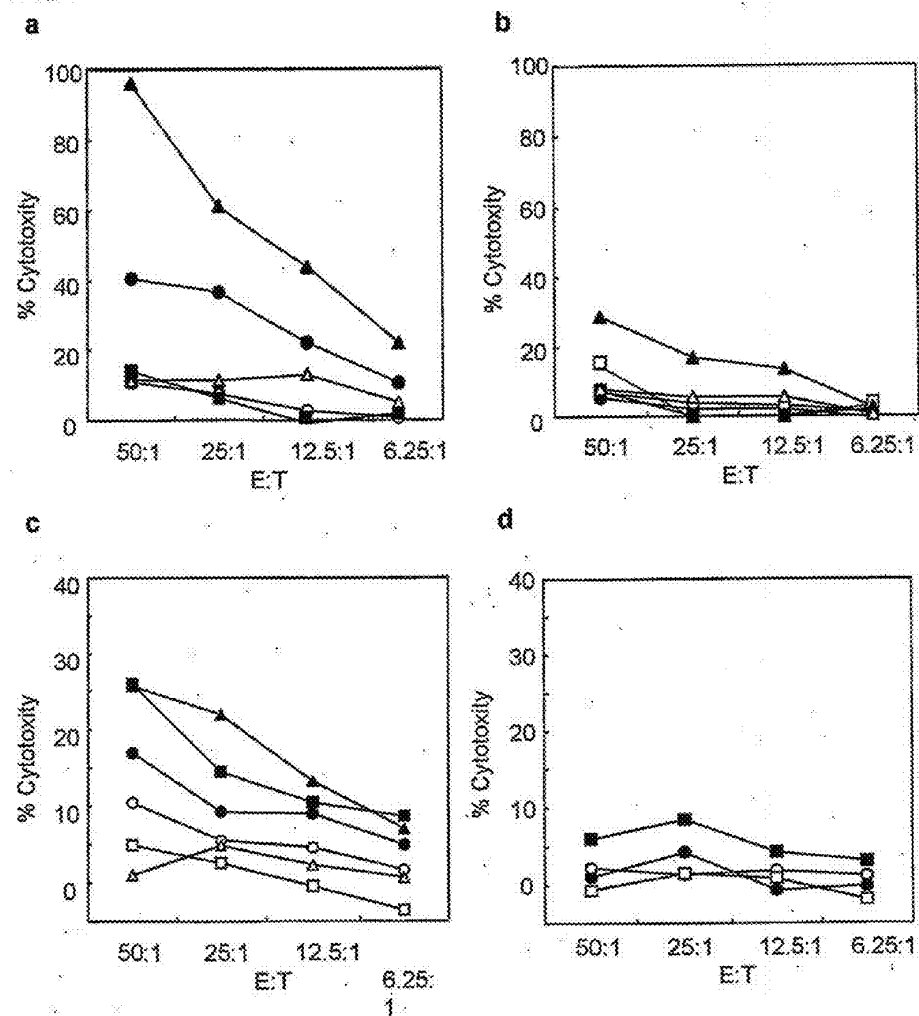


Fig. 8



TUMOR-TARGETING MONOCLONAL ANTIBODIES TO FZD10 AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a Divisional of co-pending application Ser. No. 12/308,095, filed on Dec. 8, 2008 and for which priority is claimed under 35 U.S.C. §120. Application Ser. No. 12/308,095 is the national phase of PCT International Application No. PCT/JP2006/317155 filed on Aug. 24, 2006 under 35 U.S.C. §371. The entire contents of each of the above-identified applications are hereby incorporated by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to an antibody or a fragment thereof which is capable of binding to a Frizzled homologue 10 (FZD10) protein, such as a mouse monoclonal antibody, a chimeric antibody and a humanized antibody. Also, the present invention relates to a method for treating and/or preventing FZD10-associated disease; a method for diagnosis or prognosis of FZD10-associated disease; and a method for in vivo imaging of FZD10 in a subject.

BACKGROUND OF THE INVENTION

[0003] Monoclonal antibodies against cancer-specific molecules have been proved to be useful in cancer treatment (Harris, M. (2004). *Lancet Oncol*, 5, 292-302). In addition to successful examples of clinical application of the humanized or chimeric antibodies such as trastuzumab (Baselga, J. (2004). *Oncology*, 61, Suppl 2 14-21), rituximab (Maloney, D. G., et al. (1997). *Blood*, 90, 2188-95) and bevacizumab (Ferrara, N., et al. (2004). *Nat Rev Drug Discov*, 3, 391-400) for breast cancer, malignant lymphoma and colon cancer, a number of monoclonal antibodies against other molecular targets are in development and being evaluated their antitumor activities. These monoclonal antibodies are expected to provide a hope to patients having tumors that have no effective treatment. One of the other important issues for these monoclonal antibodies is achievement of selective therapeutic effects to cancer cells without severe toxicity due to their specific reaction to cells expressing target molecules (Crist, W. M., et al. (2001). *J Clin Oncol*, 19, 3091-102; Wunder, J. S., et al. (1998). *J Bone Joint Surg Am*, 80, 1020-33; Ferguson, W. S. and Goorin, A. M. (2001). *Cancer Invest*, 19, 292-315).

[0004] Among soft tissue sarcomas, osteosarcoma, Ewing's sarcoma and rhabdomyosarcoma are sensitive to chemotherapy and these diseases can be well managed by chemotherapy. On the other hand, spindle cell sarcomas are resistant to chemo- and radiotherapy and patients with them usually exhibit poor prognosis. For synovial sarcoma (SS), surgical treatment is effective for patients at an early stage, but no effective therapeutic drug is available to those at an advanced stage. Hence, development of novel therapeutic modalities is expected to improve patients' prognosis better.

[0005] Genome-wide gene expression analysis in tumors provides the useful information to identify the new molecular targets for development of novel anticancer drugs and tumor markers. In previous study, the present inventors have analyzed gene-expression profile of several soft tissue sarcomas using genome-wide cDNA microarray consisting of 23,040

genes and demonstrated that Frizzled homologue10 (FZD10) (GenBank Accession NOs. AB027464 (SEQ ID NO:1) and BAA84093 (SEQ ID NO:2)) was up-regulated specifically and frequently in SSs (Nagayama, S., et al. (2002). *Cancer Res*, 62, 5859-66; and WO2004/020668). FZD10 gene product is a member of Frizzled family and a putative WNT signal receptor (Koike, J., et al. (1999). *Biochem Biophys Res Commun*, 262, 39-43). Further analysis showed that FZD10 is expressed specifically in SS, and at no or hardly-detectable level in other normal organs except the placenta, suggesting that therapeutics targeting this molecule would cause no or little adverse reaction (Nagayama, S., et al. (2002). *Cancer Res*, 62, 5859-66). RNAi experiments implicated that FZD10 was significantly involved in the tumor growth of SS (WO2006/013733). Furthermore, the present inventors generated the rabbit polyclonal antibody against the extracellular domain of FZD10 (FZD10-ECD), and found that this antibody had antitumor activity in mouse xenograft model of SS (Nagayama, S., et al. (2005). *Oncogene*, 24, 6201-12; and WO2005/004912). Together, the antibody therapy against FZD10 could be expected to improve the clinical outcome of SS.

SUMMARY OF THE INVENTION

[0006] Hereinbelow, it is reported that generation of the murine monoclonal antibodies against FZD10 by means of cell-immunization method for possible clinical application. In vivo tumor-binding activity of these antibodies was evaluated using fluorescent in vivo imaging system with near-infrared fluorescence in addition to the conventional method with radionuclides. Here, we reveal the binding specificity of anti-FZD10 monoclonal antibodies both in vitro and in vivo as well as internalization of these antibodies in the cells expressing FZD10, and found that SYO-1-bearing xenograft mice treated with a single tail vein of ⁹⁰Y-labeled anti-FZD10 Mab at 100 μCi dose was observed significant antitumor effect.

[0007] Based on the above findings, the present inventors concluded that the murine monoclonal antibodies against FZD10 have therapeutic potential in the treatment and diagnosis of SS and other FZD10-overexpressing tumors.

[0008] Therefore, in the first aspect, the present invention provides an antibody or a fragment thereof, which comprises an H (heavy) chain V (variable) region comprising a complementarity determining region (CDR) having the amino acid sequences shown in SEQ ID NOs: 15, 17 and 19 or a CDR functionally equivalent thereto and an L (light) chain V region comprising a CDR having the amino acid sequences shown in SEQ ID NOs: 23, 25 and 27 a CDR functionally equivalent thereto, and which is capable of binding to a Frizzled homologue 10 (FZD10) protein or a partial peptide thereof.

[0009] In one embodiment, the antibody or fragment thereof is selected from the group consisting of a mouse antibody, a chimeric antibody, a humanized antibody, an antibody fragment, and single-chain antibody.

[0010] In a preferred embodiment, the antibody is a mouse antibody. Preferably, the mouse antibody comprises an H chain having the amino acid sequence shown in SEQ ID NO: 57 and/or an L chain having the amino acid sequence shown in SEQ ID NO: 59. For example, the mouse antibody can be produced by the hybridoma clone 92-13 (FERM BP-10628).

[0011] In an alternative preferred embodiment, the antibody is a chimeric antibody. Preferably, the chimeric antibody comprises an H chain V region having the amino acid

sequence shown in SEQ ID NO: 13, for example, the chimeric antibody may comprise an H chain having the amino acid sequence shown in SEQ ID NO: 46. Preferably, the chimeric antibody comprises an L chain V region having the amino acid sequence shown in SEQ ID NO: 21, for example, the chimeric antibody may comprise an L chain having the amino acid sequence shown in SEQ ID NO: 48.

[0012] More preferably, the chimeric antibody comprises an H chain V region having the amino acid sequence shown in SEQ ID NO: 13 and an L chain V region having the amino acid sequence shown in SEQ ID NO: 21. For example, the chimeric antibody comprises an H chain having the amino acid sequence shown in SEQ ID NO: 46 and an L chain having the amino acid sequence shown in SEQ ID NO: 48.

[0013] In one embodiment, the chimeric antibody further comprises a human antibody C (constant) region.

[0014] In an alternative preferred embodiment, the antibody is a humanized antibody. In one embodiment, the humanized antibody further comprises a human antibody FR (framework) region and/or a human antibody C region.

[0015] In the second aspect, the present invention provides an antibody or a fragment thereof, which comprises an H (heavy) chain V (variable) region comprising a complementarity determining region (CDR) having the amino acid sequences shown in SEQ ID NOs: 31, 33 and 35 or a CDR functionally equivalent thereto and an L (light) chain V region comprising a CDR having the amino acid sequences shown in SEQ ID NOs: 39, 41 and 43 or a CDR functionally equivalent thereto, and which is capable of binding to a Frizzled homologue 10 (FZD10) protein or a partial peptide thereof.

[0016] In one embodiment, the antibody or fragment thereof is selected from the group consisting of a mouse antibody, a chimeric antibody, a humanized antibody, an antibody fragment, and single-chain antibody.

[0017] In a preferred embodiment, the antibody is a mouse antibody. Preferably, the mouse antibody comprises an H chain having the amino acid sequence shown in SEQ ID NO: 61 and/or an L chain having the amino acid sequence shown in SEQ ID NO: 63. For example, the mouse antibody can be produced by the hybridoma clone 93-22 (FERM BP-10620).

[0018] In an alternative preferred embodiment, the antibody is a chimeric antibody. Preferably, the chimeric antibody comprises an H chain V region having the amino acid sequence shown in SEQ ID NO: 29, for example, the chimeric antibody comprises an H chain having the amino acid sequence shown in SEQ ID NO: 50. Preferably, the chimeric antibody comprises an L chain V region having the amino acid sequence shown in SEQ ID NO: 37, for example, the chimeric antibody comprises an L chain having the amino acid sequence shown in SEQ ID NO: 52.

[0019] More preferably, the chimeric antibody comprises an H chain V region having the amino acid sequence shown in SEQ ID NO: 29 and an L chain V region having the amino acid sequence shown in SEQ ID NO: 37. For example, the chimeric antibody comprises an H chain having the amino acid sequence shown in SEQ ID NO: 50 and an L chain having the amino acid sequence shown in SEQ ID NO: 52.

[0020] In one embodiment, the chimeric antibody further comprises a human antibody C (constant) region.

[0021] In an alternative preferred embodiment, the antibody is a humanized antibody. In one embodiment, the humanized antibody further comprises a human antibody FR (framework) region and/or a human antibody C region.

[0022] In yet an alternative embodiment, the antibody or fragment thereof can be labeled with a radioisotope label or a fluorescent label. Such radioisotope label includes ⁹⁰yttrium (⁹⁰Y), ¹²⁵iodine (¹²⁵I) and ¹¹¹indium (¹¹¹In).

[0023] In the third aspect, the present invention provides a hybridoma clone 92-13 (FERM BP-10628) which produces the mouse monoclonal antibody 92-13.

[0024] In the forth aspect, the present invention provides a hybridoma clone 93-22 (FERM BP-10620) which produces the mouse monoclonal antibody 93-22.

[0025] In the fifth aspect, the present invention provides a method for treating or preventing a disease that is associated with Frizzled homologue 10 (FZD10) in a subject, comprising administering to the subject an effective amount of the antibody or fragment above. In one embodiment, the disease that is associated with FZD10 is selected from synovial sarcoma (SS), colorectal cancer, gastric cancer, chronic myeloid leukemia (CML), and acute myeloid leukemia (AML).

[0026] In the sixth aspect, the present invention provides a method for diagnosis or prognosis of a disease that is associated with Frizzled homologue 10 (FZD10) or of a predisposition to develop the disease in a subject, comprising

[0027] (a) contacting a sample or a specimen from the subject with the antibody or fragment above;

[0028] (b) detecting the FZD10 protein in the sample or specimen; and

[0029] (c) judging whether or not the subject suffers from or is at risk of developing the disease based on the relative abundance of the FZD10 protein compared to a control.

[0030] In one embodiment, the disease that is associated with FZD10 is selected from synovial sarcoma (SS), colorectal cancer, gastric cancer, chronic myeloid leukemia (CML), and acute myeloid leukemia (AML).

[0031] In the seventh aspect, the present invention provides a method for in vivo imaging of Frizzled homologue 10 (FZD10) protein in a subject, comprising administering to the subject an effective amount of the antibody or fragment above.

[0032] In the eighth aspect, the present invention provides a pharmaceutical composition for treating or preventing a disease associated with Frizzled homologue 10 (FZD10), comprising the antibody or fragment above and a pharmaceutically acceptable carrier or excipient.

[0033] In the ninth aspect, the present invention provides a kit for diagnosis or prognosis of a disease associated with Frizzled homologue 10 (FZD10), comprising the antibody or fragment above.

[0034] In the tenth aspect, the present invention provides a pharmaceutical composition for in vivo imaging of Frizzled homologue 10 (FZD10) protein, comprising the antibody or fragment above.

[0035] In the eleventh aspect, the present invention provides use of the antibody or fragment above in the manufacture of a kit for diagnosis or prognosis of a disease associated with Frizzled homologue 10 (FZD10).

[0036] In the twelfth aspect, the present invention provides use of the antibody or fragment above in the manufacture of a composition for prevention or treatment of a disease associated with Frizzled homologue 10 (FZD10).

[0037] The term "disease that is associated with FZD10" (FZD10-associated disease) refers to a disease that is associated with over-expression of FZD10 protein. Such diseases include, but are not limited to, synovial sarcoma (SS), col-

orectal cancer, gastric cancer, chronic myeloid leukemia (CML), and acute myeloid leukemia (AML).

[0038] The term "fragment" means any antibody fragment that can be prepared from the antibody against FZD10 protein and contains defined CDRs. Such fragment includes, but not limited to, Fab fragment, F(ab')₂ fragment, and Fv fragment.

[0039] The term "modified antibody" means any antibody that can be derived from the antibody against FZD10 and contains defined CDRs. Such modified antibody includes, but not limited to, a PEG-modified antibody. The antibody fragment or modified fragment can be readily recognized by a person skilled in the art and produced by using any methods known in the art.

[0040] The term "subject" herein refers to a subject who has suffered from FZD10-associated disease and also a subject suspected to have FZD10-associated disease. The subject in the present invention may be animals including mammals and avian animals. For example, mammals may include humans, mice, rats, monkeys, rabbits, and dogs.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the office upon request and payment of the necessary fee.

[0042] FIGS. 1a to 1f show characterization of binding specificity for two anti-FZD10 monoclonal antibodies.

[0043] FIG. 1a shows flow-cytometric analysis of the four antibodies, 39-2 and 39-10 (disclosed in WO2005/004912), 92-13 and 93-22, using five SS lines (SYO-1, YaFuSS, HS-SY-2, Fuji and 1973/99) and one colon-cancer cell line (LoVo). Solid lines show the fluorescent intensity detected by each mAbs; broken lines depict the fluorescent intensities of cells incubated with non-immunized mouse IgG as a negative control.

[0044] FIG. 1b shows semi-quantitative RT-PCR of FZD10 in the same tumor-cell lines as used in FIG. 1a. Expression of β 2-microglobulin gene (β 2MG) served as an internal control.

[0045] FIG. 1c shows flow-cytometric analysis of 92-13 (top panels) and 93-22 (lower panels) against exogenous FZD10 were indicated. Colon cancer cell line, SNU-05 was transfected with pCAGGS empty vector (left panels) or pCAGGS-FZD10-myc/His (right panels) and analyzed 48 hours after transfection. Solid lines show the fluorescent intensity detected by each mAbs; broken lines depict the fluorescent intensities of cells incubated with non-immunized mouse IgG as a negative control.

[0046] FIG. 1d shows binding of ¹²⁵I-labeled 39-10, 39-2, 92-13 and 93-22 to normal human blood cells. Radio-labeled Mabs were incubated with each normal human fresh blood of three individuals (A, B and C) in the absence (open bar) or presence (closed bar) of non-labeled identical antibodies.

[0047] FIG. 1e shows binding activity of ¹²⁵I-labeled Mabs. A constant amount of radio-labeled Mabs was incubated with SYO-1 cell and increasing amount of non-labeled antibodies. The percent radioactivity bound to cells was plotted against the amount of non-labeled antibody. Closed circle; 92-13, Open circle; 93-22.

[0048] FIG. 1f shows flow-cytometric analysis of self-block and cross-block. Alexa-488-labeled 92-13 (Top panels) and 93-22 (Lower panels) were incubated with SYO-1 cell in (i) PBS, or in the presence of 100 μ g of (ii) non-labeled 92-13 and (iii) non-labeled 93-22. Shaded histogram show the fluo-

rescent intensity detected by each Alexa488-labeled Mabs; broken lines depict the fluorescent intensities of cells incubated with PBS as a negative control.

[0049] FIG. 2 shows immunohistochemical analyses in SS and normal human frozen tissue sections with no antibody (a, d, g, j, and m), 92-13 (b, e, h, k, and n) and 93-22 (c, f, i, l, and o). (a-c), synovial sarcoma; (d-f), kidney; (g-i), liver, (j-l), heart; (m-o), brain. Original magnification: $\times$ 100.

[0050] FIG. 3 shows biodistribution of ¹¹¹In-labeled and ¹²⁵I-labeled antibodies. 10 kBq of (a), ¹¹¹In-labeled 92-13, (b), ¹²⁵I-labeled 92-13, (c), ¹¹¹In-labeled 93-22 and (d), ¹²⁵I-labeled 93-22 were injected intravenously into SYO-1 tumor bearing BALB/c nude mice. The organs and tumor were dissected at one hour (open bar), 24 hours (hatched bar) and 48 hours (closed bar), and the radioactivities were measured. The data shown is the representative data in two independent experiments.

[0051] FIG. 4a shows in vivo fluorescence imaging of SYO-1 tumor-bearing mice after injection of Alexa 647-labeled 92-13 or 93-22. Fluorescence-labeled Mabs were administered at a dose of 20 μ g per mouse intraperitoneally. All fluorescence images were acquired with a 60-second exposure time (t/stop=2) before injection, immediately after injection (0 hour), 24, 48 and 96 hours. The arrows indicate the position of the tumor. S.C. tumor is located in dorsal for 92-13 (top panels) and in trunk for 93-22 (lower panels). Fluorescence signal from Alexa647 was pseudo-colored according to the color bar indicated on right. In 93-22 (lower panel), the arrowheads indicate the position of injection.

[0052] FIGS. 4b and 4c show representative images of dissected organs and tumors from mice shown in FIGS. 4a, 4b; 92-13, and 4c; 93-22. i, SYO-1 tumor; ii, liver; iii, spleen; iv, kidney; v, pancreas; vi, colon.

[0053] FIG. 5a shows in vivo fluorescence imaging of LoVo tumor-bearing mice after injection of Alexa647-labeled 92-13 or 93-22. Fluorescence-labeled Mabs were administered as FIG. 4. All fluorescence images were acquired with a 60-second exposure time (t/stop=2) immediately after injection (0 hour), 48, 72, 96 and 120 hours (h). Arrow indicates the position of the tumor. S.c. tumor is located in right forearm both for 92-13 (Top panels) and 93-22 (lower panels).

[0054] FIGS. 5b and 5c show representative images of dissected organs and tumors of mice shown in FIGS. 5a, 5b; 92-13 and 5c; 93-22. i, LoVo tumor; ii, liver; iii, spleen; iv, kidney; v, pancreas; vi, colon.

[0055] FIG. 6 shows internalization of 92-13 and 93-22 was assessed by confocal microscopy. Cells were treated with PBS (a, d, and g), 50 μ g/ml of 92-13 (b, e, and h) or 93-22 (c, f, i) for 3 hours in 37° C., 5% CO₂. Antibodies bound to the cell surface were acid-stripped with 0.1M glycine buffer (pH2.5). Cells were fixed, permeabilized and then blocked with 3% BSA. Intracellular antibodies were detected with goat anti-mouse IgG-Alexa488 and nucleus was stained with DAPI. (a-c), SYO-1; (d-f), YaFuSS; (g-i) Lovo.

[0056] FIG. 7 shows the effect of ⁹⁰Y-labeled 92-13 on tumor growth. When tumors were established (0.4-2.7 cm³), mice were given a single tail vein of 100 μ Ci of ⁹⁰Y-labeled 92-13.

[0057] FIG. 8 shows both chimeric 92-13 and 93-22 induced ADCC specifically to the FZD10-overexpressing SYO-1 cells. 1 μ g/ml of chimeric 93-22 antibody (ch93-22) or chimeric 92-13 antibody (ch92-13) at various Effector: Target ratio. PBMC from various donors were used as Effector cell; (a), (c) ADCC of chimeric 92-13 against SYO-1 cell

with five healthy human PBMC donors. (b), (d) ADCC of chimeric 93-22 against LoVo cell with two healthy human PBMC donors. Quantification of cytotoxicity with LDH activity is described in (Nagayama, S., et al. Oncogene, 24, 6201-12).

DETAILED DESCRIPTION AND PREFERRED EMBODIMENTS OF THE PRESENT INVENTION

[0058] Frizzled homologue 10 (FZD10) is a member of Frizzled family, which is a receptor of Wnt signaling. As described hereinbelow, we successfully established murine monoclonal antibodies and chimeric antibodies against FZD10 protein that may be useful for medical use.

[0059] The murine monoclonal FZD10-specific antibodies (92-13 and 93-22 Mabs) are established by immunizing mice with FZD10-transfected cells. Both 92-13 and 93-22 Mabs were shown to have specific binding activity against FZD10 in SS cell line, SYO-1 cells and FZD10-transfected COST cells by using flow cytometry (FACS) analysis. To validate the specific binding activity of those antibodies in vivo, the present inventors injected fluorescent-labeled Mabs intraperitoneally or intravenously into the mice carrying SS xenografts and found that these Mabs were bound to the FZD10-expressing tumors, but not to any other normal mouse tissues by the use of the in vivo fluorescent imaging system and radioactivities. Subsequent immunohistochemical analyses with the Mabs confirmed an absence or hardly-detectable level of FZD10 protein in normal human organs except the placenta. Furthermore, interestingly the present inventors found that the Mabs were internalized into the SS cell line, SYO-1, but not into FZD10-negative cell line, LoVo using confocal laser scanning microscopy. Surprisingly, SYO-1-bearing xenograft mice treated with a single tail vein of ^{90}Y -labeled anti-FZD10 (92-13) Mab at 100 μCi dose was observed significant antitumor effect. Taken together, we conclude that these specific Mabs against FZD10 could be utilized as the novel diagnostic marker or treatment of SS with minimal or no risk of adverse reactions.

[0060] Due to their complicated protein structure, it is often very difficult to generate antibodies against seven-transmembrane proteins. In previous study, the present inventors demonstrated that FZD10 formed homo-oligomer (Nagayama, S., et al. (2005). Oncogene, 24, 6201-12). After failure of multiple attempts to generate anti-FZD10 monoclonal antibodies that could recognize a native form of FZD10 by the use of full-length or partial recombinant FZD10 proteins, we finally applied to immunization by injection of living COS-7 cells overexpressing FZD10 into the foot-pad of Balb/c-mice and successfully obtained the two hybridomas producing anti-FZD10 antibodies that had an ability to recognize the native FZD10 form in living cells by FACS analysis. Since those antibodies did not detect FZD10 protein in western blotting, the present inventors assume that those Mabs recognize the tertiary structure of FZD10.

[0061] To investigate the in vivo distribution of 92-13 and 93-22 Mabs, the present inventors applied two methods; one based on the radionuclide modalities using ^{125}I and ^{111}In -labeled antibodies, and the other based on the fluorescence imaging using near-infrared-labeled (Alexa647) antibodies. Near-infrared fluorescent, mostly indocyanine dye, is now widely used in the in vivo imaging for diagnostic purpose because the light of this wavelength penetrates living tissue quite efficiently (Chen, X., et al. (2004). Cancer Res, 64,

8009-14). The results obtained two approaches were very concordant and indicated that 92-13 and 93-22 bound to SYO-1 tumor cells, but not to other normal tissues. To confirm whether those antibodies can be applied for clinical use, the present inventors further examined the binding activity of antibodies against normal blood cells. The binding activity of ^{125}I -labeled 92-13 and 93-22 against normal human blood cells were undetectable in all of three individual donors (FIG. 1d). These results were consistent with those of FACS analysis using human peripheral blood mononuclear cell (data not shown), suggesting clinical applicability of these two antibodies with little possibility of adverse effect to SS patients because of very specific binding affinity to the FZD10 molecule. Furthermore, in vitro experiments using confocal microscopy revealed that the specific binding of 92-13 and 93-22 Mabs to cell-surface FZD10 induced the internalization of the antibodies (FIG. 6). As described previously (Stein, R., et al. (2001). Crit Rev Oncol Hematol, 39, 173-80; Stein, R., et al. (2005). Clin Cancer Res, 11, 2727-34), if labeled Mabs is internalized after binding, ^{125}I -labeled antibody is metabolized in the lysosomes and diffused from target tumor cells where ^{111}In -labeled antibody remains in the lysosomes. As observed in FIG. 3, the radioactivities of ^{111}In -labeled antibody and ^{125}I -labeled antibody in tumors were significantly different (FIGS. 3, a and b, c and d). These findings suggest that 92-13 (and 93-22) Mabs can specifically internalize into the SS cells via FZD10 protein.

[0062] When antibodies are applied to cancer therapy, the following three mechanisms are thought to exert the antitumor activity; (i) in case that the target molecule is involved in growth enhancement, neutralization of antibodies would block the growth signal transduction and then suppress the growth of tumor cells; (ii) The second possibility is the effector activities to induce antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). (iii) The third case is radionuclides or antitumor drug that is conjugated to antibodies and is delivered to the target tumor cells effectively. Although the present inventors previously demonstrated that the target molecule FZD10 is involved in the SS tumor growth, neither Mabs 92-13 nor 93-22 showed the neutralizing effect in vitro when added to the cell culture media (data not shown) or in vivo when injected to the tumor-bearing mice (data not shown).

[0063] Conjugating radionuclide or anti-cancer drug to antibodies such as Zevalin (anti-CD20 antibody conjugated with ^{90}Y trium) and Mylotarg (anti-CD33 antibody conjugated with calicheamicin), has been proven to be highly effective to confer cytotoxicity to the antibodies (Wiseman, G. A. and Witzig, T. E. (2005). Cancer Biother Radiopharm, 20, 185-8; van der Velden, V. H., et al. (2001). Blood, 97, 3197-204; Carter, P. (2001). Nat Rev Cancer, 1, 118-29). Mylotarg exerts its antitumor activity by releasing antitumor drug, calicheamicin within the cancer cell after it was internalized (van der Velden, V. H., et al. (2001). Blood, 97, 3197-204). In the Examples, for therapeutic experiments, ^{90}Y trium-DTPA-92-13 conjugate was generated and its antitumor activity was investigated. In mouse xenograft model, tumors quickly diminished after treatment of ^{90}Y trium-DTPA-92-13 (FIG. 7). Noticeably, the tumors including larger volume ($>1\text{ cm}^3$) of tumor showed no refracture until 34 days after administration and no strong toxicity was observed. Since anti-FZD10 antibodies 92-13 and 93-22 were likely to be effectively internalized into antigen-positive cells as shown in FIG. 6, conjugation of anti-cancer drug to both Mabs 92-13 and 93-22 is

also expected to exert the high anti-cancer effect to SS cells. Referring to the effector activity, both chimeric 92-13 and 93-22 induced ADCC specifically to the FZD10-overexpressing SYO-1 cells (FIGS. 8, *a* and *c*), but not to the FZD10-negative LoVo cells (FIGS. 8, *b* and *d*). Particularly, chimeric 92-13 showed higher induction of cytotoxicity as compared with chimeric 93-22, however, their activity depends on effector cell donor, possibly caused by polymorphism of Fc receptor. In conclusion, the present inventors successfully produced monoclonal antibodies which were able to bind specific to FZD10 on FZD10-overexpressing tumor cells in vitro and in vivo. Together, the present inventors are confident that anti-FZD10 monoclonal antibodies have great potential for development of novel drug therapies for treatment of SS and other tumors that over-express FZD10.

1. Production of an Antibody

[0064] Antibodies that can be used in the present invention specifically react against an FZD10 protein derived from an FZD10-associated disease. The term “antibody” used herein means an antibody molecule as a whole, or its fragments such as Fab fragments, F(ab')₂ fragments and Fv fragments, which can bind to the protein or its partial peptides as the antigen. The antibody can be either a polyclonal antibody or a monoclonal antibody. It can also be a humanized or chimeric antibody, or a single chain Fv (scFv) antibody. The antibodies (polyclonal antibodies and monoclonal antibodies) for use in the present invention can be prepared, for example, by the following process.

(1) Monoclonal antibody

[0065] Initially, an antigen is prepared for the production of an antibody useful in the present invention. FZD10 protein or its partial peptide can be used as an immunogenic protein. Alternatively, a cell expressing FZD10 protein or its partial peptide can also be used as an immunogen. The amino acid sequence of FZD10 protein used as the immunogen in the present invention and the cDNA sequence encoding the protein are publicly available in GenBank as Accession Nos. BAA84093 (SEQ ID NO: 1) and AB027464 (SEQ ID NO: 2), respectively. The FZD10 protein or its partial peptide for use as the immunogen can be synthetically prepared according to a procedure known in the art such as a solid-phase peptide synthesis process, using the available amino acid sequence information. The partial peptides of FZD10 protein include, but are not limited to, a peptide containing residues 1-225 of the amino acid sequence shown in SEQ ID NO: 1, which corresponds to the N-terminal extracellular domain of FZD10 protein (FZD10-ECD).

[0066] The protein or its partial peptide, or the cell expressing them can be prepared by using the sequence information of cDNA encoding FZD10 protein or its partial peptide according to a known gene recombination procedure. The production of the protein or its partial peptide as well as the cell expressing them according to such a gene recombination procedure will be illustrated below.

[0067] A recombinant vector for the production of protein can be obtained by linking the above cDNA sequence to an appropriate vector. A transformant can be obtained by introducing the recombinant vector for the production of protein into a host so that the target FZD10 protein or its partial peptide can be expressed.

[0068] As the vector, a phage or plasmid that is capable of autonomously replicating in a host is used. Examples of a plasmid DNA include pCAGGS, pET28, pGEX4T, pUC118,

pUC 119, pUC18, pUC19, and other plasmid DNAs derived from *Escherichia coli*; pUB110, pTP5, and other plasmid DNAs derived from *Bacillus subtilis*; and YEp13, YEp24, YCp50 and other plasmid DNAs derived from yeast. Examples of a phage DNA include lambda phages such as λgt11 and λZAP. In addition, animal virus vectors such as retrovirus vector and vaccinia virus vector can be used, and insect virus vectors such as baculovirus vector can also be used.

[0069] The DNA encoding the FZD10 protein or its partial peptide (hereinafter referred to as FZD10 DNA) is inserted into the vector, for example, by the following method. In this method, purified DNA is cleaved by an appropriate restriction enzyme and inserted into a restriction enzyme site or a multi-cloning site of an appropriate vector DNA to ligate into the vector.

[0070] In addition to a promoter and the FZD10 DNA, any of enhancers and other cis elements, splicing signals, poly A addition signals, selective markers, ribosome binding site (RBS), and other elements can be ligated into the recombinant vector for the production of protein for use in mammalian cells, if desired.

[0071] For ligating the DNA fragment to the vector fragment, a known DNA ligase can be used. The DNA fragment and the vector fragment are annealed and ligated, thereby producing a recombinant vector for the production of a protein.

[0072] The host for use in transformation is not specifically limited as long as it allows the FZD10 protein or its partial peptide to be expressed therein. Examples of the host include bacteria, for example, *E. coli*, and *Bacillus*; yeast, for example, *Saccharomyces cerevisiae*; animal cells, for example, COS cells, Chinese Hamster Ovary (CHO) cells, and insect cells.

[0073] For example, when a bacterium is used as the host, the recombinant vector for the protein production should preferably be capable of autonomously replicating in the host bacterium and comprise a promoter, a ribosome binding site, the FZD10 DNA, and a transcription termination sequence. The recombinant vector may further comprise a gene for regulating the promoter. An example of *Escherichia coli* includes *Escherichia coli* BRL, and an example of *Bacillus* is *Bacillus subtilis*. Any promoter that can be expressed in the host such as *Escherichia coli* can be used herein.

[0074] The recombinant vector can be introduced into the host bacterium by any procedures known in the art. Such procedures include, for example, a method using calcium ions and an electroporation. When yeast cell, an animal cell, or an insect cell is used as the host, a transformant can be produced according to a known procedure in the art, and then the FZD10 protein or its partial peptide can be produced in the host (transformant).

[0075] The FZD10 protein or its partial peptide for use as the immunogen in the present invention can be obtained from a culture of the above-generated transformant. The “culture” refers to any of culture supernatant, cultured cells, cultured microorganisms, and homogenates thereof. The transformant is cultured in a culture medium by a conventional process of culturing a host.

[0076] The culture medium for culturing the transformant obtained by using *Escherichia coli*, yeast, or other microorganisms as the host can be either a natural medium or a synthetic medium, as long as it comprises a carbon source,

nitrogen source, inorganic salts, and other components utilizable by the microorganism and enables the transformant to grow efficiently.

[0077] The transformant is generally cultured by shaking culture or aeration culture with stirring under aerobic conditions at 25° C. to 37° C. for 3 to 6 hours. During culturing, pH is held at a level near neutrality by adjustment with, for example, an inorganic or organic acid, and an alkaline solution. During culturing, antibiotics such as ampicillin or tetracycline may be added to the medium according to the selective marker inserted into the recombinant expression vector, if necessary.

[0078] After culturing, when the FZD10 protein or its partial peptide is produced within the microorganism or cell, the protein or its partial peptide is extracted by homogenizing the microorganism or cell. When the FZD10 protein or its partial peptide is secreted from the microorganism or cell, the culture medium is used as is, or debris of the microorganism or cell is removed from the culture medium, for example, by centrifugation. Thereafter, the FZD10 protein or its partial peptide can be isolated from the culture and purified by a conventional biochemical method for the isolation and purification of proteins, such as ammonium sulfate precipitation, gel chromatography, ion-exchange chromatography, and affinity chromatography, either individually or in combination.

[0079] Whether or not the FZD10 protein or its partial peptide has been obtained can be confirmed, for example, by SDS polyacrylamide gel electrophoresis.

[0080] Next, the obtained FZD10 protein or its partial peptide, or the transformant is dissolved in a buffer to prepare an immunogen. Where necessary, an adjuvant can be added thereto for effective immunization. Such adjuvants include, for example, commercially available Freund's complete adjuvant and Freund's incomplete adjuvant. Any of these adjuvants can be used alone or in combination.

[0081] The immunogen so prepared is administered to a mammal such as a rabbit, rat, or mouse. The immunization is performed mainly by intravenous, subcutaneous, or intraperitoneal injection. The interval of immunization is not specifically limited and the mammal is immunized one to 3 times at intervals ranging from several days to weeks. Antibody-producing cells are collected 1 to 7 days after the last immunization. Examples of the antibody-producing cells include spleen cells, lymph node cells, and peripheral blood cells.

[0082] To obtain a hybridoma, an antibody-producing cell and a myeloma cell are fused. As the myeloma cell to be fused with the antibody-producing cell, a generally available established cell line can be used. Preferably, the cell line used should have drug selectivity and properties such that it can not survive in a HAT selective medium (containing hypoxanthine, aminopterin, and thymidine) in unfused form and can survive only when fused with an antibody-producing cell. Possible myeloma cells include, for example, mouse myeloma cell lines such as P3×63-Ag.8.U1 (P3U1), and NS-1.

[0083] Next, the myeloma cell and the antibody-producing cell are fused. For the fusion, these cells are mixed, preferably at the ratio of the antibody-producing cell to the myeloma cell of 5:1, in a culture medium for animal cells which does not contain serum, such as DMEM and RPMI-1640 media, and fused in the presence of a cell fusion-promoting agent such as polyethylene glycol (PEG). The cell fusion may also be carried out by using a commercially available cell-fusing device using electroporation.

[0084] Then, the hybridoma is picked up from the cells after above fusion treatment. For example, a cell suspension is appropriately diluted with, for example, the RPMI-1640 medium containing fetal bovine serum and then plated onto a microtiter plate. A selective medium is added to each well, and the cells are cultured with appropriately replacing the selective medium. As a result, the cells that grow about 30 days after the start of culturing in the selective medium can be obtained as the hybridoma.

[0085] The culture supernatant of the growing hybridoma is then screened for the presence of an antibody that reacts with the FZD10 protein or its partial peptide. The screening of hybridoma can be performed according to a conventional procedure, for example, using enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA) or radioimmunoassay (RIA). The fused cells are cloned by the limiting dilution to establish a hybridoma, which produces the monoclonal antibody of interest.

[0086] The monoclonal antibody can be collected from the established hybridoma, for example, by a conventional cell culture method or by producing the ascites. If necessary, the antibody can be purified in the above-described antibody collecting method according to a known procedure such as ammonium sulfate precipitation, ion-exchange chromatography, gel filtration, affinity chromatography, or a combination thereof.

[0087] The globulin type of the monoclonal antibodies useful in the present invention is not specifically limited, as long as they are capable of specifically binding to the FZD10 protein and can be any of IgG, IgM, IgA, IgE, and IgD. Among them, IgG and IgM are preferred.

[0088] In the present invention, murine monoclonal antibodies 93-22 and 92-13 are successfully established and preferably used. The hybridoma clone 93-22 producing mouse monoclonal antibody 93-22 was deposited by Shuichi Nakatsuru internationally at the IPOD International Patent Organism Depository of the National Institute of Advanced Industrial Science and Technology (AIST Tsukuba Central 6, 1-1, Higashi 1-chome, Tsukuba-shi, Ibaraki-Ken, 305-8566 Japan) as of Jun. 14, 2006 under the deposit number of FERM BP-10620. Also, hybridoma clone 92-13 producing mouse monoclonal antibody 92-13 was deposited by Shuichi Nakatsuru internationally at the IPOD International Patent Organism Depository of the National Institute of AIST as of Jun. 28, 2006 under the deposit number of FERM BP-10628. The monoclonal antibody produced by the hybridoma may be preferably used in the present invention.

[0089] In the present invention, a recombinant-type monoclonal antibody may also be used, which can be produced by cloning an antibody gene from the hybridoma, integrating the antibody gene into a suitable vector, introducing the vector into a host, and producing the antibody from the host according to a conventional genetic recombination technique (see, for example, Vandamme, A. M. et al., *Eur. J. Biochem.* (1990) 192, 767-75).

[0090] More specifically, mRNA encoding variable (V) region of the anti-FZD10 mouse monoclonal antibody is isolated from the antibody-producing hybridoma (for example, those described above). The isolation of the mRNA is performed by preparing a total RNA by any known method, such as guanidium ultracentrifugation method (Chirgwin, J. M. et al., *Biochemistry* (1979) 18, 5294-9) and AGPC method (Chomczynski, P. et al., *Anal. Biochem.* (1987) 162, 156-9), and then producing the desired mRNA from the total RNA

using mRNA Purification Kit (Pharmacia) or the like. Alternatively, the mRNA may also be prepared directly using QuickPrep mRNA Purification Kit (Pharmacia).

[0091] Next, cDNA for the antibody V-region is synthesized from the mRNA with a reverse transcriptase. The synthesis of the cDNA may be performed using a commercially available kit, for example, Gene Racer™ Kit (Invitrogen). The cDNA may also be synthesized or amplified by 5'-RACE method (Frohman, M. A. et al., Proc. Natl. Acad. Sci. USA (1988) 85, 8998-9002; Belyaysky, A. et al., Nucleic Acids Res. (1989) 17, 2919-32) using 5'-Ampli FINDER RACE Kit (Clontech) in combination with a PCR method.

[0092] The amino acid sequences of H chain and L chain of mouse monoclonal antibody 92-13 are shown in SEQ ID NO: 57 and 59, respectively (encoded by the nucleotide sequence as shown in SEQ ID NO: 58 and 60, respectively). The amino acid sequences of H chain and L chain of mouse monoclonal antibody 93-22 are shown in SEQ ID NO: 61 and 63, respectively (encoded by the nucleotide sequence as shown in SEQ ID NO: 62 and 64, respectively). Based on the sequence information, primers used for amplifying the H chain or L chain of mouse monoclonal antibody of interest can be designed using a conventional method.

[0093] A DNA fragment of interest is isolated and purified from the resultant PCR product and then ligated to a vector DNA to obtain a recombinant vector. The recombinant vector is introduced into a host such as *E. coli*, and a colony containing a desired recombinant vector is selected. The nucleotide sequence of the DNA of interest in the recombinant vector is confirmed using, for example, an automated sequencer.

[0094] Once DNA encoding the anti-FZD10 antibody V-region is obtained, the DNA is integrated into an expression vector containing DNA encoding the antibody constant (C) region.

[0095] For the production of the anti-FZD10 antibody used in the present invention, the antibody gene is integrated into an expression vector so that the antibody gene can be expressed under the control of expression control elements (e.g., enhancer, promoter). A host cell is transformed with the expression vector to express the antibody.

[0096] In the expression of the antibody gene, DNA encoding heavy (H) chain and DNA encoding light (L) chain of the antibody may be integrated into separate expression vectors, and then a host cell is co-transformed with the resultant recombinant expression vectors. Alternatively, both DNA encoding H-chain and DNA encoding L-chain of the antibody may be integrated together into a single expression vector, and then a host cell is transformed with the resultant recombinant expression vector (for example, WO 94/11523).

[0097] The antibody gene can be expressed by known methods. For the expression in a mammalian cell, a conventional useful promoter, the antibody gene to be expressed and a poly(A) signal (located downstream to the 3' end of the antibody gene) may be operably linked. For example, as the useful promoter/enhancer system, a human cytomegalovirus immediate early promoter/enhancer system may be used.

[0098] Other promoter/enhancer systems, for example, those derived from viruses (e.g., retrovirus, polyoma virus, adenovirus and simian virus 40 (SV40)) and those derived from mammalian cells (e.g., human elongation factor 1 alpha (HEF1 alpha)), may also be used for the expression of the antibody in the present invention.

[0099] When SV40 promoter/enhancer system is used, the gene expression may be performed readily by the method of Mulligan et al. (Nature (1979) 277, 108-14). When HEF1 alpha promoter/enhancer system is used, the gene expression may be performed readily by the method of Mizushima et al. (Nucleic Acids Res. (1990) 18, 5322).

[0100] For the expression in *E. coli*, a conventional useful promoter, a signal sequence for secreting the antibody of interest and the antibody gene may be operably linked. As the promoter, lacZ promoter or araB promoter may be used. When lacZ promoter is used, the gene expression may be performed by the method of Ward et al. (Nature (1998) 341, 544-6; FASEB J. (1992) 6, 2422-7), while when araB promoter is used, the gene expression may be performed by the method of Better et al. (Science (1988) 240, 1041-3).

[0101] With respect to the signal sequence for secretion of the antibody, when the antibody of interest is intended to be secreted in a periplasmic space of the *E. coli*, pelB signal sequence (Lei, S. P. et al., J. Bacteriol. (1987) 169, 4379-83) may be used. The antibody secreted into the periplasmic space is isolated and then refolded so that the antibody takes an appropriate configuration.

[0102] The replication origin derived from viruses (e.g., SV40, polyoma virus, adenovirus, bovine papilloma virus (BPV)) or the like may be used. In order to increase the gene copy number in the host cell system, the expression vector may further contain a selective marker gene, such as an aminoglycoside phosphotransferase (APH) gene, a thymidine kinase (TK) gene, an *E. coli* xanthine-guanine phosphoribosyltransferase (Ecogpt) gene and a dihydrofolate reductase (dhfr) gene.

[0103] For the production of the antibody used in the present invention, any expression system including eukaryotic and prokaryotic cell systems may be used. The eukaryotic cell includes established cell lines of animals (e.g., mammals, insects, molds and fungi, yeast). The prokaryotic cell includes bacterial cells such as *E. coli* cells. It is preferable that the antibody used in the present invention be expressed in a mammalian cell, such as a CHO, COS, myeloma, BHK, Vero and HeLa cell.

[0104] Next, the transformed host cell is cultured in vitro or in vivo to produce the antibody of interest. The cultivation of the host cell may be performed by any known method. The culture medium that can be used herein may be DMEM, MEM, RPMI 1640 or IMDM medium. The culture medium may contain a serum supplement, such as fetal calf serum (FCS).

[0105] In the production of the recombinant antibody, besides the above-mentioned host cells, a transgenic animal may also be used as a host. For example, the antibody gene is inserted into a predetermined site of a gene encoding a protein inherently produced in the milk of an animal (e.g., beta-casein) to prepare a fusion gene. A DNA fragment containing the antibody gene-introduced fusion gene is injected into an embryo of a non-human animal, and the embryo is then introduced into a female animal. The female animal having the embryo therein bears a transgenic non-human animal. The antibody of interest is secreted in the milk from the transgenic non-human animal or a progeny thereof. For the purpose of increasing the amount of the antibody-containing milk, an appropriate hormone may be administered to the transgenic animal (Ebert, K. M. et al., Bio/Technology (1994) 12, 699-702).

[0106] The antibody expressed and produced as described above may be isolated from the cells or the host animal body and purified. The isolation and purification of the antibody used in the present invention may be performed on an affinity column. Other methods conventionally used for the isolation and purification of an antibody may be also be used; thus the method is not particularly limited. For example, various chromatographies, filtration, ultrafiltration, salting out and dialysis may be used singly or in combination to isolate and purify the antibody of interest (Antibodies A Laboratory Manual, Ed. Harlow, David Lane, Cold Spring Harbor Laboratory, 1988).

(2) Chimeric Antibody and Humanized Antibody

[0107] In the present invention, an artificially modified recombinant antibody may be used, including a chimeric antibody and a humanized antibody. These modified antibodies can be prepared by any known method. For example, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81: 6851-5; Neuberger et al., 1984, Nature, 312: 604-8; Takeda et al., 1985, Nature, 314: 452-4) can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., "humanized antibodies".

[0108] A chimeric antibody according to the present invention can be prepared by ligating the DNA encoding the antibody V-region to DNA encoding a human antibody C-region, integrating the ligation product into an expression vector, and introducing the resultant recombinant expression vector into a host to produce the chimeric antibody.

[0109] A humanized antibody is also referred to as "reshaped human antibody", in which the complementarity determining regions (CDRs) of an antibody of a non-human mammal (e.g., a mouse) are grafted to those of a human antibody. The general genetic recombination procedure for producing such humanized antibody is also known (for example, EP 125023; WO 96/02576).

[0110] Specifically, a DNA sequence in which mouse antibody CDRs are ligated through framework regions (FRs) is designed, and synthesized by a PCR method using several oligonucleotides as primers which were designed to have regions overlapping to the terminal regions of the CDRs and the FRs. The resultant DNA is ligated to DNA encoding the human antibody C-region, and the ligation product is integrated into an expression vector. The resultant recombinant expression vector is introduced into a host, thereby producing the humanized antibody (for example, WO 96/02576).

[0111] The FRs ligated through the CDRs are selected so that the CDRs can form a functional antigen binding site. If necessary, an amino acid(s) in the FRs of the antibody V-region may be replaced so that the CDRs of the reshaped human antibody can form an appropriate antigen binding site (Sato, K. et al., Cancer Res. (1993) 53, 851-6).

[0112] The chimeric antibody is composed of V-regions derived from a non-human mammal antibody and C-regions derived from a human antibody. The humanized antibody is composed of CDRs derived from a non-human mammal antibody and FRs and C-regions derived from a human antibody. The humanized antibody may be useful for clinical use, because the antigenicity of the antibody against a human body is reduced.

[0113] A specific example of a chimeric antibody or a humanized antibody used in the present invention is an antibody in which the CDRs are derived from the mouse monoclonal antibody 92-13 or an antibody in which the CDRs are derived from the mouse monoclonal antibody 93-22. The method for producing such chimeric antibodies and humanized antibodies are described below.

[0114] To clone DNA comprising a nucleotide sequence coding for V region of the anti-FZD10 mouse monoclonal antibody, mRNA can be isolated from hybridomas and each cDNA in the V regions of L and H chains can be synthesized with the use of a reverse transcriptase as described above. In the synthesis of cDNA, Oligo-dT primer or other appropriate primer which hybridizes to L or H chain C region may be used. For example, but not limited to, CH1 (IgG2a) primer having the nucleotide sequence as shown in SEQ ID NO: 3 for H chain V region and CL1 (kappa) primer having the nucleotide sequence as shown in SEQ ID NO: 4 for L chain V region can be used.

[0115] Amplification of cDNA of both L and H chains can be performed by PCR (polymerase chain reaction) using a commercially available kit (for example, GeneRacer™ kit from Invitrogen) or using a known method including 5'-RACE method (Frohman, M. A. et al., Proc. Natl. Acad. Sci. USA, 85, 8998-9002, 1988; Belyaysky, A. et al., Nucleic Acids Res., 17, 2919-32, 1989).

[0116] The specific primers for amplifying DNA for V regions of the mouse monoclonal antibody 92-13 include primers having the nucleotide sequences shown in SEQ ID NOs: 5 and 6 for H chain V region and primers having the nucleotide sequences shown in SEQ ID NOs: 7 and 8 for L chain V region. Using these primers, a DNA encoding H chain V region having an amino acid sequence as shown in SEQ ID NO: 13 and a DNA encoding L chain V region having an amino acid sequence as shown in SEQ ID NO: 21 can be amplified. The specific primers for amplifying DNA for V regions of the mouse monoclonal antibody 93-22 include primers having the nucleotide sequences shown in SEQ ID NOs: 53 and 54 for H chain V region and primers having the nucleotide sequences shown in SEQ ID NOs: 55 and 56 for L chain V region. Using these primers, a DNA encoding H chain V region having an amino acid sequence as shown in SEQ ID NO: 29 and a DNA encoding L chain V region having an amino acid sequence as shown in SEQ ID NO: 37 can be amplified.

[0117] Then, the amplified products are subjected to agarose gel electrophoresis according to conventional procedures, and DNA fragments of interest are excised, recovered, purified and ligated to a vector DNA.

[0118] The obtained DNA and vector DNA can be ligated using a known ligation kit to construct a recombinant vector. A vector DNA may be prepared in a known method: J. Sambrook, et al., "Molecular Cloning", Cold Spring Harbor Laboratory Press, 1989. The vector DNA is digested with restriction enzyme(s), and the nucleotide sequence of a desired DNA can be determined by a known method or using an automated sequencer.

[0119] Once DNA fragments coding for L and H chain V regions of mouse monoclonal antibody (hereinafter L or H chain of an antibody may sometimes be referred to as "mouse L or H chain" for mouse antibodies and "human L or H chain" for human antibodies) are cloned, the DNAs coding for

mouse V regions and DNAs coding for human antibody constant regions are ligated and expressed to yield chimeric antibodies.

[0120] A standard method for preparing chimeric antibodies involves ligating a mouse leader sequence and V region sequence present in a cloned cDNA to a sequence coding for a human antibody C region already present in an expression vector of a mammalian cell. Alternatively, a mouse leader sequence and V region sequence present in a cloned cDNA are ligated to a sequence coding for a human antibody C region followed by ligation to a mammalian cell expression vector.

[0121] The polypeptide comprising human antibody C region can be any of H or L chain C regions of human antibodies, including, for example, C gamma 1, C gamma 2, C gamma 3 or C gamma 4 for human H chains or C lambda or C kappa for L chains.

[0122] To prepare a chimeric antibody, two expression vectors are first constructed; that is, an expression vector containing DNAs coding for mouse L chain V region and human L chain C region under the control of an expression control element such as an enhancer/promoter system, and an expression vector containing DNAs coding for mouse H chain V region and human H chain C region under the control of an expression control element such as an enhancer/promoter system, are constructed. Then, host cells such as mammalian cells (for example, COS cell) are cotransformed with these expression vectors and the transformed cells are cultivated in vitro or in vivo to produce a chimeric antibody: see, for example, WO91/16928.

[0123] Alternatively, the mouse leader sequence present in the cloned cDNA and DNAs coding for mouse L chain V region and human L chain C region as well as the mouse leader sequence and DNAs coding for mouse H chain V region and human H chain C region are introduced into a single expression vector (see, for example, WO94/11523) and said vector is used to transform a host cell; then, the transformed host is cultured in vivo or in vitro to produce a desired chimeric antibody.

[0124] The vector for the expression of H chain of a chimeric antibody can be obtained by introducing cDNA comprising a nucleotide sequence coding for mouse H chain V region (hereinafter referred to also as "cDNA for H chain V region") into a suitable expression vector containing the genomic DNA comprising a nucleotide sequence coding for H chain C region of human antibody (hereinafter referred to also as "genomic DNA for H chain C region") or cDNA coding for said region (hereinafter referred to also as "cDNA for H chain C region"). The H chain C region includes, for example, C gamma 1, C gamma 2, C gamma 3 or C gamma 4 regions.

[0125] The expression vectors having the genomic DNA coding for H chain C region, in particular, those coding for C gamma 1 region, include, for example, HEF-PMh-g gamma (WO92/19759) and DHER-INCREMENT E-RVh-PM1-f (WO92/19759). Alternatively, human constant region library can be prepared using cDNA from human PBMC (peripheral blood mononuclear cells) as described previously (Liu, A. Y. et al., Proc. Natl. Acad. Sci. USA, Vol. 84, 3439-43, 1987; Reff, M. E. et al., Blood, Vol. 83, No. 2, 435-45, 1994).

[0126] When cDNA coding for mouse H chain V region is inserted into these expression vectors, an appropriate nucleotide sequence can be introduced into said cDNA through PCR method. For instance, PCR may be effected using a PCR

primer which is designed such that said cDNA has a recognition sequence for a suitable restriction enzyme at its 5'-end and Kozak consensus sequence immediately before the initiation codon thereof so as to improve the transcription efficiency, as well as a PCR primer which is designed such that said cDNA has a recognition sequence for a suitable restriction enzyme at its 3'-end and a splice donor site for properly splicing the primary transcription products of the genomic DNA to give a mRNA, to introduce these appropriate nucleotide sequences into the expression vector.

[0127] The thus constructed cDNA coding for mouse H chain V region is treated with a suitable restriction enzyme(s), then it is inserted into said expression vector to construct a chimeric H chain expression vector containing the genome DNA coding for H chain C region (C gamma 1 region).

[0128] The thus constructed cDNA coding for mouse H chain V region is treated with a suitable restriction enzyme(s), ligated to cDNA coding for said H chain C region C gamma 1, and inserted into an expression vector such as pQCXIH (Clontech) to construct an expression vector containing the cDNA coding for a chimeric H chain.

[0129] The vector for the expression of L chain of a chimeric antibody can be obtained by ligating a cDNA coding for mouse L chain V region and a genomic DNA or cDNA coding for L chain C region of a human antibody and introducing into a suitable expression vector. The L chain C region includes, for example, kappa chain and lambda chain.

[0130] When an expression vector containing cDNA coding for mouse L chain V region is constructed, appropriate nucleotide sequences such as a recognition sequence or Kozak consensus sequence can be introduced into said expression vector through PCR method.

[0131] The entire nucleotide sequence of cDNA coding for human L lambda chain C region may be synthesized by a DNA synthesizer and constructed through PCR method. The human L lambda chain C region is known to have at least 4 different isotypes and each isotype can be used to construct an expression vector.

[0132] The constructed cDNA coding for human L lambda chain C region and the above constructed cDNA coding for mouse L chain V region can be ligated between suitable restriction enzyme sites and inserted into an expression vector such as pQCXIH (Clontech), to construct an expression vector containing cDNA coding for a L lambda chain of a chimeric antibody.

[0133] The DNA coding for human L kappa chain C region to be ligated to the DNA coding for mouse L chain V region can be constructed from, for example, HEF-PM1k-gk containing the genomic DNA (see WO92/19759). Alternatively, human constant region library can be prepared using cDNA from human PBMC (peripheral blood mononuclear cells) as described previously (Liu, A. Y. et al., Proc. Natl. Acad. Sci. USA, Vol. 84, 3439-43, 1987; Reff, M. E. et al., Blood, Vol. 83, No. 2, 435-45, 1994).

[0134] Recognition sequences for suitable restriction enzymes can be introduced, through PCR method, into 5'- and 3'-ends of DNA coding for L kappa chain C region, and the DNA coding for mouse L chain V region as constructed above and the DNA coding for L kappa chain C region can be ligated to each other and inserted into an expression vector such as pQCXIH (Clontech) to construct an expression vector containing cDNA coding for L kappa chain of a chimeric antibody.

[0135] In order to make a humanized antibody in which CDR of a mouse monoclonal antibody is grafted to a human antibody, it is desirable that there exists a high homology between FR of the mouse monoclonal antibody and FR of the human antibody. Accordingly, a comparison is made between V regions of H and L chains of mouse anti-FZD10 monoclonal antibody and the V regions of all the known antibodies whose structures have been elucidated with the use of Protein Data Bank. Further, they are simultaneously compared with the human antibody subgroups (HSG: Human subgroup) classified by Kabat et al. based on the length of antibody FR, the homology of amino acids, and the like: Kabat, E. A. et al, US Dep, Health and Human Services, US Government Printing Offices, 1991.

[0136] The first step for designing DNA coding for a humanized antibody V region is to select a human antibody V region as a basis for the designing. For example, FR of a human antibody V region having a homology of higher than 80% with FR of a mouse antibody V region can be used in the production of a humanized antibody.

[0137] In the humanized antibody, the C region and the framework (FR) regions of the V region of said antibody are originated from human and the complementarity determining regions (CDR) of the V region are originated from mouse. A polypeptide comprising the V region of the humanized antibody can be produced in the manner called CDR-grafting by PCR method so long as a DNA fragment of a human antibody would be available as a template. The "CDR-grafting" refers to a method wherein a DNA fragment coding for a mouse-derived CDR is made and replaced for the CDR of a human antibody as a template.

[0138] If a DNA fragment of a human antibody to be used as a template is not available, a nucleotide sequence registered in a database may be synthesized in a DNA synthesizer and a DNA for a V region of a humanized antibody can be produced by the PCR method. Further, when only an amino acid sequence is registered in the database, the entire nucleotide sequence may be deduced from the amino acid sequence on the basis of knowledge on the codon usage in antibodies as reported by Kabat, E. A. et al. in US Dep. Health and Human Services, US Government Printing Offices, 1991. This nucleotide sequence is synthesized in a DNA synthesizer and a DNA of a humanized antibody V region can be prepared by PCR method and introduced into a suitable host followed by expression thereof to produce the desired polypeptide.

[0139] General procedures of CDR-grafting by PCR method are described below when a DNA fragment of a human antibody as a template is available.

[0140] First, mouse derived DNA fragments corresponding to respective CDRs are synthesized. CDRs 1 to 3 are synthesized on the basis of the nucleotide sequences of the previously cloned mouse H and L chain V regions. For example, when a humanized antibody is produced based on the mouse monoclonal antibody 92-13, CDR sequences of H chain V region can be the amino acid sequences as shown in SEQ ID NOs: 15 (VH CDR1), 17 (VH CDR2) and 19 (VH CDR3); and CDR sequences of L chain V region can be the amino acid sequences as shown in SEQ ID NOs: 23 (VL CDR1), 25 (VL CDR2) and 27 (VL CDR3). When a humanized antibody is produced based on the mouse monoclonal antibody 93-22, CDR sequences of H chain V region can be the amino acid sequences as shown in SEQ ID NOs: 31 (VH CDR1), 33 (VH CDR2) and 35 (VH CDR3); and CDR sequences of L chain V

region can be the amino acid sequences as shown in SEQ ID NOs: 39 (VL CDR1), 41 (VL CDR2) and 43 (VL CDR3).

[0141] The DNA for H chain V region of a humanized antibody may be ligated to DNA for any human antibody H chain C region, for example, human H chain C gamma 1 region. As mentioned above, the DNA for H chain V region may be treated with a suitable restriction enzyme and ligated to a DNA coding for a human H chain C region under an expression control element such as an enhancer/promoter system to make an expression vector containing DNAs for a humanized H chain V region and a human H chain C region.

[0142] The DNA for L chain V region of a humanized antibody may be ligated to DNA for any human antibody L chain C region, for example, human L chain C lambda region. The DNA for L chain V region may be treated with a suitable restriction enzyme and ligated to a DNA coding for a human L chain C region under an expression control element such as an enhancer/promoter system to make an expression vector containing DNAs coding for a humanized L chain V region and a human L chain C region.

[0143] The DNA coding for H chain V region of a humanized antibody and a human H chain C region and the DNA coding for a humanized L chain V region and human L chain C region may also be introduced into a single expression vector such as that disclosed in WO94/11523, said vector may be used to transform a host cell, and the transformed host may be cultivated in vivo or in vitro to produce a desired humanized antibody.

[0144] To produce a chimeric or humanized antibody, two expression vectors as above mentioned should be prepared. Thus, with respect to a chimeric antibody, an expression vector comprising a DNA coding for a mouse H chain V region and a human H chain C region under the control of an expression control element such as an enhancer/promoter, and an expression vector comprising a DNA coding for a mouse L chain V region and a human L chain C region under the control of an expression control element are constructed. With respect to a humanized antibody, an expression vector comprising a DNA coding for a humanized H chain V region and a human H chain C region under the control of an expression control element, and an expression vector comprising a DNA coding for a humanized L chain V region and a human L chain C region under the control of an expression control element are constructed.

[0145] Then, a host cell such as a mammalian cell (for example, COS cell) may be cotransformed with these expression vectors and the resulting transformed cell may be cultured in vitro or in vivo to produce the chimeric or humanized antibody (see, for example, WO91/16928).

[0146] Alternatively, a DNA coding for H chain V and C regions and a DNA coding for L chain V and C regions may be ligated to a single vector and transformed into a suitable host cell to produce an antibody. Thus, in the expression of a chimeric antibody, a DNA coding for a mouse leader sequence present in the cloned cDNA, a mouse H chain V region and a human H chain C region as well as a DNA coding for a mouse leader sequence, a mouse L chain V region and a human L chain C region, can be introduced into a single expression vector such as one disclosed in e.g. WO94/11523. In the expression of a humanized antibody, a DNA coding for a humanized H chain V region and a human H chain C region and a DNA coding for a humanized L chain V region and a human L chain C region may be introduced into a single expression vector such as one disclosed in e.g. WO94/11523.

Such a vector is used to transform a host cell and the transformed host is cultured in vivo or in vitro to produce a chimeric or humanized antibody of interest.

[0147] Any expression system may be used to produce the chimeric or humanized antibody against FZD10 protein according to the present invention. For example, eukaryotic cells include animal cells such as established mammalian cell lines, fungal cells, and yeast cells; prokaryotic cells include bacterial cells such as *Escherichia coli*. Preferably, the chimeric or humanized antibody of the present invention is expressed in a mammalian cell such as COS or CHO cell.

[0148] Any conventional promoters useful for the expression in mammalian cells may be used. For example, human cytomegalovirus (HCMV) immediate early promoter is preferably used. In addition, promoters for gene expression in mammalian cells may include virus promoters, such as those of retrovirus, polyoma virus, adenovirus and simian virus (SV) 40, and mammalian cell derived promoters, such as those of human polypeptide chain elongation factor-1 alpha (HEF-1 alpha). For example, SV40 promoter may be readily used according to Mulligan et al. method (Nature, 277, 108-14, 1979); Mizushima, S. et al. method (Nucleic Acids Research, 18, 5322, 1990) may be easily used with HEF-1 alpha promoter.

[0149] Replication origin includes those derived from SV40, polyoma virus, adenovirus or bovine papilloma virus (BPV). Further, the expression vector may comprise a gene for phosphotransferase AHP(3') II or I (neo), thymidine kinase (TK), *E. coli* xanthine-guanine phosphoribosyltransferase (Ecogpt) or dihydrofolate reductase (DHFR) as a selective marker for increasing the gene copy number in a host cell system.

[0150] The chimeric or humanized antibody of interest which is thus produced by culturing the transformant transformed with a DNA coding for the chimeric or humanized antibody may be isolated from the cell and then purified.

[0151] The isolation and purification of the chimeric or humanized antibody of interest may be carried out by using a protein A agarose column, but may also be performed by any methods used in isolation and purification of a protein and thus is not limited. For instance, a chromatography, ultrafiltration, salting out and dialysis may optionally be selected or combined to isolate and purify the chimeric or humanized antibody.

[0152] After isolating the chimeric antibody or humanized antibody, the concentration of the resulting purified antibody can be determined by ELISA.

[0153] The determination of the antigen-binding activity or other activities including binding activity to a normal cell of the chimeric antibody or humanized antibody may be performed by any known methods (Antibodies A Laboratory Manual, Ed. Harlow, David Lane, Cold Spring Harbor Laboratory, 1988).

[0154] As the method for the determination of the antigen-binding activity of an antibody, techniques such as ELISA (enzyme-linked immunosorbent assay), EIA (enzyme immunoassay), RIA (radioimmunoassay) or fluorescent assay may be employed.

(3) Antibody Fragment and Modified Antibody

[0155] The antibody used in the present invention may be any fragment thereof or a modified antibody, as long as it can bind to FZD10 protein and inhibit its activity. For example, the fragment of the antibody includes Fab, F(ab')₂, Fv, or a

single chain Fv (scFv) composed of a H-chain Fv fragment or a L-chain Fv fragment linked together through a suitable linker. Specifically, such antibody fragments can be produced by cleaving the antibody with an enzyme (e.g., papain, pepsin) into antibody fragments, or by constructing a gene encoding the antibody fragment and inserting the gene into an expression vector and introducing the resultant recombinant expression vector into a suitable host cell, thereby expressing the antibody fragment (see, for example, Co, M. S., et al., J. Immunol. (1994), 152, 2968-76; Better, M. & Horwitz, A. H., Methods in Enzymology (1989), 178, 476-96, Academic Press, Inc.; Pluckthun, A. & Skerra, A., Methods in Enzymology (1989) 178, 497-515, Academic Press, Inc.; Lamoyi, E., Methods in Enzymology (1989) 121, 652-63; Rousseaux, J. et al., Methods in Enzymology (1989) 121, 663-9; and Bird, R. E. et al., Trends Biotechnol. (1991) 9, 132-7). Alternatively, Fab expression libraries may be constructed (Huse et al., 1989, Science, 246: 1275-81) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

[0156] A scFv can be produced by ligating the H-chain V-region to the L-chain V-region through a linker, preferably a peptide linker (Huston, J. S. et al., Proc. Natl. Acad. Sci. USA (1988) 85, 5879-83). The H-chain V-region and the L-chain V-region in the scFv may be derived from any one of the antibodies described herein. The peptide linker which binds the V-regions may be any single chain peptide, for example, of 12-19 amino acid residues.

[0157] As a modified antibody, for example, anti-FZD10 antibody or fragment thereof conjugated to any molecule (e.g., polyethylene glycol) may also be used. Such modified antibodies are also encompassed in the "antibody" of the present invention. The modified antibodies can be prepared by chemical modifications of the antibodies. The chemical modification techniques suitable for this purpose have already been established in the art.

2. Therapeutic Uses

[0158] Described below are methods and pharmaceutical compositions for treating and/or preventing FZD10-associated disease using the antibody of the present invention. The outcome of a treatment is to at least produce in a treated subject a healthful benefit, which in the case of tumors, includes but is not limited to remission of the tumors, palliation of the symptoms of the tumors, and control of metastatic spread of the tumors.

[0159] Specifically, the method for treating and/or preventing FZD10-associated disease in a subject according to the present invention comprises administering to a subject in need thereof the antibody or the fragment described above.

[0160] The term "subject" herein refers to a subject who has suffered from FZD10-associated disease and also a subject suspected to have FZD10-associated disease. The subject in the present invention may be animals including mammals and avian animals. For example, mammals may include humans, mice, rats, monkeys, rabbits, and dogs.

[0161] The term "FZD10-associated disease" herein refers to a disease associated with the over-expression of FZD10 protein. Specifically, FZD10-associated diseases include, but are not limited to, synovial sarcoma (SS), colorectal cancer, gastric cancer, chronic myeloid leukemia (CML), and acute myeloid leukemia (AML).

[0162] The antibody or fragment thereof described herein can specifically bind to FZD10 protein, so when the antibody

or fragment thereof is administered to a subject, it binds to FZD10 protein in the subject and the activity of FZD10 protein may be inhibited. Alternatively, when the antibody or fragment thereof may be conjugated with a therapeutic moiety and administered to a subject, it is delivered to a region that expresses FZD10 protein (i.e. suffered region) in a subject and the therapeutic moiety can be selectively delivered to the suffered region and acted thereon. Such therapeutic moiety may be any therapeutics that are known or will be developed for having a therapeutic efficacy on FZD10-associated disease and includes, but not limited to, a radioisotope label and chemotherapeutic agent. A radioisotope label which can be used as therapeutics can be selected depending on a variety of elements including β -ray energy and its emission efficiency, the presence or absence of γ -ray emitted, its energy and emission efficiency, physical half-life, and labeling procedure. Generally, the radioisotope label based on yttrium (such as ^{90}Y) and iodine (such as ^{125}I and ^{131}I) may be used. A chemotherapeutic agent may be any agent that is known or will be developed for treating FZD10-associated disease and includes, but not limited to, methotrexate, taxol, mercaptopurine, thioguanine, cisplatin, carboplatin, mitomycin, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, vinblastine, vincristine, vinorelbine, paclitaxel, and docetaxel. The antibody or fragment thereof described herein can selectively bind to FZD10 protein and not bind to a normal cell, so side effect which is caused by the antibody or fragment thereof, or radioisotope or chemotherapeutic agent can be effectively avoided and therefore the therapeutic potency may be high.

[0163] The antibody or fragment thereof described herein can be administered to a subject at effective doses to treat or prevent the FZD10-associated disease. An effective dose refers to that amount of an antibody or a fragment thereof sufficient to result in a healthful benefit in the treated subject. Formulations and methods of administration that can be employed when the pharmaceutical composition contains an antibody of the present invention are described below.

[0164] Pharmaceutical compositions for use in accordance with the present invention can be formulated in conventional manner using one or more pharmaceutically acceptable carriers or excipients.

[0165] The antibodies or fragments thereof can be formulated for parenteral administration (i.e., intravenous or intramuscular) by injection, via, for example, bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the antibody can be in lyophilized powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0166] Toxicity and therapeutic efficacy of the antibody or fragment, or the therapeutic moiety conjugated thereto can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD/ED.

[0167] Antibodies or therapeutic moieties that exhibit large therapeutic indices are preferred. While antibodies or moi-

eties that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such antibodies or moieties to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[0168] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosages for use in humans. The dosage of such antibodies lies preferably within a range of circulating plasma concentrations that include the ED50 with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed, the route of administration utilized and types and amounts of the therapeutic moiety conjugated. For any antibody used in the method of the invention, the effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the 1050 (i.e., the concentration of the test antibody that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

[0169] While depending on the conditions and age of the subject and/or administration route, one skilled in the art can select an appropriate dose of the pharmaceutical composition of the present invention. For example, the pharmaceutical composition of the present invention is administered in an amount such that the antibody according to the present invention is administered to the subject in a day in an amount of about 3 to about 15 μg per kg body weight of subject, and preferably of about 10 to about 15 μg per kg body weight of subject. The administration interval and times can be selected in consideration of the condition and age of the subject, administration route, and response to the pharmaceutical composition. For example, the pharmaceutical composition can be administered to the subject one to 5 times, preferably 1 times a day for 5 to 10 days.

[0170] The pharmaceutical composition can be administered systemically or locally. It is preferably administered in a targeting delivery manner so as to deliver the active component to an affected site.

[0171] In particular embodiments, the methods and compositions of the present invention are used for the treatment or prevention of FZD10-associated disease together with one or a combination of chemotherapeutic agents including, but not limited to, methotrexate, taxol, mercaptopurine, thioguanine, cisplatin, carboplatin, mitomycin, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, vinblastine, vincristine, vinorelbine, paclitaxel, and docetaxel.

[0172] With respect to radiation therapy, any radiation therapy protocol can be used depending upon the type of FZD10-associated disease to be treated. For example, but not by way of limitation, X-ray radiation can be administered. Gamma ray emitting radioisotopes, such as radioactive isotopes of radium, cobalt, and other elements may also be administered to expose tissues.

[0173] In another embodiment, chemotherapy or radiation therapy is administered, preferably at least an hour, five hours, 12 hours, a day, a week, a month, and more preferably several months (e.g., up to three months) subsequent to using the methods and compositions containing the antibody of the present invention. The chemotherapy or radiation therapy administered prior to, concurrently with, or subsequent to the

treatment using the methods and compositions according to the present invention can be administered by any method known in the art.

3. Diagnostic and Prognostic Uses

[0174] Antibodies directed against FZD10 protein or fragments thereof may also be used as diagnostics and prognostics, as described herein. Such diagnostics methods may used to detect the presence or absence of FZD10-associated disease and the risk of having the disease. The method for diagnosis and/or prognosis of an FZD10-associated disease of the present invention comprises immunologically detecting or determining the FZD10 protein derived from the disease in a sample using an antibody or a fragment thereof according to the present invention. Specifically, a method for diagnosis or prognosis of FZD10-associated disease or of a predisposition to develop the disease in a subject according to the present invention comprises:

[0175] (a) contacting a sample from the subject with an antibody against FZD10 protein or a fragment thereof;

[0176] (b) detecting the FZD10 protein in the sample; and

[0177] (c) judging whether or not the subject suffers from or is at risk of developing the disease based on the relative abundance of the FZD10 protein compared to a control.

[0178] The method for diagnosis and/or prognosis of the present invention can be performed based on any procedures, as long as it is an assay using an antibody, i.e., an immunological assay. Thereby one can detect the FZD10 protein using the antibody or a fragment thereof of the present invention as the antibody used in the assay. For example, the FZD10 protein can be detected by using an immunohistochemical staining, immunoassay such as enzyme immunoassays (ELISA and EIA), immunofluorescent assay, radioimmunoassay (RIA), or Western blotting.

[0179] A sample to be tested in the method for diagnosis and/or prognosis of FZD10-associated disease of the present invention is not specifically limited, as long as it is a biological sample that may contain the FZD10 protein derived from the FZD10-associated disease. Examples of the sample include extract of a cell or organ, and tissue sections, as well as blood, sera, plasma, lymphocyte cultivated supernatant, urine, spinal fluid, saliva, sweat, and ascites. The abundance of the FZD10 protein as determined in samples such as tumor tissue, tumor biopsy, and metastasis tissue by using the antibody or a fragment thereof of the present invention is specifically useful as an index of an FZD10-associated disease.

[0180] For example, antibodies and fragments thereof described herein may be used to quantitatively or qualitatively detect the FZD10 protein. The antibodies (or fragment thereof) of the present invention may, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for in situ detection of FZD10 protein. In situ detection may be accomplished by removing a histological sample from a subject, such as paraffin-embedded sections of tissues (such as surgical specimens) and applying thereto a labeled antibody of the present invention. The antibody (or fragment thereof) is preferably applied by overlaying a sample with the labeled antibody (or fragment thereof). Using the present invention, those skilled in the art will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

[0181] Immunoassays for FZD10 protein will typically comprise incubating a sample from a subject to be examined, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells that have been incubated in cell culture, in the presence of a detectably labeled antibody of the present invention, and detecting the bound antibody by any of a number of techniques well-known in the art.

[0182] The sample may be brought into contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or another solid support which is capable of immobilizing cells, cell particles, or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled antibody against FZD10. The solid phase support may then be washed with the buffer a second time to remove unbound antibody. The amount of bound label on the solid support may then be detected by conventional means.

[0183] The term "solid phase support or carrier" means any support capable of binding an antigen or an antibody. Those skilled in the art will know many suitable carriers for binding antibodies or antigens, or will be able to ascertain the same by use of routine experimentation.

[0184] The binding activity of a given lot of anti-FZD10 antibody may be determined according to well-known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

[0185] To detect a reaction between the antibody (or its fragment) of the present invention and the FZD10 protein derived from an FZD10-associated disease affected site in a sample easily, the reaction can be directly detected by labeling the antibody of the present invention or indirectly detected by using a labeled secondary antibody. The latter indirect detection procedure, such as a sandwich assay or competitive assay of ELISA, is preferably used in the method of the present invention for better sensitivity.

[0186] Examples of labels for use herein are as follows. Peroxidases (PODs), alkaline phosphatases, β -galactosidase, urease, catalase, glucose oxidase, lactate dehydrogenase, amylases, and biotin-avidin complexes can be used in an enzyme immunoassay.

[0187] Fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (TRITC), substituted rhodamine isothiocyanate, dichlorotriazine isothiocyanate and Alexa488 can be used in an immunofluorescent assay. Tritium, iodine (such as ^{125}I , and ^{131}I), and indium (such as ^{111}In) can be used in a radioimmunoassay. NADH-FMN $_2$ -luciferase assay, luminol-hydrogen peroxide-POD system, acridinium esters, and dioxetane compounds can be used in an immunoluminescent assay.

[0188] The label can be attached to the antibody according to a conventional procedure. For example, the label can be attached to the antibody by a glutaraldehyde method, maleimide method, pyridyl disulfide method, or periodate method in the enzyme immunoassay, and by a chloramine T method or Bolton-Hunter method in the radioimmunoassay.

[0189] The assay can be performed according to a known procedure (Ausubel, F. M. et al. Eds., Short Protocols in Molecular Biology, Chapter 11 "Immunology" John Wiley & Sons, Inc. 1995).

[0190] For example, when the antibody of the present invention is directly labeled with the label described above, the sample is brought into contact with the labeled antibody to thereby form a complex between the FZD10 protein and the

antibody. Then, unbound labeled antibody is separated, and the level of the FZD10 protein in the sample can be determined based on the amount of the bound labeled antibody or that of the unbound labeled antibody.

[0191] When a labeled secondary antibody is used, the antibody of the present invention is allowed to react with the sample in a primary reaction, and the resulting complex is allowed to react with the labeled secondary antibody in a secondary reaction. The primary reaction and the secondary reaction can be performed in reverse order, concurrently with some interval of time therebetween. The primary and secondary reactions yield a complex of [FZD10 protein]-[the antibody of the invention]-[the labeled secondary antibody] or a complex of [the antibody of the invention]-[FZD10 protein]-[the labeled secondary antibody]. Unbound labeled secondary antibody is then separated, and the level of the FZD10 protein in the sample can be determined based on the abundance of the bound labeled secondary antibody or that of the unbound labeled secondary antibody.

[0192] According to another embodiment, the antibody of the present invention is labeled with a radioisotope or a fluorescent label, and the labeled antibody is parenterally administered to a subject. Thus, the localization of a primary tumor and the related metastasized tumor of FZD10-associated disease can be rapidly found in a non-invasive manner. Such a diagnosis method is known as tumor in vivo imaging, and one skilled in the art can easily understand the procedures thereof. The labeled antibody can be administered to the subject systemically or locally, preferably through a parenteral route such as intravenous injection, intramuscular injection, intraperitoneal injection, or subcutaneous injection.

[0193] The antibodies according to the present invention specifically react with a FZD10 protein as mentioned above and can thereby be used in kits for diagnosis and/or prognosis of an FZD10-associated disease.

[0194] The kit for diagnosis and/or prognosis of the present invention comprises an antibody or a fragment thereof described herein. By detecting the FZD10 protein in a sample from a subject who is suspected to suffer from an FZD10-associated disease with the use of the kit for diagnosis and/or prognosis of the present invention, whether or not the subject suffers from the FZD10-associated disease can be rapidly and easily ascertained. Kits for diagnosis and/or prognosis of diseases using such immunological reactions have been widely known, and one skilled in the art can easily select appropriate components other than the antibody. The kits for diagnosis and/or prognosis of the present invention can be used in any means, as long as it is a means for immunoassay.

EXAMPLES

[0195] The present invention will be further illustrated by the following non-limiting examples.

[0196] Cell lines and tissue specimens used in the following examples were prepared as described below. Specifically, cell lines derived from synovial sarcomas (HS-SY-2, YaFuSS, 1973/99, Fuji and SYO-1), colon cancers (LoVo, SNU-C4 and SNU-05), HEK293 and COST cells were grown in monolayers in appropriate media supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution, and maintained at 37° C. in air containing 5% CO₂. Primary synovial sarcoma (SS) samples were obtained after informed consent, and snap-frozen in liquid nitrogen immediately after resection and stored at -80° C.

Example 1

Generation of Anti-FZD10 Monoclonal Antibodies

[0197] (1) Generating Monoclonal Antibodies with Cell Immunization

[0198] Mouse anti-FZD10 monoclonal antibodies (Mabs) were generated by immunizing four weeks old female Balb/c mice in their foot pads with 2×10^7 COS-7 cells transfected with 2×10^7 of pCAGGS/neo-FZD10-myc/His (Medical and Biological Laboratories, Nagoya, Japan). Construction of pCAGGS/neo-FZD10-myc/His was reported previously (Nagayama, S., et al. (2005). *Oncogene*, 24, 6201-12) and this expresses the entire coding sequence of FZD10 cDNA and Myc and His epitope tags at its C terminus. The mice had been immunized with Freund complete adjuvant (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan) in one day prior to the cell immunization. Spleen cells from the immunized mice were harvested and fused with the myeloma cell line. The hybridomas were subcloned and assayed by Cell ELISA for the ability to secrete immunoglobulin that binds to the extracellular domain of FZD10 (amino acid residues 1-225 of FZD10). For cell ELISA, COS-7 cells expressing FZD10-myc/His (the entire coding sequence of FZD10 cDNA and Myc and His epitope tags at its C terminus) were seeded into 96-well plates. Subsequently, 50 μ l of the culture supernatants obtained from hybridomas were added to the plate and incubated for 30 minutes at room temperature. After washing the cells, goat anti-mouse IgG-POD (Medical and Biological Laboratories, Nagoya, Japan) was added at 1:10000 dilution, incubated for 30 minutes at room temperature. Bound antibodies were detected at OD_{450-620nm}. Positive clones were further analyzed for specific binding activity. These clones includes: clones 39-2 and 39-10 (disclosed in WO2005/004912, referred to as 5F2) as well as 92-13 and 93-22. All Mabs were of the IgG2a isotype as determined by means of the IsoStrip Mouse Monoclonal antibody isotyping kit (Roche). The Mabs were affinity purified on protein G-sepharose for further characterization.

[0199] The hybridoma clone 93-22 producing mouse monoclonal antibody 93-22 was deposited by Shuichi Nakatsuru internationally at the IPOD International Patent Organism Depository of the National Institute of Advanced Industrial Science and Technology (AIST Tsukuba Central 6, 1-1, Higashi 1-chome, Tsukuba-shi, Ibaraki-Ken, 305-8566 Japan) as of Jun. 14, 2006 under the deposit number of FERM BP-10620. Also, hybridoma clone 92-13 producing mouse monoclonal antibody 92-13 was deposited by Shuichi Nakatsuru internationally at the IPOD International Patent Organism Depository of the National Institute of AIST as of Jun. 28, 2006 under the deposit number of FERM BP-10628.

(2) Labeling Antibodies with Radionuclides

[0200] ¹²⁵I-labeled Mabs were prepared by chloramine T method (Arano, Y., et al. (1999). *Cancer Res*, 59, 128-34). 740 kBq/2 μ l of Na¹²⁵I was added to 10 μ g of Mab in 100 μ l of 0.3M sodium phosphate buffer. One μ g of chloramine-T in 3 μ l of 0.3M sodium phosphate buffer was further added, incubated for 5 min at room temperature. Labeled antibody was purified using Biospin column 6 (Bio-Rad).

[0201] For labeling Mabs with ¹¹¹In, 1 mg of Mab in 100 μ l of 50 mM borate buffer (pH8.5) was conjugated to isothiocyanato benzyl diethylenetriaminepentaacetic acid (SCN-BZ-DTPA; Macrocylics) in dimethylformamide at molar ratio 1:3. After incubation at 37° C. for 20 hours, Mab conjugates were purified using Biospin column 6. 40 μ l of ¹¹¹In

was incubated in 60 μ l of 0.25M acetic acid buffer (pH5.5) and incorporated into 10 μ g/ μ l of Mab-DTPA conjugates for one hour at room temperature. Labeled antibody was purified using Biospin column 6.

[0202] For generating ^{90} Y-conjugated 92-13, 92-13 was conjugated with DTPA to lysine residues. DTPA-92-13 was labeled with yttrium to a specific activity 100 μ Ci/mg, and the immunoreactivity of the ^{90} Y-DTPA-92-13 was approximately 70%.

(3) Synthesis of Alexa647-Labeled Mabs.

[0203] Labeling Mabs with Alexa-Fluoro647 was carried out according to manufacturer's instruction using Alexa647 Monoclonal Antibody Labeling Kit (Molecular Probes, Eugene, Oreg.). The Alexa647 reactive dye has a succinimidyl ester moiety that reacts with primary amines of proteins, and resulting Mabs-dye conjugates were purified by size exclusion column.

Example 2

Binding Activities of Anti-FZD10 Monoclonal Antibodies

[0204] The present inventors applied two methods for evaluation of the binding affinity of mouse-monoclonal antibodies; flow cytometrical analysis with fluorescent dyes and radioactive measurement using ^{125} I.

(1) Flow Cytometry (FACS) Analysis

[0205] To investigate the cell-binding affinities of the four antibodies, 39-2 and 39-10 (disclosed in WO2005/004912), 92-13 and 93-22, we performed flow cytometry (FACS) experiments. For flow cytometrical analysis with indirect fluorescence, suspensions of 5×10^6 cells were incubated with 10 μ g/ml of Mabs or non-immunized mouse IgG (Beckman Coulter) for 30 min at 4° C. After washing with PBS, 2 μ g of fluorescent goat anti-mouse IgG (Alexa Fluor 488, Molecular Probes, Eugene, Oreg.) was added, and the cell suspension was incubated for 30 min at 4° C. for analysis by FACScan (Becton Dickinson, Franklin Lakes, N.J.). For direct immunofluorescence assays, cells were incubated with 2 μ g of Alexa488-Mabs in the presence or absence of excess amount (100 μ g) of non-labeled Mabs for 30 min at 4° C. and subjected to analysis by FACScan.

[0206] In order to confirm the expression of FZD10 in cell lines, we performed RT-PCR. For RT-PCR experiments, total RNAs were extracted from cell lines using TRIzol reagent (Invitrogen, Carlsbad, Calif., USA), and 3 μ g aliquot of each total RNA was reversely transcribed. PCR amplification was performed using the cDNAs as templates with the following primers: 5'-TATCGGGCTCTTCTCTGTGC-3' (SEQ ID NO: 9) and 5'-GACTGGGCAGGGATCTCATA-3' (SEQ ID NO: 10) for FZD10 and 5'-TTAGCTGTGCTCGCGCTACT-3' (SEQ ID NO: 11) and 5'-TCACATGGTTACACGGCAG-3' (SEQ ID NO: 12) for β 2-microglobulin (β 2MG), the internal control.

[0207] As shown in FIG. 1a, all of four Mabs, 39-2, 39-10, 92-13 and 93-22 bound to four FZD10-expressing SS cell lines, SYO-1, YafuSS, HS-SY-2, and Fuji in FZD10-dose dependent manner, but not bound to two cell lines, 1973/99 and LoVo, in which no transcript of FZD10 was detected. Table 1 below indicates correlation between relative Mean fluorescent Intensities (MFI) of these Mabs and the expres-

sion levels of FZD10 shown in FIG. 1b. In addition, particularly, we demonstrated that 92-13 and 93-22 Mabs also bound to the SNU-05 transfected with FZD10-myc/His construct, while no binding was detected with SNU-05 cells transfected with empty vector (FIG. 1c), suggesting specific binding of those 92-13 and 93-22 Mabs against FZD10 protein.

TABLE 1

Binding of anti-FZD10 mAbs to human SS cell lines SYO-1, YaFuSS, HS-SY-II, Fuji, 1973/99 and human colon cancer cell line, LoVo.						
	SYO-1	YaFuSS	HS-SY-II	Fuji	1973/99	LoVo
39-2	17.6	11.6	9.4	7.1	5.5	1.1
39-10	18.4	11.8	9.9	6.9	4.9	1.0
92-13	4.7	3.0	3.0	1.3	0.9	1.0
93-22	3.3	2.7	2.4	1.1	1.0	1.1

The MFI of FZD10 is measured by flow cytometry as described above.

(2) Binding Activity Against Normal Blood Cells

[0208] To confirm whether those antibodies can be applied for clinical use, the present inventors further examined the binding activity of antibodies against normal blood cells. To evaluate the non-specific binding activity of Mabs against normal blood cell, ^{125} I-labeled Mabs were incubated with 100 μ l of healthy fresh blood. After incubation for one hour at room temperature, the radioactivities of cell pellet were measured as described above.

[0209] The binding activity of ^{125} I-labeled 92-13 and 93-22 Mabs against normal human blood cells were undetectable in all of three individual donors, whereas those of 39-2 and 39-10 Mabs were detected in all of three individual donors (FIG. 1d). These results were consistent with those of FACS analysis using human peripheral blood mononuclear cell (data not shown), suggesting clinical applicability of only 92-13 and 93-22 antibodies with little possibility of adverse effect to SS patients because of very specific binding affinity to the FZD10 molecule. Therefore, we focused on only 92-13 and 93-22 antibodies for further analysis.

(3) Additional Analyses

[0210] Furthermore, binding assay was performed using ^{125} I-labeled Mabs (see Example 1 (2)) to evaluate the binding affinity against FZD10 molecules on cell surface. For radioactive analysis, 0.5 kBq (0.001 μ g antibody) ^{125} I-labeled Mabs prepared in Example 1 (2) were added to 100 μ l of cell suspension with various amounts of non-labeled identical Mabs. After incubation for one hour at room temperature, the cell suspension was centrifuged at 800xg. Supernatant was removed and the radioactivity of cell pellet was measured.

[0211] The results showed higher binding affinity of 92-13 antibody than 93-22 antibody; approximately 33% of 92-13 bound to the cells and approximately 9% of 93-22 antibody bound to the cells under the same condition (FIG. 1e). The amount of the bound antibody decreased as non-labeled antibodies were added in a dose-dependent manner.

[0212] We subsequently performed binding competition analysis of 92-13 with 93-22 Mabs using flow cytometry. Cell binding of the both of Alexa488-labeled antibodies were completely blocked by high amount of non-labeled antibodies (FIG. 1f; ii and iii) to each other, suggesting that 92-13 and 93-22 Mabs are likely to recognize very similar or same

epitope of FZD10. These findings suggest that these Mabs is able to specifically recognize FZD10 expressed on cell surface of SS cells.

Example 3

Immunohistochemistry

[0213] To evaluate the binding specificity of 92-13 and 93-22 to human tissues, we performed immunohistochemical analysis using frozen tissue sections. Tissue sections of frozen normal adult human organs (BioChain, Hayward, Calif.) were fixed with 4% paraformaldehyde at 4° C. for 15 min, and incubated with 5 µg/ml Mabs for one hour at room temperature. Subsequently, mouse ENVISION Polymer Reagent (DAKO) was added and visualized with peroxidase substrate (3,3'-Diaminobenzidine Tetrahydrochloride).

[0214] The results are shown in FIG. 2. FIG. 2 shows immunohistochemical analyses in SS and normal human frozen tissue sections with no antibody (a, d, g, j, and m), 92-13 (b, e, h, k, and n) and 93-22 (c, f, i, l, and o). (a-c), synovial sarcoma; (d-f), kidney; (g-i), liver; (j-l), heart; (m-o), brain. Expectedly, we observed strong immunoreactivity to FZD10 in SS specimen (FIG. 2, a, b, and c) and placenta (data not shown), but did not detect in normal kidney, heart, brain and liver (FIG. 2, d-o), as concordant with the results of northern-blot and RT-PCR experiments (Nagayama, S., et al. (2005). *Oncogene*, 24, 6201-6212).

Example 4

Biodistribution of Anti-FZD10 Mabs in Balb/c Mice Xenograft Model

[0215] Distribution of 92-13 and 93-22 in in vivo model was examined in BALB/c mice by means of two independent methods, radionuclide imaging and fluorescent imaging.

(1) In Vivo Radionuclide Imaging

[0216] In vivo experiments were performed in the animal facility in accordance with institutional guidelines. BALB/cA Jcl-nu mice (female, 7 weeks old) were injected subcutaneously (s.c.) with SYO-1 tumor cells (5×10^6 cells), in 0.1 ml PBS, in the flanks. For biodistribution studies, mice with fully established tumors were given 10 kBq (0.5-1 µg) of ^{125}I -labeled Mabs and 10 kBq (0.5-1 µg) of ^{111}In -labeled Mabs via tail vein. At 1, 24, 48 hours, animals were euthanized and the weight and radioactivity of tissues were measured. The distribution was expressed as % of injected dose/g of tissue for all samples. For optical imaging of biodistribution, LoVo-tumor bearing mice were used in addition to SYO-1 tumor mice. LoVo tumor cells (1×10^7 cells) were injected s.c. into BALB/cA Jcl-nu mice as described above. When tumors were fully established, the mice were subjected to the imaging study.

[0217] The results in FIG. 3a demonstrates that the radioactivity of ^{111}In -92-13 associated with the blood decreased from 35% injected dose per gram (% ID/g) at one hour postinjection to 12% after 48 hours. Radioactivities of ^{111}In -92-13 associated liver, kidney, intestine, spleen, pancreas, lung, heart, stomach and muscle remained fairly constant or decreasing throughout the observation (FIG. 3a). Radioactivity of ^{111}In -92-13 associated with tumor accumulated throughout the experiment, from 2% ID/g at one hour postinjection to 11% ID/g after 48 hours. On the other hand, FIG. 3b

demonstrates that radioactivity of ^{125}I -labeled 92-13 associated with tumor did not increased significantly although blood-associated radioactivity fell from 25% at one hour to 7% after 48 hours and radioactivities associated with other normal organs remained constant. The ^{125}I -labeled antibodies were possibly degraded inside the cell after internalization. ^{111}In -labeled 93-22 was also accumulated into SYO-1 tumor at 48 hours postinjection (FIG. 3c) and ^{125}I -labeled 93-22 showed poor accumulation (FIG. 3d), suggesting its internalization as well as 92-13.

(2) In Vivo Fluorescence Imaging

[0218] In vivo fluorescence imaging was performed with IVIS™ Imaging System 100 series (Xenogen, Alameda, Calif.). An optimized Cy_{5.5} filter was used to acquire Alexa647-Mabs fluorescence in vivo. SYO-1 tumor-bearing mice were injected 20 µg of Alexa647-labeled Mabs intraperitoneally and subjected to fluorescent imaging at various time points. The mice were fed with food that is not containing alfalfa for four days in prior to injecting Mabs in order to reduce the background fluorescence. When acquiring images, mice were anesthetized with 2% of isoflurane (Abbott Laboratories) and placed in the IVIS system. The mice were euthanized at four days after the Mab injection, the tumor and major organs were dissected, and fluorescence image was obtained.

[0219] As shown in FIG. 4a, significant amount of fluorescence was detected at the location of tumor at 24 hours after the injection. The tumor-bound fluorescence was observed for both Mabs, 92-13 and 93-22; the signals reached at maximum level at about 48 hours after the injection, and could be detectable at 96 hours after the injection. The present inventors sacrificed these mice at 120 hours postinjection and measured their fluorescence intensity in the tumor and also important normal organs (liver, spleen, kidney, pancreas, colon) (FIGS. 4b and 4c). Very strong fluorescence signal was observed in the dissected tumor, whereas no fluorescence signal was detected in normal organs. To validate the binding specificity, the present inventors generated xenografts using antigen-negative cell line, LoVo, in nude mice and injected Alexa647-labeled Mabs, performed fluorescence imaging analysis. In LoVo-bearing mice, fluorescent was detected neither at the location of the tumor (FIG. 5a), nor in the dissected tumor or other organs (FIGS. 5b and 5c). These results demonstrated that these Mabs are also able to bind specifically to FZD10-expressed tumor cells in vivo.

Example 5

Internalization of Anti-FZD10 Mabs into Antigen-Positive Cells

[0220] To investigate molecular behavior of these Mabs after binding to the cell surfaces, their localization was traced using in vitro imaging system.

[0221] Cells were plated into 8-well chamber slides (Nalge Nunc International, Naperville, Ill.) at density of 5×10^4 cells per well. Cells were incubated with Mabs for three hours at 37° C. in air chamber containing 5% CO₂. Mabs bound to the cell surface were removed by acid stripping buffer (0.1M Glycine, 500 mM NaCl, pH2.5) at 4° C. for 10 min and neutralized with 500 mM Tris (pH7.5). Cells were then fixed with 3.7% formaldehyde for 15 min at room temperature, and permeabilized by exposure to 0.2% TritonX-100 for 10 min,

followed by blocking with 3% bovine serum albumin for one hour at room temperature. To detect the Mabs internalized into the cell, samples were incubated with Alexa488-labeled goat-anti mouse IgG (1:700 dilution) for one hour at room temperature. The slides were mounted with DAPI (Vectashield, Vector Laboratories, Burlingame, Calif.) and analyzed under Leica TCS SP1 confocal optics.

[0222] As shown in FIG. 6, both Mabs 92-13 and 93-22 were efficiently incorporated into the cytosol of SYO-1 cells and YaFuSS cells at 3 hours after the incubation of Mab with cells by confocal microscope imaging detected using Alexa488-labeled goat anti-mouse IgG (FIG. 6, *a-f*). On the other hand, the fluorescence signals of these Mabs were hardly detectable in LoVo cells without FZD10 expression (FIG. 6, *g-i*), demonstrating that the specific binding of Mabs to cell-surface FZD10 induced the internalization of the antibodies.

Example 6

Specific Cytotoxicity of Mabs

[0223] 92-13 and 93-22 had no effect on tumor cell growth when added directly into the cultured cell (data not shown). For therapy studies, SYO-1 tumors were grown in BALB/cA Jcl-nu mice in the same manner as in Example 4. The diameters of the tumors were measured by calipers and the tumor volumes were determined using the following formula: $0.5 \times (\text{larger diameter}) \times (\text{smaller diameter})^2$ as described previously (Nagayama, S., et al. (2005). *Oncogene*, 24, 6201-12). When the tumor volumes reached more than 0.4-2.8 cm³, Balb/c-nude mice bearing subcutaneous SYO-1 tumor were randomly assigned to treatment groups and received intravenous injections of the 100 μ Ci of ⁹⁰Y-labeled Mabs or control Mabs via tail vein. Mice were weighed and tumor diameters were recorded.

[0224] FIG. 7 showed that tumor volumes were markedly reduced immediately after treatment, almost to traces within one week in all mice. When 50 μ Ci of ⁹⁰Y-DTPA-92-13 were given to the mice, tumors >1 cm³ volumes refracted two weeks after treatment although they showed marked reduction of tumor size immediately after treatment. The mice showed temporary decrease of the weight (10~15%), however, they recovered in one week and no visible toxic signs were observed (data not shown).

Example 7

Generation of Chimeric Antibodies

[0225] Chimeric antibodies corresponding to mouse 92-13 and 93-22 antibodies, ch92-13 and ch93-22 were generated by replacement of the variable region sequence of each mouse antibody to the human IgG1 constant region under the control of CMV promoter. Total RNAs were extracted from hybridoma clones 92-13 and 93-22. cDNA was synthesized from the total RNA using GeneRacer™ Kit (Invitrogen). The sequences of variable regions of monoclonal antibodies were amplified using forward primer (GeneRacer™ 5'Primer) and reverse primer; CH1 (IgG2a); 5'-AATTTTCTGTCCACCT-TGGTG-3' (SEQ ID NO: 3) for heavy chain and CL1 (kappa); 5'-CTAACACTCATTCTGTGAAGCTCT-3' (SEQ ID NO: 4) for light chain. PCR products were sequenced and the sequences coding the m92-13 and m93-22 variable region were determined.

[0226] As a result, the amino acid sequence of mouse Ig H-chain variable regions and L-chain variable regions were determined as follows:

92-13, H-Chain Variable Region:

[0227]

MKCSWVIFFLMAVVTGVNSEVQLQQSGAELVKP
GASVKLSCTASGFNINDTYMH WVKQRPEQGLEWI-
GRIDPANGNTKYDPKFQGGKATI-
TADTSSNTAYLQLSSLTSEDV AVYYCARGARGSRFAY-
WGQGTLLTVSA (SEQ ID NO: 13) encoded by the
nucleotide sequence of SEQ ID NO: 14, and

92-13, L-chain variable region:

MSVPTQVLGLLLWLTDARCDIQMTQSPASLSVSV
GETVTITCRASENIYSNLAWY QQQKGKSPQLLVY-
VATNLADGVPSRFSGSGSGTQYSLKIN-
SLQSEDFGSYYCQHF WGTPTYFGGGTKL (SEQ ID
NO: 21) encoded by the nucleotide sequence of SEQ ID NO:
22; and

93-22, H-chain variable region:

MGWSRIFLFLSITAGVHCQVQLQQSGPELVKPGA
SVKISKASGYAFSSSWMNW VKQRPQGGLLEWI-
GRIYPGDGDITNYNGKFKGKATL-
TADKSSSTAYMQLSSLTSDV AVYFCARGGNYGW-
FAYWGQGTLLTVSAGS (SEQ ID NO: 29) encoded by the
nucleotide sequence of SEQ ID NO: 30, and

93-22, L-chain variable region:

METDTLLLVWVLLWVPGSTGDIVLTQSPASLAVS
LGQRATISCRASKSVSTSGYSY MHWYQQKPGQPPK-
WYLASNLESGVPARFSGSGSGTDFTLNI-
HPVEEEDAATYY CQHSRELYTFGGGKLG (SEQ ID
NO: 37) encoded by the nucleotide sequence of SEQ ID NO:
38. Underlines indicate the signal sequences.

[0228] The CDR (complementarity determining region) sequences of the antibodies were determined as follows:

92-13,	(SEQ ID NO: 15)
INDTYMH as VH CDR1,	
RIDPANGNTKYD as VH CDR2,	(SEQ ID NO: 17)
and	
GSRFAY as VH CDR3,	(SEQ ID NO: 19)
RASENIYSNLA as VL CDR1,	(SEQ ID NO: 23)
VATNLAD as VL CDR2,	(SEQ ID NO: 25)
and	
QHFWGTPY as VL CDR3;	(SEQ ID NO: 27)
and	
93-22,	(SEQ ID NO: 31)
SSWMN as VH CDR1,	
RIYPGDGDITNYN as VH CDR2,	(SEQ ID NO: 33)
and	
GGNYGWFAF as VH CDR3,	(SEQ ID NO: 35)

-continued

(SEQ ID NO: 39)
RASKSVSTSGYSYMH as VL CDR1,

(SEQ ID NO: 41)
LASNLES as VL CDR2,
and

(SEQ ID NO: 43)
QHSRELY as VL CDR3.

[0229] In addition, the amino acid sequences of the H chains and L chains of mouse monoclonal antibodies 92-13, 93-22 and 39-10 are determined as follows:

92-13, H chain: SEQ ID NO: 58 (encoded by the nucleotide sequence of SEQ ID NO: 57);

92-13, L chain: SEQ ID NO: 60 (encoded by the nucleotide sequence of SEQ ID NO: 59);

93-22, H chain: SEQ ID NO: 62 (encoded by the nucleotide sequence of SEQ ID NO: 61);

93-22, L chain: SEQ ID NO: 64 (encoded by the nucleotide sequence of SEQ ID NO: 63);

39-10, H chain: SEQ ID NO: 66 (encoded by the nucleotide sequence of SEQ ID NO: 65);

39-10, L chain: SEQ ID NO: 68 (encoded by the nucleotide sequence of SEQ ID NO: 67).

[0230] According to the determined sequence, specific primers for m92-13 variable region were designed: 5'-AAT-AGCGGCCGCACCATGAAATGCAGCTGGGTTATCTT-3' (SEQ ID NO: 5) and 5'-AATAGCTAGCTGCAGAGACAGTGACCAAGTCC-3' (SEQ ID NO: 6) for heavy chain and 5'-AATAGCGGCCGCACCATGAGTGTGCCACTCAGG-3' (SEQ ID NO: 7) and 5'-TTCCAGCTTG-TTCCCCC-3' (SEQ ID NO: 8) for light chain. Also, specific primers for m93-22 variable region were designed, 5'-AATAGCGGCCGCACCATGGGATGGAGC-CGGATCTTT-3' (SEQ ID NO: 53) and 5'-AATAGGATCCT-GCAGAGACAGTGACCAAGTCCCTT-3' (SEQ ID NO: 54) for heavy chain and 5'-AATAGCGGCCGCACCATG-GAGACAGACACTCCT-3' (SEQ ID NO: 55) and 5'-AATAGGATCCAGCTTGGTCCCCCTCCGAACGT-3' (SEQ ID NO: 56) for light chain. To construct the expression vector for chimeric antibodies, two cassette vectors were prepared. The DNA fragment coding human IgG1 (CH1-CH3) was inserted into pQCXIH (Clontech) (pQCXCHIH) and the DNA fragment coding human Igk (CL1) was inserted into pQCXIP (pQCXCLIP). For obtaining DNA fragments coding human IgG1 or human Igk, human constant region library was prepared using cDNA from human PBMC (peripheral blood mononuclear cells) by the reported method (Liu, A. Y. et al., Proc. Natl. Acad. Sci. USA, Vol. 84, 3439-43, 1987; Reff, M. E. et al., Blood, Vol. 83, No. 2, 435-45, 1994). The DNAs coding variable region of m92-13 and m93-22 heavy chain and light chain were PCR amplified, sequenced and subcloned into pQCXCHIH and pQCXCLIP respectively using NotI and BamHI site. These vectors were co-transfected into CHO cells. Transfected cells were cultured in F-12 medium containing 500 µg/ml Hygromycin and 10 µg/ml Puromycin. When cells grow sub-confluently, the medium was exchanged to serum-free medium (CHO-S-FM II; GIBCO) and chimeric antibody was purified from the supernatant of cultured cells using protein A-affinity column (GE Amersham) and was sequenced. The sequence of heavy chain of chimeric antibody ch92-13 comprises SEQ ID NO: 46 encoded by the nucleotide sequence of SEQ ID NO: 45;

and the sequence of light chain of chimeric antibody ch92-13 comprises SEQ ID NO: 48 encoded by the nucleotide sequence of SEQ ID NO: 47. The sequence of heavy chain of chimeric antibody ch93-22 comprises SEQ ID NO: 49 encoded by the nucleotide sequence of SEQ ID NO: 50; and the sequence of light chain of chimeric antibody ch93-22 comprises SEQ ID NO: 52 encoded by the nucleotide sequence of SEQ ID NO: 51.

Example 8

Binding Activity of Chimeric Antibodies

[0231] Antibody-dependent cell cytotoxicity (ADCC) activities induced by chimeric 92-13 and 93-22 were determined using LDH activity as described previously (Nagayama, S., et al. (2005). Oncogene, 24, 6201-6212). Fresh effector cells were isolated from heparinized peripheral blood of a healthy donor by Ficoll-Plaque (Amersham Bioscience). Effector cells (E) and target cells (T) (each, 5×10^3 /well) were co-incubated for 6 h at 37° C. in quadruplicate at various E:T ratios, together with chimeric 92-13, chimeric 93-22 or non-immunized human IgG, in 0.1 ml of phenol red-free RPMI 1640 supplemented with 5% FBS in a 96-well plate. LDH released in the culture supernatants was determined by absorbance at 490 nm. The percentage of specific cytotoxicity was calculated according to the manufacturer's instructions.

[0232] Referring to the effector activity, both chimeric 92-13 and 93-22 induced ADCC specifically to the FZD10-overexpressing SYO-1 cells (FIGS. 8, *a* and *c*), but not to the FZD10-negative LoVo cells (FIGS. 8, *b* and *d*). Particularly, chimeric 92-13 showed higher induction of cytotoxicity as compared with chimeric 93-22; however, their activity depends on effector cell donor, possibly caused by polymorphism of Fc receptor.

[0233] Each of the applications and patents mentioned in this document, and each document cited or referenced in each of the above applications and patents are hereby incorporated herein by reference. Furthermore, all documents cited in this text, and all documents cited or referenced in documents cited in this text, and any manufacturer's instructions or catalogues for any products cited or mentioned in this text, are hereby incorporated herein by reference.

[0234] Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments and that many modifications and additions thereto may be made within the scope of the invention. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the claims. Furthermore, various combinations of the features of the following dependent claims can be made with the features of the independent claims without departing from the scope of the present invention.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 68

<210> SEQ ID NO 1

<211> LENGTH: 2811

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

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tgggctcgtg cgccgccatc agtcccatgg acatggagcg cccgggcgac ggcaaatgcc      120
agcccatcga gatcccgatg tgcaaggaca tcggctacaa catgactcgt atgcccaacc      180
tgatgggcca cgagaaccag cgcgaggcag ccatccagtt gcacgagttc gcgcccgtgg      240
tggagtacgg ctgccacggc cacctccgct tcttctctgt ctcgtgttac gcgcgatgt      300
gcaccgagca ggtctctacc cccatcccg cctgcgggt catgtgcgag caggcccggc      360
tcaagtgtc cccgattatg gagcagttca acttcaagtg gcccgactcc ctggactgcc      420
ggaaactccc caacaagaac gaccccaact acctgtgcat ggaggcgccc aacaacggct      480
cggacgagcc caccggggc tcgggcctgt tcccggcct gtccggcgcg cagcgggccc      540
acagcgcgca ggagcaccgc ctgaaggacg ggggcccccg gcgcgcgcg tcgcacaacc      600
cgggcaagtt ccaccacgtg gagaagagcg cgtcgtgcgc gccgctctgc acgcccggcg      660
tggacgtgta ctggagccgc gaggacaagc gcttcgcagt ggtctggctg gccatctggg      720
cgggtcgtgt cttcttctcc agcgccttca ccgtgctcac cttcctcacc gaccggccc      780
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acctggcagc ctgggccatc ccggcggtga agaccatcct gatcctggtc atgcgcaggg      1140
tggcggggga cgagctcacc ggggtctgct acgtgggcag catggacgtc aacgcgtca      1200
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taagatgtg tgcttttctt ggttggtgtt ttctttcttc ttcttctttt ttttttttt      1860
ataaaagcaa aagagaaata cataaaaaag tgtttacctt gaaattcagg atgctgtgat      1920
acactgaaag gaaaaatgta cttaaagggt tttgttttgt tttggttttc cagcgaagg      1980

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aagctcctcc agtgaagtag cctcttgtgt aactaatttg tggtaaagta gttgattcag 2040
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ggctttgcta cccatttaca aataagagga cagataactg ctttgcaaat tcaagagcct 2160
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aatgatttcc acaaacagat caggaagcac taggttgga gagacacttt gtctagtgtg 2700
ttctcttcac agtgccagga aagagtgggt tctgcgtgtg tatatttgta atatatgata 2760
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<210> SEQ ID NO 2

<211> LENGTH: 581

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

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Met  Gln  Arg  Pro  Gly  Pro  Arg  Leu  Trp  Leu  Val  Leu  Gln  Val  Met  Gly
 1          5          10          15
Ser  Cys  Ala  Ala  Ile  Ser  Ser  Met  Asp  Met  Glu  Arg  Pro  Gly  Asp  Gly
          20          25          30
Lys  Cys  Gln  Pro  Ile  Glu  Ile  Pro  Met  Cys  Lys  Asp  Ile  Gly  Tyr  Asn
          35          40          45
Met  Thr  Arg  Met  Pro  Asn  Leu  Met  Gly  His  Glu  Asn  Gln  Arg  Glu  Ala
          50          55          60
Ala  Ile  Gln  Leu  His  Glu  Phe  Ala  Pro  Leu  Val  Glu  Tyr  Gly  Cys  His
          65          70          75          80
Gly  His  Leu  Arg  Phe  Phe  Leu  Cys  Ser  Leu  Tyr  Ala  Pro  Met  Cys  Thr
          85          90          95
Glu  Gln  Val  Ser  Thr  Pro  Ile  Pro  Ala  Cys  Arg  Val  Met  Cys  Glu  Gln
          100          105          110
Ala  Arg  Leu  Lys  Cys  Ser  Pro  Ile  Met  Glu  Gln  Phe  Asn  Phe  Lys  Trp
          115          120          125
Pro  Asp  Ser  Leu  Asp  Cys  Arg  Lys  Leu  Pro  Asn  Lys  Asn  Asp  Pro  Asn
          130          135          140
Tyr  Leu  Cys  Met  Glu  Ala  Pro  Asn  Asn  Gly  Ser  Asp  Glu  Pro  Thr  Arg
          145          150          155          160
Gly  Ser  Gly  Leu  Phe  Pro  Pro  Leu  Phe  Arg  Pro  Gln  Arg  Pro  His  Ser
          165          170          175
Ala  Gln  Glu  His  Pro  Leu  Lys  Asp  Gly  Gly  Pro  Gly  Arg  Gly  Gly  Cys
          180          185          190
Asp  Asn  Pro  Gly  Lys  Phe  His  His  Val  Glu  Lys  Ser  Ala  Ser  Cys  Ala
          195          200          205
Pro  Leu  Cys  Thr  Pro  Gly  Val  Asp  Val  Tyr  Trp  Ser  Arg  Glu  Asp  Lys

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210	215	220
Arg Phe Ala Val Val Trp	Leu Ala Ile Trp	Ala Val Leu Cys Phe Phe
225	230	235 240
Ser Ser Ala Phe Thr Val	Leu Thr Phe Leu Ile Asp	Pro Ala Arg Phe
245	250	255
Arg Tyr Pro Glu Arg Pro	Ile Ile Phe Leu Ser Met	Cys Tyr Cys Val
260	265	270
Tyr Ser Val Gly Tyr Leu	Ile Arg Leu Phe Ala Gly	Ala Glu Ser Ile
275	280	285
Ala Cys Asp Arg Asp Ser	Gly Gln Leu Tyr Val	Ile Gln Glu Gly Leu
290	295	300
Glu Ser Thr Gly Cys Thr	Leu Val Phe Leu Val	Leu Tyr Tyr Phe Gly
305	310	315 320
Met Ala Ser Ser Leu Trp	Trp Val Val Leu Thr	Leu Thr Trp Phe Leu
325	330	335
Ala Ala Gly Lys Lys Trp	Gly His Glu Ala Ile	Glu Ala Asn Ser Ser
340	345	350
Tyr Phe His Leu Ala Ala	Trp Ala Ile Pro Ala	Val Lys Thr Ile Leu
355	360	365
Ile Leu Val Met Arg Arg	Val Ala Gly Asp Glu	Leu Thr Gly Val Cys
370	375	380
Tyr Val Gly Ser Met Asp	Val Asn Ala Leu Thr	Gly Phe Val Leu Ile
385	390	395 400
Pro Leu Ala Cys Tyr Leu	Val Ile Gly Thr Ser	Phe Ile Leu Ser Gly
405	410	415
Phe Val Ala Leu Phe His	Ile Arg Arg Val Met	Lys Thr Gly Gly Glu
420	425	430
Asn Thr Asp Lys Leu Glu	Lys Leu Met Val Arg	Ile Gly Leu Phe Ser
435	440	445
Val Leu Tyr Thr Val Pro	Ala Thr Cys Val Ile	Ala Cys Tyr Phe Tyr
450	455	460
Glu Arg Leu Asn Met Asp	Tyr Trp Lys Ile Leu	Ala Ala Gln His Lys
465	470	475 480
Cys Lys Met Asn Asn Gln	Thr Lys Thr Leu Asp	Cys Leu Met Ala Ala
485	490	495
Ser Ile Pro Ala Val Glu	Ile Phe Met Val Lys	Ile Phe Met Leu Leu
500	505	510
Val Val Gly Ile Thr Ser	Gly Met Trp Ile Trp	Thr Ser Lys Thr Leu
515	520	525
Gln Ser Trp Gln Gln Val	Cys Ser Arg Arg Leu	Lys Lys Lys Ser Arg
530	535	540
Arg Lys Pro Ala Ser Val	Ile Thr Ser Gly Gly	Ile Tyr Lys Lys Ala
545	550	555 560
Gln His Pro Gln Lys Thr	His His Gly Lys Tyr	Glu Ile Pro Ala Gln
565	570	575
Ser Pro Thr Cys Val		
580		

<210> SEQ ID NO 3
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:

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<223> OTHER INFORMATION: An artificially synthesized primer for RT-PCR

<400> SEQUENCE: 3

aattttcttg tccaccttg tg 22

<210> SEQ ID NO 4

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: An artificially synthesized primer for RT-PCR

<400> SEQUENCE: 4

ctaactca ttctgtga agctct 26

<210> SEQ ID NO 5

<211> LENGTH: 38

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: An artificially synthesized primer for PCR

<400> SEQUENCE: 5

aatagcgcc gcacatgaa atgcagctgg gttatctt 38

<210> SEQ ID NO 6

<211> LENGTH: 33

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: An artificially synthesized primer for PCR

<400> SEQUENCE: 6

aatagctagc tgcagagaca gtgaccagag tcc 33

<210> SEQ ID NO 7

<211> LENGTH: 34

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: An artificially synthesized primer for PCR

<400> SEQUENCE: 7

aatagcgcc gcacatgag tgtgccact cagg 34

<210> SEQ ID NO 8

<211> LENGTH: 18

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: An artificially synthesized primer for PCR

<400> SEQUENCE: 8

ttccagcttg gtccccc 18

<210> SEQ ID NO 9

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: An artificially synthesized primer sequence for RT-PCR

<400> SEQUENCE: 9

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tatcgggctc ttctctgtgc 20

<210> SEQ ID NO 10
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: An artificially synthesized primer sequence for RT-PCR

<400> SEQUENCE: 10
 gactgggcag ggatctcata 20

<210> SEQ ID NO 11
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: An artificially synthesized primer sequence for RT-PCR

<400> SEQUENCE: 11
 ttagctgtgc tcgcgtact 20

<210> SEQ ID NO 12
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: An artificially synthesized primer sequence for RT-PCR

<400> SEQUENCE: 12
 tcacatgggt cacacggcag 20

<210> SEQ ID NO 13
 <211> LENGTH: 137
 <212> TYPE: PRT
 <213> ORGANISM: Mouse

<400> SEQUENCE: 13

Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val Val Thr Gly
 1 5 10 15

Val Asn Ser Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys
 20 25 30

Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile
 35 40 45

Asn Asp Thr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
 50 55 60

Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp
 65 70 75 80

Pro Lys Phe Gln Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn
 85 90 95

Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val
 100 105 110

Tyr Tyr Cys Ala Arg Gly Ala Arg Gly Ser Arg Phe Ala Tyr Trp Gly
 115 120 125

Gln Gly Thr Leu Val Thr Val Ser Ala
 130 135

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<210> SEQ ID NO 14
<211> LENGTH: 411
<212> TYPE: DNA
<213> ORGANISM: Mouse

<400> SEQUENCE: 14

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gttcagctgc agcagctctgg ggcagagctt gtgaagccag gggcctcagt caagttgtcc 120
tgcacagctt ctgggttcaa cattaacgac acctatatgc actgggtgaa gcagaggcct 180
gaacagggcc tggagtggat tggaaggatt gatcctgcga atggtaatac taaatatgac 240
ccgaagttcc agggcaaggc cactataaca gcagacacat cctccaacac agcctacctg 300
cagctcagca gcctgacatc tgaggacact gccgtctatt actgtgctag aggagcacgg 360
gggagtagat ttgcttactg gggccaaggg actctgggtca ctgtctctgc a 411

<210> SEQ ID NO 15
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mouse

<400> SEQUENCE: 15

Ile Asn Asp Thr Tyr Met His
1 5

<210> SEQ ID NO 16
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Mouse

<400> SEQUENCE: 16

attaacgaca cctatatgca c 21

<210> SEQ ID NO 17
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Mouse

<400> SEQUENCE: 17

Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp
1 5 10

<210> SEQ ID NO 18
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Mouse

<400> SEQUENCE: 18

aggattgatc ctgcgaatgg taatactaaa tatgac 36

<210> SEQ ID NO 19
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Mouse

<400> SEQUENCE: 19

Gly Ser Arg Phe Ala Tyr
1 5

<210> SEQ ID NO 20
<211> LENGTH: 18

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<212> TYPE: DNA
<213> ORGANISM: Mouse

<400> SEQUENCE: 20

gggagtagat ttgcttac 18

<210> SEQ ID NO 21
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Mouse

<400> SEQUENCE: 21

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

Asp Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser
20 25 30

Val Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn
35 40 45

Ile Tyr Ser Asn Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro
50 55 60

Gln Leu Leu Val Tyr Val Ala Thr Asn Leu Ala Asp Gly Val Pro Ser
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn
85 90 95

Ser Leu Gln Ser Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp
100 105 110

Gly Thr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu
115 120

<210> SEQ ID NO 22
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Mouse

<400> SEQUENCE: 22

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gacatccaga tgactcagtc tccagcctcc ctatctgtat ctgtgggaga aactgtcacc 120

atcacatgtc gagcaagtga gaatatttac agtaatttag catgggtatca gcagaaacag 180

ggaaaatctc ctcagctcct ggtctatggt gcaacaaact tagcagatgg tgtgccatca 240

aggttcagtg gcagtggtatc aggcacacag tattccctca agatcaacag cctgcagtct 300

gaagattttg ggagttatta ctgtcaacat ttttggggta ctccgtacac gttcggaggg 360

gggaccaagc tg 372

<210> SEQ ID NO 23
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mouse

<400> SEQUENCE: 23

Arg Ala Ser Glu Asn Ile Tyr Ser Asn Leu Ala
1 5 10

<210> SEQ ID NO 24
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Mouse

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<400> SEQUENCE: 24

cgagcaagtg agaataattta cagtaattta gca

33

<210> SEQ ID NO 25

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Mouse

<400> SEQUENCE: 25

Val Ala Thr Asn Leu Ala Asp

1 5

<210> SEQ ID NO 26

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Mouse

<400> SEQUENCE: 26

gtctatgttg caacaaactt agcagat

27

<210> SEQ ID NO 27

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Mouse

<400> SEQUENCE: 27

Gln His Phe Trp Gly Thr Pro Tyr

1 5

<210> SEQ ID NO 28

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Mouse

<400> SEQUENCE: 28

caacattttt ggggtactcc gtac

24

<210> SEQ ID NO 29

<211> LENGTH: 139

<212> TYPE: PRT

<213> ORGANISM: Mouse

<400> SEQUENCE: 29

Met Gly Trp Ser Arg Ile Phe Leu Phe Leu Leu Ser Ile Thr Ala Gly
1 5 10 15Val His Cys Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
20 25 30Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe
35 40 45Ser Ser Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
50 55 60Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn
65 70 75 80Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser
85 90 95Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp Ser Ala Val
100 105 110

Tyr Phe Cys Ala Arg Gly Gly Asn Tyr Gly Trp Phe Ala Tyr Trp Gly

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115	120	125
Gln Gly Thr Leu Val Thr Val Ser Ala Gly Ser		
130	135	
 <210> SEQ ID NO 30		
<211> LENGTH: 417		
<212> TYPE: DNA		
<213> ORGANISM: Mouse		
 <400> SEQUENCE: 30		
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gtccagctgc agcagctctgg acctgagctg gtgaagcctg gggcctcagt gaagatttcc		120
tgcaaagctt ctggctacgc attcagtagc tcttgatga actgggtgaa gcagaggcct		180
ggacagggtc ttgagtggat tggacggatt tatcctggag atggagatac taactacaat		240
gggaagtcca agggcaaggc cactctgact gcagacaaat cctccagcac agcctacatg		300
caactcagca gcttgacctc tgtggactct gcggtctatt tctgtgcaag agggggtaac		360
tacggctggt ttgcttactg gggccaaggg actctggtca ctgtctctgc aggatcc		417
 <210> SEQ ID NO 31		
<211> LENGTH: 5		
<212> TYPE: PRT		
<213> ORGANISM: Mouse		
 <400> SEQUENCE: 31		
Ser Ser Trp Met Asn		
1	5	
 <210> SEQ ID NO 32		
<211> LENGTH: 18		
<212> TYPE: DNA		
<213> ORGANISM: Mouse		
 <400> SEQUENCE: 32		
agtagctctt ggatgaac		18
 <210> SEQ ID NO 33		
<211> LENGTH: 12		
<212> TYPE: PRT		
<213> ORGANISM: Mouse		
 <400> SEQUENCE: 33		
Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn		
1	5	10
 <210> SEQ ID NO 34		
<211> LENGTH: 36		
<212> TYPE: DNA		
<213> ORGANISM: Mouse		
 <400> SEQUENCE: 34		
cggatttata ctggagatgg agatactaac tacaat		36
 <210> SEQ ID NO 35		
<211> LENGTH: 9		
<212> TYPE: PRT		
<213> ORGANISM: Mouse		
 <400> SEQUENCE: 35		

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Gly Gly Asn Tyr Gly Trp Phe Ala Tyr
1 5

<210> SEQ ID NO 36
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Mouse

<400> SEQUENCE: 36

gggggtaact acggctgggt tgcttac

27

<210> SEQ ID NO 37
<211> LENGTH: 129
<212> TYPE: PRT
<213> ORGANISM: Mouse

<400> SEQUENCE: 37

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
20 25 30

Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser
35 40 45

Val Ser Thr Ser Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro
50 55 60

Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser
65 70 75 80

Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys
100 105 110

Gln His Ser Arg Glu Leu Tyr Thr Phe Gly Gly Gly Thr Lys Leu Gly
115 120 125

Ser

<210> SEQ ID NO 38
<211> LENGTH: 456
<212> TYPE: DNA
<213> ORGANISM: Mouse

<400> SEQUENCE: 38

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gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctggggca gagggccacc 120

atctcatgca gggccagcaa aagtgtcagt acatctggct atagttatat gcactgggtac 180

caacagaaac caggacagcc acccaaaact ctcactctatc ttgcatccaa cctagaatct 240

ggggtccctg ccaggttcag tggcagtggg tctgggacag acttcacct caacatccat 300

cctgtggagg aggaggatgc tgcaacctat tactgtcagc acagtaggga gctgtacacg 360

ttcggagggg ggaccaagct gggatccgaa atcaaacgaa ctgtggctgc accatctgtc 420

ttcatcttcc cgccatctga tgagcagttg aaatct 456

<210> SEQ ID NO 39
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Mouse

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<400> SEQUENCE: 39

Arg	Ala	Ser	Lys	Ser	Val	Ser	Thr	Ser	Gly	Tyr	Ser	Tyr	Met	His
1			5						10				15	

<210> SEQ ID NO 40

<211> LENGTH: 45

<212> TYPE: DNA

<213> ORGANISM: Mouse

<400> SEQUENCE: 40

agggccagca	aaagtgtcag	tacatctggc	tatagttata	tgac	45
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<210> SEQ ID NO 41

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Mouse

<400> SEQUENCE: 41

Leu	Ala	Ser	Asn	Leu	Glu	Ser
1			5			

<210> SEQ ID NO 42

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Mouse

<400> SEQUENCE: 42

cttgcatcca	acctagaatc	t	21
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<210> SEQ ID NO 43

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Mouse

<400> SEQUENCE: 43

Gln	His	Ser	Arg	Glu	Leu	Tyr
1			5			

<210> SEQ ID NO 44

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Mouse

<400> SEQUENCE: 44

cagcacagta	gggagctgta	c	21
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<210> SEQ ID NO 45

<211> LENGTH: 1404

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: A chemically synthesized sequence of H chain of anti-REG4 chimeric antibody with signal sequence.

<400> SEQUENCE: 45

atgaaatgca	gctgggttat	cttcttctg	atggcagtgg	ttacaggggt	caattcagag	60
gttcagctgc	agcagtcctg	ggcagagctt	gtgaagccag	gggcctcagt	caagttgtcc	120
tgacagctt	ctggcttcaa	cattaacgac	acctatatgc	actgggtgaa	gcagaggcct	180
gaacagggcc	tggagtggat	tggaaggatt	gacctctgca	atggtaatac	taaatatgac	240
cgaagtcc	agggaaggc	cactataaca	gcagacacat	cctccaacac	agcctacctg	300

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cagctcagca gcttgacatc tgaggacact gccgtctatt actgtgctag aggagcacgg 360
gggagtagat ttgcttactg gggccaaggg actctgggtca ctgtctctgc agctagcacc 420
aaggggcccat cgggtcttccc cctggcacc cctccaaga gcacctctgg gggcacagcg 480
gccctgggct gccgtgtcaa ggactacttc cccgaaccgg tgacgggtgc gtggaactca 540
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcttc aggactctac 600
tccctcagca gcgtgggtgac cgtgccctcc agcagcttgg gcaccagac ctacatctgc 660
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 720
gacaaaaact acacatgccc accgtgccc gcacctgaac tcctgggggg accgtcagtc 780
ttctcttccc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 840
tgcggtgtgg tggacgtgag ccacgaagac cctgagggtca agttcaactg gtacgtggac 900
ggcggtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 960
cgtgtgtgtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 1020
tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaacctctc caaagccaaa 1080
gggcagcccc gagaaccaca ggtgtacacc ctgccccat cccgggatga gctgaccaag 1140
aaccaggtca gcctgacctg cctgggtcaaa ggtctctatc ccagcgacat cgccgtggag 1200
tgggagagca atgggcagcc ggagaacaac tacaagacca cgctcccgt gctggactcc 1260
gacggctcct tcttctctta tagcaagctc accgtggaca agagcaggtg gcagcagggg 1320
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1380
ctctccctgt ccccggttaa atga 1404

```

<210> SEQ ID NO 46

<211> LENGTH: 467

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: A chemically synthesized sequence of H chain of anti-REG4 chimeric antibody with signal sequence.

<400> SEQUENCE: 46

```

Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val Val Thr Gly
1           5           10          15

```

```

Val Asn Ser Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys
20          25          30

```

```

Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile
35          40          45

```

```

Asn Asp Thr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
50          55          60

```

```

Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp
65          70          75          80

```

```

Pro Lys Phe Gln Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn
85          90          95

```

```

Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val
100         105         110

```

```

Tyr Tyr Cys Ala Arg Gly Ala Arg Gly Ser Arg Phe Ala Tyr Trp Gly
115         120         125

```

```

Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser
130         135         140

```

-continued

Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	145	150	155	160
Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	165	170	175	
Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	180	185	190	
Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	195	200	205	
Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	210	215	220	
Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	225	230	235	240
Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	245	250	255	
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	260	265	270	
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	275	280	285	
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	290	295	300	
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	305	310	315	320
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	325	330	335	
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	340	345	350	
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	355	360	365	
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	370	375	380	
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	385	390	395	400
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	405	410	415	
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	420	425	430	
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	435	440	445	
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	450	455	460	
Pro	Gly	Lys														465			

<210> SEQ ID NO 47

<211> LENGTH: 705

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: A chemically synthesized sequence of L chain of anti-REG4 chimeric antibody with signal sequence.

<400> SEQUENCE: 47

atgagtgtgc ccactcaggt cctgggggttg ctgctgctgt ggcttacaga tgccagatgt 60

gacatccaga tgactcagtc tccagcctcc ctatctgtat ctgtgggaga aactgtcacc 120

-continued

```

atcacatgtc gagcaagtga gaatatttac agtaatttag catggtatca gcagaaacag    180
ggaaaatctc ctcagctcct ggtctatgtt gcaacaaact tagcagatgg tgtgccatca    240
agggtcagtg gcagtggtac aggcacacag tattccctca agatcaacag cctgcagtct    300
gaagattttg ggagttatta ctgtcaacat ttttggggta ctccgtacac gttcggaggg    360
gggaccaagc tggaatcaa acgaactgtg gctgcacat ctgtcttcac cttcccgcca    420
tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat    480
cccagagagg ccaaagtaca gtggaagggtg gataacgccc tccaatcggg taactccag    540
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgaca    600
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc    660
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag                    705

```

<210> SEQ ID NO 48

<211> LENGTH: 234

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: A chemically synthesized sequence of L chain of anti-REG4 chimeric antibody with signal sequence.

<400> SEQUENCE: 48

```

Met Ser Val Pro Thr Gln Val Leu Gly Leu Leu Leu Trp Leu Thr
1           5           10           15
Asp Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser
20          25          30
Val Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn
35          40          45
Ile Tyr Ser Asn Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro
50          55          60
Gln Leu Leu Val Tyr Val Ala Thr Asn Leu Ala Asp Gly Val Pro Ser
65          70          75          80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn
85          90          95
Ser Leu Gln Ser Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp
100         105         110
Gly Thr Pro Tyr Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys Arg
115         120         125
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
130         135         140
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
145         150         155         160
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
165         170         175
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
180         185         190
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
195         200         205
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
210         215         220
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225         230

```

-continued

<210> SEQ ID NO 49
 <211> LENGTH: 1398
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: A chemically synthesized sequence of H chain of anti-REG4 chimeric antibody with signal sequence.

<400> SEQUENCE: 49

```

atgggatgga gccgatctt tctcttctc ctgtcaataa ctgcagggtg ccattgccag      60
gtccagctgc agcagctctg acctgagctg gtgaagcctg gggcctcagt gaagatttcc    120
tgcaaaagctt ctggctacgc attcagtagc tcttggatga actgggtgaa gcagaggcct    180
ggacagggtc ttgagtggat tggacggatt tatcctggag atggagatac taactacaat    240
gggaagtta agggcaaggc cacactgact gcagacaaat cctccagcac agcctacatg    300
caactcagca gcttgacctc tgtggactct gcggtctatt tctgtgcaag agggggtaac    360
tacggctggt ttgcttactg gggccaaggg actctgggtc ctgtctctgc aggatccgcc    420
tccaccaagg gcccatcggt ctccccctg gcgccctgct ccaggagcac ctccgagagc    480
acagcggccc tgggtgcct ggtaaggac tacttccccg aaccgggtgac ggtgtcgtgg    540
aactcaggcg ctctgaccag cggcgtgcac accttcccg ctgtcctaca gtctcagga    600
ctctactccc tcagcagcgt ggtgaccgtg cctccagca acttcggcac ccagacctac    660
acctgcaacg taaatcaca gccacgcaac accaagggtg acaagacagt tgagcgcaa    720
tgttgtgtcg agtgcaccac gtgcccagca ccacctgtgg caggaccgtc agtcttctc    780
tccccccaa aacccaagga caccctcatg atctccccga cccctgaggt cagtgctgtg    840
gtggtggacg tgagccacga agaccccag gtccagttca actggtacgt ggacggcgtg    900
gaggtgcata atgccaaagc aaagccgagg gaggagcagt acaacagcac gtaccgtgtg    960
gtcagcgtcc tcacctctc gcaccaggac tggctgaatg gcaaggagta caagtgcaag   1020
gtctccaaca aagccctccc agcccccatc gagaaaacca tctccaaagc caaagggcag   1080
ccccgagaac cacaggtgta caccctgccc ccatcccggt atgagctgac caagaaccag   1140
gtcagcctga cctgcctggt caaaggcttc tatcccagcg acatcgccgt ggagtgggag   1200
agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc   1260
tccttcttcc tctacagcaa gctcacctg gacaagagca ggtggcagca ggggaacgtc   1320
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gacctctcc   1380
ctgtctccgg gtaaatga                                     1398

```

<210> SEQ ID NO 50
 <211> LENGTH: 465
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: A chemically synthesized sequence of H chain of anti-REG4 chimeric antibody with signal sequence.

<400> SEQUENCE: 50

```

Met Gly Trp Ser Arg Ile Phe Leu Phe Leu Leu Ser Ile Thr Ala Gly
1           5           10          15

Val His Cys Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
20          25          30

Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe

```

-continued

35					40					45					
Ser	Ser	Ser	Trp	Met	Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu
50					55					60					
Glu	Trp	Ile	Gly	Arg	Ile	Tyr	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn
65				70						75				80	
Gly	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Lys	Ser	Ser	Ser
			85					90					95		
Thr	Ala	Tyr	Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Val	Asp	Ser	Ala	Val
			100					105					110		
Tyr	Phe	Cys	Ala	Arg	Gly	Gly	Asn	Tyr	Gly	Trp	Phe	Ala	Tyr	Trp	Gly
		115					120					125			
Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala	Gly	Ser	Ala	Ser	Thr	Lys	Gly
	130					135					140				
Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser
145					150					155				160	
Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val
			165						170				175		
Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe
		180						185					190		
Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val
		195					200					205			
Thr	Val	Pro	Ser	Ser	Asn	Phe	Gly	Thr	Gln	Thr	Tyr	Thr	Cys	Asn	Val
	210					215					220				
Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Thr	Val	Glu	Arg	Lys
225					230					235				240	
Cys	Cys	Val	Glu	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Pro	Val	Ala	Gly	Pro
		245							250				255		
Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser
		260						265					270		
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp
		275					280					285			
Pro	Glu	Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn
	290					295					300				
Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val
305				310						315				320	
Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu
			325					330					335		
Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys
		340						345					350		
Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr
		355					360					365			
Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr
	370						375				380				
Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu
385				390						395				400	
Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu
			405					410					415		
Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys
		420						425					430		
Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu
		435					440					445			

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Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 450 455 460

Lys
 465

<210> SEQ ID NO 51
 <211> LENGTH: 720
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: A chemically synthesized sequence of L chain of
 anti-REG4 chimeric antibody with signal sequence.

<400> SEQUENCE: 51

```

atggagacag acacactcct gttatgggta ctgctgctct gggttccagg ttccactggt      60
gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctggggca gagggccacc      120
atctcatgca gggccagcaa aagtgtcagt acatctggct atagttatat gcactgggtac      180
caacagaaac caggacagcc acccaaacct ctcactatc ttgcatccaa cctagaatct      240
ggggtcctcg ccaggttcag tggcagtggg tctgggacag acttcaccct caacatccat      300
cctgtggagg aggaggatgc tgcaacctat tactgtcagc acagtaggga gctgtacacg      360
ttcggagggg ggaccaagct gggatccgaa atcaaacgaa ctgtggctgc accatctgtc      420
ttcatcttcc cgccatctga tgagcagttg aaatctggaa ctgcctctgt tgtgtgcctg      480
ctgaataact tctatccccc agaggccaaa gtacagtgga aggtggataa cgccctccaa      540
tcgggtaact cccaggagag tgtcacagag caggacagca aggacagcac ctacagcctc      600
agcagcaccg tgacgctgag caaagcagac tacgagaaac acaaagtcta cgctgcgaa      660
gtcaccatc agggcctgag ctgcccgcgc acaaagagct tcaacagggg agagtgttag      720

```

<210> SEQ ID NO 52
 <211> LENGTH: 239
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: A chemically synthesized sequence of L chain of
 anti-REG4 chimeric antibody with signal sequence.

<400> SEQUENCE: 52

```

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
  1          5          10          15
Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
  20          25          30
Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser
  35          40          45
Val Ser Thr Ser Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro
  50          55          60
Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser
  65          70          75          80
Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
  85          90          95
Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys
  100         105         110
Gln His Ser Arg Glu Leu Tyr Thr Phe Gly Gly Gly Thr Lys Leu Gly
  115         120         125
Ser Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro

```

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130	135	140	
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu			
145	150	155	160
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp			
	165	170	175
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp			
	180	185	190
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys			
	195	200	205
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln			
	210	215	220
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys			
225	230	235	
<210> SEQ ID NO 53			
<211> LENGTH: 36			
<212> TYPE: DNA			
<213> ORGANISM: Artificial sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: An artificially synthesized primer for PCR			
<400> SEQUENCE: 53			
aatagcggcc gcaccatggg atggagccgg atcttt			36
<210> SEQ ID NO 54			
<211> LENGTH: 36			
<212> TYPE: DNA			
<213> ORGANISM: Artificial sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: An artificially synthesized primer for PCR			
<400> SEQUENCE: 54			
aataggatcc tgcagagaca gtgaccagag tccctt			36
<210> SEQ ID NO 55			
<211> LENGTH: 35			
<212> TYPE: DNA			
<213> ORGANISM: Artificial sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: An artificially synthesized primer for PCR			
<400> SEQUENCE: 55			
aatagcggcc gcaccatgga gacagacaca ctctc			35
<210> SEQ ID NO 56			
<211> LENGTH: 34			
<212> TYPE: DNA			
<213> ORGANISM: Artificial sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: An artificially synthesized primer for PCR			
<400> SEQUENCE: 56			
aataggatcc cagcttggtc cccctccga acgt			34
<210> SEQ ID NO 57			
<211> LENGTH: 1404			
<212> TYPE: DNA			
<213> ORGANISM: Artificial sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Synthetic construct			
<220> FEATURE:			
<221> NAME/KEY: CDS			

-continued

<222> LOCATION: (1) .. (1404)

<400> SEQUENCE: 57

atg aaa tgc agc tgg gtt atc ttc ttc ctg atg gca gtg gtt aca ggg	48
Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val Val Thr Gly	
1 5 10 15	
gtc aat tca gag gtt cag ctg cag cag tct ggg gca gag ctt gtg aag	96
Val Asn Ser Glu Val Gln Leu Gln Ser Gly Ala Glu Leu Val Lys	
20 25 30	
cca ggg gcc tca gtc aag ttg tcc tgc aca gct tct ggc ttc aac att	144
Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile	
35 40 45	
aac gac acc tat atg cac tgg gtg aag cag agg cct gaa cag ggc ctg	192
Asn Asp Thr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu	
50 55 60	
gag tgg att gga agg att gat cct gcg aat ggt aat act aaa tat gac	240
Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp	
65 70 75 80	
ccg aag ttc cag ggc aag gcc act ata aca gca gac aca tcc tcc aac	288
Pro Lys Phe Gln Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn	
85 90 95	
aca gcc tac ctg cag ctc agc agc ctg aca tct gag gac act gcc gtc	336
Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val	
100 105 110	
tat tac tgt gct aga gga gca cgg ggg agt aga ttt gct tac tgg ggc	384
Tyr Tyr Cys Ala Arg Gly Ala Arg Gly Ser Arg Phe Ala Tyr Trp Gly	
115 120 125	
caa ggg act ctg gtc act gtc tct gca gcc aaa aca aca gcc cca tcg	432
Gln Gly Thr Leu Val Thr Val Ser Ala Ala Lys Thr Thr Ala Pro Ser	
130 135 140	
gtc tat cca ctg gcc cct gtg tgt gga gat aca act ggc tcc tcg gtg	480
Val Tyr Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val	
145 150 155 160	
act cta gga tgc ctg gtc aag ggt tat ttc cct gag cca gtg acc ttg	528
Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu	
165 170 175	
acc tgg aac tct gga tcc ctg tcc agt ggt gtg cac acc ttc cca gct	576
Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala	
180 185 190	
gtc ctg cag tct gac ctc tac acc ctc agc agc tca gtg act gta acc	624
Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr	
195 200 205	
tcg agc acc tgg ccc agc cag tcc atc acc tgc aat gtg gcc cac ccg	672
Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro	
210 215 220	
gca agc agc acc aag gtg gac aag aaa att gag ccc aga ggg ccc aca	720
Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr	
225 230 235 240	
atc aag ccc tgt cct cca tgc aaa tgc cca gca cct aac ctc ttg ggt	768
Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly	
245 250 255	
gga cca tcc gtc ttc atc ttc cct cca aag atc aag gat gta ctc atg	816
Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met	
260 265 270	
atc tcc ctg agc ccc ata gtc aca tgt gtg gtg gtg gat gtg agc gag	864
Ile Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu	
275 280 285	
gat gac cca gat gtc cag atc agc tgg ttt gtg aac aac gtg gaa gta	912

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<210> SEQ ID NO 58
<211> LENGTH: 467
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide: 92-13 H chain

<400> SEQUENCE: 58

Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val Val Thr Gly
1          5          10          15

Val Asn Ser Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys
          20          25          30

Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile
          35          40          45

Asn Asp Thr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
          50          55          60

Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp
65          70          75          80

Pro Lys Phe Gln Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn
          85          90          95

Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val
          100          105          110

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Tyr Tyr Cys Ala Arg Gly Ala Arg Gly Ser Arg Phe Ala Tyr Trp Gly
 115 120 125
 Gln Gly Thr Leu Val Thr Val Ser Ala Ala Lys Thr Thr Ala Pro Ser
 130 135 140
 Val Tyr Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val
 145 150 155 160
 Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu
 165 170 175
 Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala
 180 185 190
 Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr
 195 200 205
 Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro
 210 215 220
 Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr
 225 230 235 240
 Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly
 245 250 255
 Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met
 260 265 270
 Ile Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu
 275 280 285
 Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val
 290 295 300
 His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu
 305 310 315 320
 Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly
 325 330 335
 Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile
 340 345 350
 Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val
 355 360 365
 Tyr Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr
 370 375 380
 Leu Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu
 385 390 395 400
 Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro
 405 410 415
 Val Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val
 420 425 430
 Glu Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val
 435 440 445
 His Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr
 450 455 460
 Pro Gly Lys
 465

<210> SEQ ID NO 59

<211> LENGTH: 705

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic construct

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<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(705)

<400> SEQUENCE: 59

atg agt gtg ccc act cag gtc ctg ggg ttg ctg ctg ctg tgg ctt aca      48
Met Ser Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr
1      5      10      15

gat gcc aga tgt gac atc cag atg act cag tct cca gcc tcc cta tct      96
Asp Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser
20     25     30

gta tct gtg gga gaa act gtc acc atc aca tgt cga gca agt gag aat     144
Val Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn
35     40     45

att tac agt aat tta gca tgg tat cag cag aaa cag gga aaa tct cct     192
Ile Tyr Ser Asn Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro
50     55     60

cag ctc ctg gtc tat gtt gca aca aac tta gca gat ggt gtg cca tca     240
Gln Leu Leu Val Tyr Val Ala Thr Asn Leu Ala Asp Gly Val Pro Ser
65     70     75     80

agg ttc agt ggc agt gga tca ggc aca cag tat tcc ctc aag atc aac     288
Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn
85     90     95

agc ctg cag tct gaa gat ttt ggg agt tat tac tgt caa cat ttt tgg     336
Ser Leu Gln Ser Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp
100    105    110

ggg act ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa cgg     384
Gly Thr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
115    120    125

gct gat gct gca cca act gta tcc atc ttc cca cca tcc agt gag cag     432
Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
130    135    140

tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg aac aac ttc tac     480
Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
145    150    155    160

ccc aaa gac atc aat gtc aag tgg aag att gat ggc agt gaa cga caa     528
Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
165    170    175

aat ggc gtc ctg aac agt tgg act gat cag gac agc aaa gac agc acc     576
Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
180    185    190

tac agc atg agc agc acc ctc acg ttg acc aag gac gag tat gaa cga     624
Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
195    200    205

cat aac agc tat acc tgt gag gcc act cac aag aca tca act tca ccc     672
His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
210    215    220

att gtc aag agc ttc aac agg aat gag tgt tag                          705
Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
225    230

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<210> SEQ ID NO 60
<211> LENGTH: 234
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide: 92-13 L chain

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<400> SEQUENCE: 60

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

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1	5	10	15													
Asp	Ala	Arg	Cys	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ser	
	20							25					30			
Val	Ser	Val	Gly	Glu	Thr	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Glu	Asn	
	35						40					45				
Ile	Tyr	Ser	Asn	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Gln	Gly	Lys	Ser	Pro	
	50					55				60						
Gln	Leu	Leu	Val	Tyr	Val	Ala	Thr	Asn	Leu	Ala	Asp	Gly	Val	Pro	Ser	
	65				70					75				80		
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Gln	Tyr	Ser	Leu	Lys	Ile	Asn	
			85					90						95		
Ser	Leu	Gln	Ser	Glu	Asp	Phe	Gly	Ser	Tyr	Tyr	Cys	Gln	His	Phe	Trp	
		100						105					110			
Gly	Thr	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	
		115					120					125				
Ala	Asp	Ala	Ala	Pro	Thr	Val	Ser	Ile	Phe	Pro	Pro	Ser	Ser	Glu	Gln	
	130					135				140						
Leu	Thr	Ser	Gly	Gly	Ala	Ser	Val	Val	Cys	Phe	Leu	Asn	Asn	Phe	Tyr	
	145				150					155				160		
Pro	Lys	Asp	Ile	Asn	Val	Lys	Trp	Lys	Ile	Asp	Gly	Ser	Glu	Arg	Gln	
			165					170						175		
Asn	Gly	Val	Leu	Asn	Ser	Trp	Thr	Asp	Gln	Asp	Ser	Lys	Asp	Ser	Thr	
		180						185					190			
Tyr	Ser	Met	Ser	Ser	Thr	Leu	Thr	Leu	Thr	Lys	Asp	Glu	Tyr	Glu	Arg	
		195				200						205				
His	Asn	Ser	Tyr	Thr	Cys	Glu	Ala	Thr	His	Lys	Thr	Ser	Thr	Ser	Pro	
	210				215					220						
Ile	Val	Lys	Ser	Phe	Asn	Arg	Asn	Glu	Cys							
	225				230											
<210> SEQ ID NO 61																
<211> LENGTH: 1404																
<212> TYPE: DNA																
<213> ORGANISM: Artificial sequence																
<220> FEATURE:																
<223> OTHER INFORMATION: Synthetic construct																
<220> FEATURE:																
<221> NAME/KEY: CDS																
<222> LOCATION: (1)..(1404)																
<400> SEQUENCE: 61																
atg	gga	tgg	agc	cgg	atc	ttt	ctc	ttc	ctc	ctg	tca	ata	act	gca	ggt	48
Met	Gly	Trp	Ser	Arg	Ile	Phe	Leu	Phe	Leu	Leu	Ser	Ile	Thr	Ala	Gly	
1		5						10					15			
gtc	cat	tgc	cag	gtc	cag	ctg	cag	cag	tct	gga	cct	gag	ctg	gtg	aag	96
Val	His	Cys	Gln	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	
		20						25				30				
cct	ggg	gcc	tca	gtg	aag	att	tcc	tgc	aaa	gct	tct	ggc	tac	gca	ttc	144
Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	
		35				40						45				
agt	agc	tct	tgg	atg	aac	tgg	gtg	aag	cag	agg	cct	gga	cag	ggt	ctt	192
Ser	Ser	Ser	Trp	Met	Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	
	50				55					60						
gag	tgg	att	gga	cgg	att	tat	cct	gga	gat	gga	gat	act	aac	tac	aat	240
Glu	Trp	Ile	Gly	Arg	Ile	Tyr	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	
	65				70					75				80		

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ggg aag ttc aag ggc aag gcc aca ctg act gca gac aaa tcc tcc agc	288
Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser	
85 90 95	
aca gcc tac atg caa ctc agc agc ctg acc tct gtg gac tct gcg gtc	336
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp Ser Ala Val	
100 105 110	
tat ttc tgt gca aga ggg ggt aac tac ggc tgg ttt gct tac tgg ggc	384
Tyr Phe Cys Ala Arg Gly Gly Asn Tyr Gly Trp Phe Ala Tyr Trp Gly	
115 120 125	
caa ggg act ctg gtc act gtc tct gca gcc aaa aca aca gcc cca tcg	432
Gln Gly Thr Leu Val Thr Val Ser Ala Ala Lys Thr Thr Ala Pro Ser	
130 135 140	
gtc tat cca ctg gcc cct gtg tgt gga gat aca act ggc tcc tcg gtg	480
Val Tyr Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val	
145 150 155 160	
act cta gga tgc ctg gtc aag ggt tat ttc cct gag cca gtg acc ttg	528
Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu	
165 170 175	
acc tgg aac tct gga tcc ctg tcc agt ggt gtg cac acc ttc cca gct	576
Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala	
180 185 190	
gtc ctg cag tct gac ctc tac acc ctc agc agc tca gtg act gta acc	624
Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr	
195 200 205	
tcg agc acc tgg ccc agc cag tcc atc acc tgc aat gtg gcc cac ccg	672
Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro	
210 215 220	
gca agc agc acc aag gtg gac aag aaa att gag ccc aga ggg ccc aca	720
Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr	
225 230 235 240	
atc aag ccc tgt cct cca tgc aaa tgc cca gca cct aac ctc ttg ggt	768
Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly	
245 250 255	
gga cca tcc gtc ttc atc ttc cct cca aag atc aag gat gta ctc atg	816
Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met	
260 265 270	
atc tcc ctg agc ccc ata gtc aca tgt gtg gtg gtg gat gtg agc gag	864
Ile Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu	
275 280 285	
gat gac cca gat gtc cag atc agc tgg ttt gtg aac aac gtg gaa gta	912
Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val	
290 295 300	
cac aca gct cag aca caa acc cat aga gag gat tac aac agt act ctc	960
His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu	
305 310 315 320	
cgg gtg gtc agt gcc ctc ccc atc cag cac cag gac tgg atg agt ggc	1008
Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly	
325 330 335	
aag gag ttc aaa tgc aag gtc aac aac aaa gac ctc cca gcg ccc atc	1056
Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile	
340 345 350	
gag aga acc atc tca aaa ccc aaa ggg tca gta aga gct cca cag gta	1104
Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val	
355 360 365	
tat gtc ttg cct cca cca gaa gaa gag atg act aag aaa cag gtc act	1152
Tyr Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr	
370 375 380	
ctg acc tgc atg gtc aca gac ttc atg cct gaa gac att tac gtg gag	1200

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Leu Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu	
385 390 395 400	
tgg acc aac aac ggg aaa aca gag cta aac tac aag aac act gaa cca	1248
Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro	
405 410 415	
gtc ctg gac tct gat ggt tct tac ttc atg tac agc aag ctg aga gtg	1296
Val Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val	
420 425 430	
gaa aag aag aac tgg gtg gaa aga aat agc tac tcc tgt tca gtg gtc	1344
Glu Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val	
435 440 445	
cac gag ggt ctg cac aat cac cac acg act aag agc ttc tcc cgg act	1392
His Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr	
450 455 460	
ccg ggt aaa tga	1404
Pro Gly Lys	
465	

<210> SEQ ID NO 62

<211> LENGTH: 467

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polypeptide: 93-22 H chain

<400> SEQUENCE: 62

Met Gly Trp Ser Arg Ile Phe Leu Phe Leu Leu Ser Ile Thr Ala Gly	
1 5 10 15	
Val His Cys Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys	
20 25 30	
Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe	
35 40 45	
Ser Ser Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu	
50 55 60	
Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn	
65 70 75 80	
Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser	
85 90 95	
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp Ser Ala Val	
100 105 110	
Tyr Phe Cys Ala Arg Gly Gly Asn Tyr Gly Trp Phe Ala Tyr Trp Gly	
115 120 125	
Gln Gly Thr Leu Val Thr Val Ser Ala Ala Lys Thr Thr Ala Pro Ser	
130 135 140	
Val Tyr Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val	
145 150 155 160	
Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu	
165 170 175	
Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala	
180 185 190	
Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr	
195 200 205	
Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro	
210 215 220	
Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr	
225 230 235 240	

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Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly
      245                      250                      255

Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met
      260                      265                      270

Ile Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu
      275                      280                      285

Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val
      290                      295                      300

His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu
      305                      310                      315                      320

Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly
      325                      330                      335

Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile
      340                      345                      350

Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val
      355                      360                      365

Tyr Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr
      370                      375                      380

Leu Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu
      385                      390                      395                      400

Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro
      405                      410                      415

Val Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val
      420                      425                      430

Glu Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val
      435                      440                      445

His Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr
      450                      455                      460

Pro Gly Lys
465

<210> SEQ ID NO 63
<211> LENGTH: 714
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(714)

<400> SEQUENCE: 63

atg gag aca gac aca ctc ctg tta tgg gta ctg ctg ctc tgg gtt cca      48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1      5      10      15

ggg tcc act ggt gac att gtg ctg aca cag tct cct gct tcc tta gct      96
Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
20      25      30

gta tct ctg ggg cag agg gcc acc atc tca tgc agg gcc agc aaa agt      144
Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser
35      40      45

gtc agt aca tct ggc tat agt tat atg cac tgg tac caa cag aaa cca      192
Val Ser Thr Ser Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro
50      55      60

gga cag cca ccc aaa ctc ctc atc tat ctt gca tcc aac cta gaa tct      240
Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser

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65	70	75	80	
ggg gtc cct gcc agg ttc agt ggc agt ggg tct ggg aca gac ttc acc				288
Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr	85	90	95	
ctc aac atc cat cct gtg gag gag gag gat gct gca acc tat tac tgt				336
Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys	100	105	110	
cag cac agt agg gag ctg tac acg ttc gga ggg ggg acc aag ctg gaa				384
Gln His Ser Arg Glu Leu Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu	115	120	125	
ata aaa cgg gct gat gct gca cca act gta tcc atc ttc cca cca tcc				432
Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser	130	135	140	
agt gag cag tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg aac				480
Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn	145	150	155	160
aac ttc tac ccc aaa gac atc aat gtc aag tgg aag att gat ggc agt				528
Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser	165	170	175	
gaa cga caa aat ggc gtc ctg aac agt tgg act gat cag gac agc aaa				576
Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys	180	185	190	
gac agc acc tac agc atg agc agc acc ctc acg ttg acc aag gac gag				624
Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu	195	200	205	
tat gaa cga cat aac agc tat acc tgt gag gcc act cac aag aca tca				672
Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser	210	215	220	
act tca ccc att gtc aag agc ttc aac agg aat gag tgt tag				714
Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys	225	230	235	

<210> SEQ ID NO 64

<211> LENGTH: 237

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polypeptide: 93-22 L chain

<400> SEQUENCE: 64

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro	1	5	10	15
Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala	20	25	30	
Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser	35	40	45	
Val Ser Thr Ser Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro	50	55	60	
Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser	65	70	75	80
Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr	85	90	95	
Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys	100	105	110	
Gln His Ser Arg Glu Leu Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu	115	120	125	
Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser				

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130	135	140	
Ser Glu Gln Leu Thr	Ser Gly Gly Ala Ser	Val Val Cys Phe Leu Asn	
145	150	155	160
Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser			
	165	170	175
Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys			
	180	185	190
Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu			
	195	200	205
Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser			
	210	215	220
Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys			
	225	230	235
<210> SEQ ID NO 65			
<211> LENGTH: 1395			
<212> TYPE: DNA			
<213> ORGANISM: Artificial sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Synthetic construct			
<220> FEATURE:			
<221> NAME/KEY: CDS			
<222> LOCATION: (1)..(1395)			
<400> SEQUENCE: 65			
atg gat tgg ctg tgg aac ttg cta ttc ctg atg gca gct gcc caa agt			48
Met Asp Trp Leu Trp Asn Leu Leu Phe Leu Met Ala Ala Ala Gln Ser			
1	5	10	15
atc caa gca cag atc cag ttg gtg cag tct gga cct gag ctg aag aag			96
Ile Gln Ala Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys			
	20	25	30
cct gga gag aca gtc aag atc tcc tgc aag gct tct ggg tat acc ttc			144
Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe			
	35	40	45
aca aac tat gga atg aac tgg gtg aag cag gct cca gga aag ggt tta			192
Thr Asn Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu			
	50	55	60
aag tgg atg ggc tgg ata aac acc aac act gga gag cca aca tat gct			240
Lys Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr Tyr Ala			
	65	70	80
gaa gag ttc aag gga cgg ttt gcc ttc tct ttg gaa acc tct gcc agc			288
Glu Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser			
	85	90	95
act gcc tat ttg cag atc aac aac ctc aaa aat gag gac acg gct aca			336
Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr			
	100	105	110
tat ttc tgt gca aga ggg ggg tac ggg gac tac tgg ggc caa ggc acc			384
Tyr Phe Cys Ala Arg Gly Gly Tyr Gly Asp Tyr Trp Gly Gln Gly Thr			
	115	120	125
act ctc aca gtc tcc tca gcc aaa aca aca gcc cca tcg gtc tat cca			432
Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro			
	130	135	140
ctg gcc cct gtg tgt gga gat aca act ggc tcc tcg gtg act cta gga			480
Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly			
	145	150	155
tgc ctg gtc aag ggt tat ttc cct gag cca gtg acc ttg acc tgg aac			528
Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp Asn			
	165	170	175

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tct gga tcc ctg tcc agt ggt gtg cac acc ttc cca gct gtc ctg cag Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln 180 185 190	576
tct gac ctc tac acc ctc agc agc tca gtg act gta acc tcg agc acc Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser Thr 195 200 205	624
tgg ccc agc cag tcc atc acc tgc aat gtg gcc cac ccg gca agc agc Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser Ser 210 215 220	672
acc aag gtg gac aag aaa att gag ccc aga ggg ccc aca atc aag ccc Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro 225 230 235 240	720
tgt cct cca tgc aaa tgc cca gca cct aac ctc ttg ggt gga cca tcc Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser 245 250 255	768
gtc ttc atc ttc cct cca aag atc aag gat gta ctc atg atc tcc ctg Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu 260 265 270	816
agc ccc ata gtc aca tgt gtg gtg gtg gat gtg agc gag gat gac cca Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro 275 280 285	864
gat gtc cag atc agc tgg ttt gtg aac aac gtg gaa gta cac aca gct Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala 290 295 300	912
cag aca caa acc cat aga gag gat tac aac agt act ctc cgg gtg gtc Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val 305 310 315 320	960
agt gcc ctc ccc atc cag cac cag gac tgg atg agt ggc aag gag ttc Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe 325 330 335	1008
aaa tgc aag gtc aac aac aaa gac ctc cca gcg ccc atc gag aga acc Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr 340 345 350	1056
atc tca aaa ccc aaa ggg tca gta aga gct cca cag gta tat gtc ttg Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu 355 360 365	1104
cct cca cca gaa gaa gag atg act aag aaa cag gtc act ctg acc tgc Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys 370 375 380	1152
atg gtc aca gac ttc atg cct gaa gac att tac gtg gag tgg acc aac Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn 385 390 395 400	1200
aac ggg aaa aca gag cta aac tac aag aac act gaa cca gtc ctg gac Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp 405 410 415	1248
tct gat ggt tct tac ttc atg tac agc aag ctg aga gtg gaa aag aag Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys 420 425 430	1296
aac tgg gtg gaa aga aat agc tac tcc tgt tca gtg gtc cac gag ggt Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly 435 440 445	1344
ctg cac aat cac cac acg act aag agc ttc tcc cgg act ccg ggt aaa Leu His Asn His His Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys 450 455 460	1392
tga	1395

<210> SEQ ID NO 66

<211> LENGTH: 464

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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide: 39-10 H chain

<400> SEQUENCE: 66

Met Asp Trp Leu Trp Asn Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys
20 25 30
Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45
Thr Asn Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu
50 55 60
Lys Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr Tyr Ala
65 70 75 80
Glu Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser
85 90 95
Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr
100 105 110
Tyr Phe Cys Ala Arg Gly Gly Tyr Gly Asp Tyr Trp Gly Gln Gly Thr
115 120 125
Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro
130 135 140
Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly
145 150 155 160
Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp Asn
165 170 175
Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
180 185 190
Ser Asp Leu Tyr Thr Leu Ser Ser Val Thr Val Thr Ser Ser Thr
195 200 205
Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser Ser
210 215 220
Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro
225 230 235 240
Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser
245 250 255
Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu
260 265 270
Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro
275 280 285
Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala
290 295 300
Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val
305 310 315 320
Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe
325 330 335
Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr
340 345 350
Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu
355 360 365
Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys

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370	375	380	
Met Val Thr Asp Phe	Met Pro Glu Asp Ile Tyr	Val Glu Trp Thr Asn	
385	390	395	400
Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp			
	405	410	415
Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys			
	420	425	430
Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly			
	435	440	445
Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys			
	450	455	460
 <210> SEQ ID NO 67			
<211> LENGTH: 717			
<212> TYPE: DNA			
<213> ORGANISM: Artificial sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Synthetic construct			
<220> FEATURE:			
<221> NAME/KEY: CDS			
<222> LOCATION: (1)..(717)			
 <400> SEQUENCE: 67			
atg gag aca gac aca ctc ctg cta tgg gtg ctg ctg ctc tgg gtt cca			48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro			
1	5	10	15
ggt tcc aca ggt gac att gtg ctg acc caa tct cca gct tct ttg gct			96
Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala			
	20	25	30
gtg tct cta ggg cag agg gcc acc ata tcc tgc aga gcc agt gaa agt			144
Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser			
	35	40	45
gtt gat agt tat ggc aat agt ttt atg cac tgg tac cag cag aaa cca			192
Val Asp Ser Tyr Gly Asn Ser Phe Met His Trp Gln Gln Lys Pro			
	50	55	60
gga cag cca ccc aaa ctc ctc atc tat cgt gca tcc aac cta gaa tct			240
Gly Gln Pro Pro Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser			
	65	70	75
ggg atc cct gcc agg ttc agt ggc agt ggg tct agg aca gac ttc acc			288
Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr			
	85	90	95
ctc acc att aat cct gtg gag gct gat gat gtt gca acc tat tac tgt			336
Leu Thr Ile Asn Pro Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys			
	100	105	110
cag caa agt aat gag gat cct cgg acg ttc ggt gga ggc acc aag ctg			384
Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu			
	115	120	125
gaa atc aaa cgg gct gat gct gca cca act gta tcc atc ttc cca cca			432
Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro			
	130	135	140
tcc agt gag cag tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg			480
Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu			
	145	150	155
aac aac ttc tac ccc aaa gac atc aat gtc aag tgg aag att gat ggc			528
Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly			
	165	170	175
agt gaa cga caa aat ggc gtc ctg aac agt tgg act gat cag gac agc			576
Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser			
	180	185	190

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aaa gac agc acc tac agc atg agc agc acc ctc acg ttg acc aag gac      624
Lys Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp
      195                200                205

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gag tat gaa cga cat aac agc tat acc tgt gag gcc act cac aag aca      672
Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr
      210                215                220

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tca act tca ccc att gtc aag agc ttc aac agg aat gag tgt tag      717
Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
      225                230                235

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<210> SEQ ID NO 68

<211> LENGTH: 238

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polypeptide: 39-10 L chain

<400> SEQUENCE: 68

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Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1           5           10           15

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Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
      20           25           30

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Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser
      35           40           45

```

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Val Asp Ser Tyr Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro
      50           55           60

```

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Gly Gln Pro Pro Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser
      65           70           75           80

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Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr
      85           90           95

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Leu Thr Ile Asn Pro Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys
      100          105          110

```

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Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu
      115          120          125

```

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Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro
      130          135          140

```

```

Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu
      145          150          155          160

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Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly
      165          170          175

```

```

Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser
      180          185          190

```

```

Lys Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp
      195          200          205

```

```

Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr
      210          215          220

```

```

Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
      225          230          235

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1. A method for treating a disease associated with Frizzled homologue 10 (FZD10), comprising administering to a patient in need thereof an antibody or fragment thereof and a pharmaceutically acceptable carrier or excipient, wherein the disease associated with FZD10 is selected from the group consisting of synovial sarcoma, colorectal cancer, gastric cancer, chronic myeloid leukemia, and acute myeloid leukemia, and wherein the antibody or fragment is selected from:

- (i) an antibody or fragment thereof, which comprises an H (heavy) chain V (variable) region comprising a complementarity determining region (CDR) having the amino acid sequences shown in SEQ ID NOs: 15, 17 and 19 and an L (light) chain V region comprising a CDR having the amino acid sequences shown in SEQ ID NOs: 23, 25 and 27, and which is capable of binding to a Frizzled homologue 10 (FZD10) protein or a partial peptide thereof; or
- (ii) an antibody or fragment thereof, which binds the FZD10 protein and in which the complementarity determining regions (CDRs) are the CDRs of the mouse monoclonal antibody 92-13 (FERM BP-10628).

2. The method of claim 1, wherein the method is for treating synovial sarcoma.

3. The method according to claim 1, wherein the antibody is selected from the group consisting of a mouse antibody, a chimeric antibody, a humanized antibody, an antibody fragment, and single-chain antibody.

4. The method according to claim 1, wherein the antibody is a mouse antibody.

5. The method according to claim 4, wherein the mouse antibody comprises an H chain having the amino acid sequence shown in SEQ ID NO: 57 and/or an L chain having the amino acid sequence shown in SEQ ID NO: 59.

6. The method according to claim 4, wherein the mouse antibody is produced by the hybridoma clone 92-13 (FERM BP-10628).

7. The method according to claim 1, wherein the antibody is a chimeric antibody.

8. The method according to claim 7, wherein the chimeric antibody comprises an H chain V region having the amino acid sequence shown in SEQ ID NO: 13.

9. The method according to claim 7, wherein the chimeric antibody comprises an H chain having the amino acid sequence shown in SEQ ID NO: 46.

10. The method according to claim 7, wherein the chimeric antibody comprises an L chain V region having the amino acid sequence shown in SEQ ID NO: 21.

11. The method according to claim 7, wherein the chimeric antibody comprises an L chain having the amino acid sequence shown in SEQ ID NO: 48.

12. The method according to claim 7, wherein the chimeric antibody comprises an H chain V region having the amino acid sequence shown in SEQ ID NO: 13 and an L chain V region having the amino acid sequence shown in SEQ ID NO: 21.

13. The method according to claim 7, wherein the chimeric antibody comprises an H chain having the amino acid sequence shown in SEQ ID NO: 46 and an L chain having the amino acid sequence shown in SEQ ID NO: 48.

14. The method according to a claim 7, wherein the chimeric antibody further comprises a human antibody C (constant) region.

15. The method according to claim 1, wherein the antibody is a humanized antibody.

16. The method according to claim 15, wherein the humanized antibody further comprises a human antibody FR (framework) region and/or a human antibody C region.

17. The method according to claim 1, wherein the antibody is a chimeric antibody that further comprises a human antibody C (constant) region or which is a humanized antibody that further comprises a human antibody FR (framework) region and/or a human antibody C region.

18. The method according to claim 1, wherein the antibody is a chimeric antibody comprising:

- (i) an H chain V region having the amino acid sequence shown in SEQ ID NO: 13 and an L chain V region having the amino acid sequence shown in SEQ ID NO: 21; or
- (ii) an H chain having the amino acid sequence shown in SEQ ID NO: 46 and an L chain having the amino acid sequence shown in SEQ ID NO: 48.

* * * * *