ANTI-CD98 ANTIBODIES AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates generally to anti-CD98 antibodies and to methods of using such antibodies.

BACKGROUND

[0002] CD98 (also referred to as CD98 heavy chain; 42F heavy chain; SLC3A2) is a type II transmembrane glycoprotein composed of 529 amino acid residues. The protein comprises a 75 amino acid N-terminal intracellular cytoplasmic domain, a single transmembrane domain, and a 426 amino acid C-terminal extracellular domain (Parmacek et al., Nucleic Acids Res. 17: 1915-1931, 1989). CD98 covalently links via a disulfide bond to one of several light chains (SLC7A5, 6, 7, 8, 10, or 11), which are L-type amino acid transporters. This interaction is required for the cell surface expression and amino acid transport function of the light chains. CD98 also associates with integrin β subunits, thereby regulating integrin signaling that controls cell proliferation, survival, migration, and epithelial adhesion/polarity (Cai et al., J. Cell Sci. 118: 889-899, 2005).

[0003] CD98 was originally identified as a cell surface antigen associated with lymphocyte activation (Haynes et al., J. Immunol. 126: 1409-1414, 1981). CD98 has since been identified in all cell types with the exception of platelets and is expressed at the highest levels in the gastrointestinal (GI) tract and the tubules of the kidney (Verrey et al., Pflugers Arch. 440: 503-512, 2000). Upregulation of CD98 has been observed in intestinal inflammation. Recently, intestinal CD98 expression was shown to have a crucial role in controlling homeostatic and innate immune responses in the gut. Modulation of CD98 expression in intestinal epethilial cells has therefore been suggested as a promising therapeutic strategy for the treatment and prevention of inflammatory intestinal diseases, such as inflammatory bowel disease (IBD) and colitis-associated cancer (Nguyen et al., J. Clin. Invest. 121: 1733-1747, 2011). CD98 is also overexpressed on the cell surface of almost all tumor cells, regardless of tissue of origin (Itoh et al., Jpn. J. Cancer Res. 92: 1313-1321, 2001).

[0004] Increased expression of one of the light chains that binds CD98, L-type amino acid transporter 1 (LAT1; also known as SLC7A5) has also been observed in many types of human cancer cells, including breast cancer, colon cancer, oral cancer, ovarian cancer, esophageal cancer, glioma and leukemia (Fan et al., Biochem. Pharmacol. 80: 811-818, 2010). Increased amino acid supply may be required to support the high growth rate of cancer cells, both by providing the amino acid building blocks for protein synthesis, and by stimulating growth via mammalian target of rapamycin (mTOR) (Fan et al., supra; Imai et al., Anticancer Res. 30: 4819-4828, 2010). The expression of LAT1 and CD98 is significantly higher in metastatic sites of human cancers than in the primary sites, suggesting that overexpression of LAT1/CD98 may be essential for progression and metatstasis of human cancers. In

particular, LAT1/CD98 overexpression appears to be required for tumor metastasis in patients with colon cancer. (Kaira et al., Cancer Sci. 99: 2380-2386, 2008).

[0005] The expression pattern and functions of CD98 and LAT1 suggest these proteins as promising targets for treatment of a variety of human cancers. Inhibitors of LAT1 activity have demonstrated antitumor activity in a number of cancer types, including non-small cell lung cancers (Imai et al., supra), colon cancer cells (Oda et al., Cancer Sci. 101: 173-179, 2010), oral cancer cells (Kim et al., Biol. Pharm. Bull. 33: 1117-1121, 2010), and breast cancer cells (Shennan and Thomson, Oncol. Rep. 20: 885-889, 2008). LAT1 has also been suggested as a target for treatment of ovarian cancer (Fan et al, supra).

[0006] A murine monoclonal antibody to CD98, identified as HBJ127, was found to inhibit lymphocyte proliferation (Yagita and Hashimoto, J. Immunol. 136: 2062-2068, 1986) and to inhibit the growth of bladder tumor and lymphoma cells (Yagita et al., Cancer Res. 46: 1478-1489, 1986). The epitope for the HJ127 antibody was found to be residues $_{442}$AFS$_{444}$ of human CD98 (Itoh et al., 2007). A different murine monoclonal antibody to CD98 was shown to significantly inhibit tumor cell growth in vitro for glioma, prostate and colon cancer cells (Papetti and Herman, Am. J. Pathol. 159: 165-178, 2001). Additional monoclonal antibodies to human CD98 have been disclosed in U.S. Publication No. 20100143367. These monoclonal antibodies bind to epitopes within amino acid regions 372-530 or 104-371 of CD98. Five of these antibodies were found to inhibit amino acid uptake in a bladder cancer cell, and three of these antibodies were shown to suppress tumor growth in a mouse model.

[0007] As disclosed herein, analysis of fresh primary acute myelogenous leukemia (AML) tumor samples from patients using surface tagged antigen profiling (sTAg) of the cell surface proteome identified the transmembrane protein CD98 as being present at high density on the surface of AML tumor cells. CD98 is therefore a target for the treatment of AML, for example, by using binding agents such as antibodies which specifically bind to CD98. Binding agents specific for CD98, such as anti-CD98 antibodies, were also shown in various *in vivo* xengraft models to have utility in treating not only AML but various cancers, such as sarcoma, lymphoma, non-small cell lung cancer (NSCLC) and colorectal cancer.

[0008] The invention provides antibodies to CD98 that are useful in the diagnosis and treatment of various types of human cancers.

SUMMARY

[0009] Using in-solution labeling of intact AML tumor cell surfaces, followed by high-resolution, solution-based liquid chromatography coupled tandem mass spectrometry (LC-MS/MS), CD98 was identified as being present at high density on the surface of a majority of AML cell subtypes as compared to normal cells including developing blood cells. Thus, the invention provides anti-CD98 antibodies and methods of using the such antibodies in the treatment of AML and other cancers, including but not limited to lymphoma, sarcoma, non-small cell lung cancer and colorectal cancer.

[0010] In an embodiment, the invention provides an isolated antibody or a functional fragment thereof that specifically binds to human CD98, wherein the antibody or functional fragment binds to an epitope comprising residues A377, D397, I398, G400 and A401 of human CD98. In some embodiments, the epitope further comprises residues D374 and L378 of human CD98. In some embodiments, the epitope further comprises residues P379 and G380 of human CD98. In some embodiments, the epitope further comprises residues F395 and P396 of human CD98. In some embodiments, the epitope further comprises residues Q381, P382 and P399 of human CD98. In some embodiments, the epitope further comprises any one or more additional residues selected from the group consisting of D374, L378, P379, G380, Q381, P382, F395, P396 and P399 of human CD98.

[0011] In an embodiment, the invention provides an isolated antibody or a functional fragment thereof that specifically binds to human CD98, wherein the antibody binds to an epitope comprising residues P379, G380, D397 and I398 of human CD98. In some embodiments, the epitope further comprises residues F395 and P396 of human CD98. In some embodiments, the epitope further comprises residues Q381, P382, P399, G400 and A401 of human CD98. In some embodiments, the epitope further comprises residues D374, A377 and L378 of human CD98. In some embodiments, the epitope further comprises any one or more additional residues selected from the group consisting of D374, A377, L378, Q381, P382, F395, P396, P399, G400 and A401 of human CD98.

[0012] In some embodiments, the invention provides an isolated antibody or a functional fragment thereof, wherein the antibody or functional fragment binds to an epitope comprising residues D374, A377, L378, P379, G380, Q381, P382, F395, P396, D397, I398, P399, G400 and A401 of human CD98.

[0013] In some embodiments, the invention provides an isolated antibody or a functional fragment thereof that specifically binds to human CD98, wherein the antibody or functional fragment binds to an epitope comprised within amino acid residues 369-405 of human CD98. In some embodiments, the invention provides an isolated antibody or a functional fragment thereof that specifically binds to human CD98, wherein the antibody or functional fragment binds to an epitope consisting of amino acid residues 369-405 of human CD98.

[0014] In some embodiments, the monoclonal antibody of the invention is a humanized, human or chimeric antibody. In some embodiments, the antibody functional fragment of the invention is an Fab, F(ab')2, Fv or scFv fragment.

[0015] n an embodiment, the invention provides an isolated antibody or a functional fragment thereof comprising all three heavy chain complementarity determining regions (CDRs) from a heavy chain variable domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 31, and SEQ ID NO: 35, and/or all three light chain CDRs from a light chain variable domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 33, and SEQ ID NO: 37.

[0016] In an embodiment, the invention provides an isolated antibody or a functional fragment thereof comprising all three heavy chain CDRs from a heavy chain variable domain having an amino acid

sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 31, and SEQ ID NO: 35, and all three light chain CDRs from a light chain variable domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 33, and SEQ ID NO: 37. In some embodiments, the antibody or functional fragment thereof comprises all heavy and light chain complementarity determining regions (CDRs) from: (a) the antibody designated 8-34B; (b) the antibody designated 18-2A 2.2; (c) the antibody designated 18-2A 7.1; (d) the antibody designated 1-47C; or (e) the antibody designated 1-115A. In some embodiments, the antibody or functional fragment thereof comprises all heavy and light chain CDRs from the antibody designated 8-34B. In some embodiments, the antibody or functional fragment thereof comprises all all heavy and light chain CDRs from the antibody designated 18-2A 2.2. In some embodiments, the antibody or functional fragment thereof comprises all all heavy and light chain CDRs from the antibody designated 18-2A 7.1. In some embodiments, the antibody or functional fragment thereof comprises all all heavy and light chain CDRs from the antibody designated 1-47C. In some embodiments, the antibody or functional fragment thereof comprises all all heavy and light chain CDRs from the antibody designated 1-115A.

[0017] In some embodiments, the antibody comprises a heavy chain variable domain sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 31, and SEQ ID NO: 35. In some embodiments, the antibody comprises a light chain variable domain sequence consisting of SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 33, and SEQ ID NO: 37. In some embodiments, the antibody comprises a heavy chain variable domain sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 31, and SEQ ID NO: 35, and further comprises a light chain variable domain sequence consisting of SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 33, and SEQ ID NO: 37.

[0018] In an embodiment, the antibody comprises the heavy chain variable domain sequence of SEQ ID NO: 4 and the light chain variable domain sequence of SEQ ID NO: 6. In an embodiment, the antibody comprises the heavy chain variable domain sequence of SEQ ID NO: 8 and the light chain variable domain sequence of SEQ ID NO: 10. In an embodiment, the antibody comprises the heavy chain variable domain sequence of SEQ ID NO: 12 and the light chain variable domain sequence of SEQ ID NO: 14. In an embodiment, the antibody comprises the heavy chain variable domain sequence of SEQ ID NO: 31 and the light chain variable domain sequence of SEQ ID NO: 33. In an embodiment, the antibody comprises the heavy chain variable domain sequence of SEQ ID NO: 35 and the light chain variable domain sequence of SEQ ID NO: 37.

[0019] In an embodiment, the invention provides humanized antibodies. In some embodiments, the humanized antibody comprises a heavy chain variable domain sequence selected from SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 22, and SEQ ID NO: 23. In some embodiments, the humanized antibody comprises a light chain variable domain sequence selected from SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 20, and SEQ ID NO: 21. In some embodiments, the humanized antibody comprises a heavy chain variable domain sequence selected from SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 22, and SEQ ID NO: 23, and further comprises a light

chain variable domain sequence selected from SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 20, and SEQ ID NO: 21. In some embodiments, the humanized antibody comprises a light chain variable domain sequence selected from SEQ ID NO: 15 and SEQ ID NO: 16, and a heavy chain variable domain sequence selected from SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19. In an embodiment, the humanized antibody comprises the light chain variable domain sequence of SEQ ID NO: 15 and the heavy chain variable domain sequence of SEQ ID NO: 18.

[0020] In an embodiment, the humanized antibody comprises the light chain variable domain sequence of SEQ ID NO: 20 and the heavy chain variable domain sequence of SEQ ID NO: 22. In an embodiment, the humanized antibody comprises the light chain variable domain sequence of SEQ ID NO: 21 and the heavy chain variable domain sequence of SEQ ID NO: 23. In an embodiment, the humanized antibody comprises the light chain variable domain sequence of SEQ ID NO: 20 and the heavy chain variable domain sequence of SEQ ID NO: 23. In an embodiment, the humanized antibody comprises the light chain variable domain sequence of SEQ ID NO: 21 and the heavy chain variable domain sequence of SEQ ID NO: 22. In an embodiment, the invention provides an antibody that bind to the same epitope as a humanized antibody comprising the light chain variable domain sequence of SEQ ID NO: 21 and the heavy chain variable domain sequence of SEQ ID NO: 22. In an alternative embodiment, the invention comprises a binding agent that binds to essentially the same epitope as an antibody from bin 1 or bins 3-7 as shown in Fig. 1.

[0021] In a further embodiment, the invention comprises a binding agent that binds to essentially the same epitope as any of the antibodies disclosed above. In some embodiments, the binding agent inhibits the growth of a tumor expressing CD98. In some embodiments, the binding agent is an antibody or a functional fragment thereof. In other embodiments, the binding agent is an anticalin, an adnectin, an affibody, a DARPin, a fynomer, an affitin, an affilin, an avimer, a cysteine-rich knottin peptide, or an engineered Kunitz-type inhibitor.

[0022] In one embodiment, the invention provides a binding agent capable of binding to CD98, wherein any one of the antibodies disclosed above displaces the binding agent in a competitive binding assay. In some embodiments, the binding agent is an antibody, or a functional fragment thereof. In another embodiment, the invention provides a binding agent capable of binding to CD98, wherein the binding agent displaces any one of the antibodies disclosed above in a competitive binding assay. In some embodiments, the binding agent is an antibody, or a functional fragment thereof.

[0023] In some embodiments, the invention provides an antibody that binds to CD98, wherein the antibody comprises a heavy chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 17, SEQ ID NO: 18; SEQ ID NO: 19, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 31, and SEQ ID NO; 35. In some embodiments, the antibody comprises a light chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence selected from the

group consisting of SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 33, and SEQ ID NO: 37. In some embodiment, the antibody comprises a heavy chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 17, SEQ ID NO: 18; SEQ ID NO: 19, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 31, and SEQ ID NO: 35, and the antibody further comprises a light chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 33, and SEQ ID NO: 37.

[0024] In some embodiments, the invention provides an antibody that is a variant of any of the above antibodies having one or more amino acid substitutions, deletions, insertions or modifications, and which retains a biological function of the antibody. In some embodiments, the invention provides an antibody that binds to CD98 expressed on the cell surface and inhibits the growth of the cell. In some embodiments, the anti-CD98 antibody binds to CD98 expressed on the cell surface and inhibits cell proliferation. In some embodiments, the anti-CD98 antibody binds to CD98 expressed on the cell surface and induces cell death. In some embodiments, the invention provides an antibody that is a variant of any one of the above antibodies having improvements in one or more of a property such as binding affinity, specificity, thermostability, expression level, effector function, glycosylation, reduced immunogenicity, or solubility as compared to the unmodified antibody.

[0025] In some embodiments, the invention provides any one of the above antibodies or functional fragments, wherein theantibody or fragment is conjugated to a cytotoxic agent. In various embodiments, the cytotoxic agent is selected from a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin, or a radioactive isotope. In some embodiments, the invention provides any one of the above antibodies or functional fragments, wherein theantibody or fragment is conjugated to a detectable marker. In various embodiments, the detectable marker is selected from a radioisotope, a metal chelator, an enzyme, a fluorescent compound, a bioluminescent compound and a chemiluminescent compound.

[0026] In an embodiment, the invention provides a hybridoma that produces a monoclonal antibody of the invention. In an embodiment, the invention provides a transgenic animal that produces a monoclonal antibody of the invention.

[0027] In some embodiments, a polynucleotide encoding any of the above antibodies is provided. In an embodiment, a vector comprising the polynucleotide is provided. In an embodiment, a host cell comprising the vector is provided. In an embodiment, the host cell is prokaryotic. In an embodiment, the host cell is an E. coli cell. In another embodiment, the host cell is eukaryotic. In an embodiment, the host cell is a Chinese Hamster Ovary (CHO) cell. In an embodiment, a method of making an anti-CD98 antibody is provided, wherein the method comprises culturing the host cell under

conditions suitable for expression of the polynucleotide encoding the antibody, and isolating the antibody.

[0028] In one embodiment, the invention provides a pharmaceutical composition comprising any of the above antibodies or functional fragments thereof, antibody conjugates, or binding agents of the invention. In a further embodiment, the invention provides a method of inhibiting growth of cancer cells that express CD98, the method comprising exposing the cells to any one or more of the above antibodies or functional fragments thereof, antibody conjugates, or binding agents of the invention. In various embodiments, the cancer cells are from a cancer selected from bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

[0029] In an embodiment, the invention provides a method for treating a cancer in a subject comprisingadministering to the subject a pharmaceutical composition comprising any of the above antibodies or functional fragments thereof, antibody conjugates, or binding agents of the invention. In various embodiments, the cancer is selected from bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers. In some embodiments, the cancer is acute myeloid leukemia. In some embodiments, the subject has relapsed or refractory acute myeloid leukemia. In some embodiments, the cancer is associated with increased expression of CD98 on the surface of a cell.

[0030] In some embodiments, the subject is administered one or more chemotherapeutic compound in combination with the antibody or functional fragment, wherein the chemotherapeutic compound is selected from bendamustine hydrochloride, cyclophosphamide, ifosfamide, fludurabine, cytarabine, gemcitabine, prednisone, prednisolone, methylprednisolone, paclitaxel, docetaxel, vinorelbine, vincristine, etoposide, irinotecan, anthracycline, adriamycin, cisplatin, carboplatin and rituximab.

[0031] In an embodiment, a method of detecting the presence of CD98 in a biological sample is provided, the method comprising contacting the biological sample with any of the above antibodies under conditions permissive for binding of the antibody to CD98, and detecting whether a complex is formed between the antibody and CD98. In some embodiments, the biological sample is from a mammal having or suspected of having a cancer of cells or tissues including, but not limited to, bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

[0032] In an embodiment, a method of diagnosing a cancer associated with increased expression of CD98 is provided, the method comprising contacting a test cell with any of the above antibodies; determining the level of expression of CD98 by detecting binding of the antibody to CD98; and comparing the level of expression of CD98 by the test cell with the level of expression of CD98 by a control cell, wherein a higher level of expression of CD98 by the test cell as compared to the control cell indicates the presence of a cancer associated with increased expression of CD98. In some embodiments, the test cell is a cell from a patient suspected of having a cancer selected from bladder, breast, colon,

rectal, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, glioma, lymphoma or leukemia,, or a metatasis of any of these cancers. In an embodiment, the method comprises determining the level of expression of CD98 on the surface of the test cell and comparing the level of expression of CD98 on the surface of the test cell with the level of expression of CD98 on the surface of the control cell. In some embodiments, the test cell is a cancer cell and the control cell is a normal cell of the same tissue type.

[0033] In an embodiment, the invention provides a use of any of the above antibodies or functional fragments in the in the manufacture of a medicament, wherein the medicament is for use in a method of inhibiting growth of cancer cells that express CD98. In various embodiments, the cells are from a cancer is selected from bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

[0034] In an embodiment, the invention provides any of the above antibodies or functional fragments for use in inhibiting the growth of cancer cells that express CD98. In various embodiments, the cells are from a cancer is selected from bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

[0035] In an embodiment, the invention provides a use of a pharmaceutical composition comprising any of the above antibodies or functional fragments in the manufacture of a medicament, wherein the medicament is for use in a method of treating cancer in a subject. In various embodiments, the cancer is selected from bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers. In some embodiments, the cancer is acute myeloid leukemia. In some embodiments, the subject has relapsed or refractory acute myeloid leukemia. In some embodiments, the cancer is associated with increased expression of CD98 on the surface of a cell. In some embodiments, the subject is administered one or more chemotherapeutic compound in combination with the antibody or functional fragment, wherein the chemotherapeutic compound is selected from bendamustine hydrochloride, cyclophosphamide, ifosfamide, fludurabine, cytarabine, gemcitabine, prednisone, prednisolone, methylprednisolone, paclitaxel, docetaxel, vinorelbine, vincristine, etoposide, irinotecan, anthracycline, adriamycin, cisplatin, carboplatin and rituximab.

[0036] In an embodiment, the invention provides a pharmaceutical composition comprising any of the above antibodies or functional fragments and a pharmaceutically acceptable carrier, for use in treating cancer in a subject. In various embodiments, the cancer is selected from bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers. In some embodiments, the cancer is acute myeloid leukemia. In some embodiments, the subject has relapsed or refractory acute myeloid leukemia. In some embodiments, the cancer is associated with increased expression of CD98 on the surface of a cell. In some embodiments, the subject is administered one or more chemotherapeutic

compound in combination with the antibody or functional fragment, wherein the chemotherapeutic compound is selected from bendamustine hydrochloride, cyclophosphamide, ifosfamide, fludurabine, cytarabine, gemcitabine, prednisone, prednisolone, methylprednisolone, paclitaxel, docetaxel, vinorelbine, vincristine, etoposide, irinotecan, anthracycline, adriamycin, cisplatin, carboplatin and rituximab.

[0037] In an embodiment, the invention provides the use of any of the above antibodies or functional fragments in the manufacture of a medicament, wherein the medicament is for use in a method for detecting the presence of of CD98 in a biological sample. In some embodiments, the method comprisescontacting the biological sample with any of the above antibodies under conditions permissive for binding of the antibody to CD98, and detecting whether a complex is formed between the antibody and CD98. In some embodiments, the biological sample is from a mammal having or suspected of having a cancer of cells or tissues including, but not limited to, bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

[0038] In an embodiment, the invention provides any of the above antibodies or functional fragments for use in a method of detecting the presence of CD98 in a biological sample. In some embodiments, the method comprisescontacting the biological sample with any of the above antibodies under conditions permissive for binding of the antibody to CD98, and detecting whether a complex is formed between the antibody and CD98. In some embodiments, the biological sample is from a mammal having or suspected of having a cancer of cells or tissues including, but not limited to, bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

[0039] In an embodiment, the invention provides the use of any of the above antibodies or functional fragments in the manufacture of a medicament, wherein the medicament is for use in a method of diagnosing a cancer associated with increased expression of CD98. In some embodiments, the method comprises contacting a test cell with any of the above antibodies; determining the level of expression of CD98 by detecting binding of the antibody to CD98; and comparing the level of expression of CD98 by the test cell with the level of expression of CD98 by a control cell, wherein a higher level of expression of CD98 by the test cell as compared to the control cell indicates the presence of a cancer associated with increased expression of CD98. In some embodiments, the test cell is a cell from a patient suspected of having a cancer selected from bladder, breast, colon, rectal, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, glioma, lymphoma or leukemia,, or a metatasis of any of these cancers. In an embodiment, the method comprises determining the level of expression of CD98 on the surface of the test cell and comparing the level of expression of CD98 on the surface of the test cell with the level of expression of CD98 on the surface of the control cell. In some embodiments, the test cell is a cancer cell and the control cell is a normal cell of the same tissue type.

[0040] In an embodiment, the invention provides any of the above antibodies or functional fragments for use in a method of diagnosing a cancer associated with increased expression of CD98. In

some embodiments, the method comprises contacting a test cell with any of the above antibodies; determining the level of expression of CD98 by detecting binding of the antibody to CD98; and comparing the level of expression of CD98 by the test cell with the level of expression of CD98 by a control cell, wherein a higher level of expression of CD98 by the test cell as compared to the control cell indicates the presence of a cancer associated with increased expression of CD98. In some embodiments, the test cell is a cell from a patient suspected of having a cancer selected from bladder, breast, colon, rectal, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, glioma, lymphoma or leukemia,, or a metatasis of any of these cancers. In an embodiment, the method comprises determining the level of expression of CD98 on the surface of the test cell and comparing the level of expression of CD98 on the surface of the test cell with the level of expression of CD98 on the surface of the control cell. In some embodiments, the test cell is a cancer cell and the control cell is a normal cell of the same tissue type.

[0041] In another embodiment of the invention, an article of manufacture, or "kit", containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, blister pack, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds an antibody or an antibody-drug conjugate (ADC) composition which is effective for treating the condition, and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibdy or ADC. The label or package insert indicates that the composition is used for treating the condition of choice, such as cancer. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

BRIEF DESCRIPTION OF THE FIGURES

[0042] Fig. 1 shows the protein expression level of CD98 that was identified and quantified by sTAg analysis in the AML, CLL, CRC specimens and relevant normal controls. Lines indicate the mean of % normalized spectral abundance factor (NSAF) in positive samples.

[0043] Fig. 2 is a graph showing the results of epitope binning for 39 anti-CD98 antibodies.

[0044] Fig. 3 shows the binding properties of chimeric anti-CD98 monoclonal antibodies 8-34B, 18-2A 2.1, 18-2A 2.2, and 18-2A 2.7. Fig. 3A is a graph showing the results of epitope binning for chimeric anti-CD98 monoclonal antibodies. The four reference antibodies are as in Fig. 1. "Isotype" is a control antibody of the same isotype that does not bind CD98. Fig. 3B shows the Kd of chimeric anti-CD98 monoclonal antibodies as determined by FACS analysis with colon cancer cell line DLD1. Fig.

3C shows the results of FACS analysis of three AML primary tumor samples and a cell line expressing cynomolgus monkey CD98 (cynCD98), stained with chimeric anti-CD98 monoclonal antibodies.

[0045] Fig. 4 shows the construction of the humanized 8-34B antibodies. Fig. 4A shows the sequences of the murine 8-34B light chain variable domain (IGN 34) aligned to the sequence of the human acceptor sequence (AC) and the humanized light chains L1 and L2. The CDRs according to Kabat numbering are shown in red, and the substitutions in L2 as compared to L1 are underlined. Fig. 4A discloses SEQ ID NOS 6, 38, 15-16 and 38, respectively, in order of appearance. Fig. 4B shows the sequences of the murine 8-34B heavy chain variable domain (IGN 34) aligned to the sequence of the human acceptor sequence (AC) and the humanized heavy chains H1, H2 and H3. The CDRs according to Kabat numbering are shown in red, and the substitutions in H2 and H3 as compared to H3 are underlined. Fig. 4B discloses SEQ ID NOS 4, 39, 17-19 and 40, respectively, in order of appearance.

[0046] Fig. 5 shows that anti-CD98 antibody treatment induces strong tumor growth inhibition in established Ramos tumors. Tumor volumes at which treatment was initiated increased from (A) ~75mm^3, (B) ~150mm^3 to (C) ~250mm^3. Dosing of the antibodies was stopped at day 29 (A) or day 22 (B) and tumor regrowth was measured for the duration of the study. Rituximab (anti-CD20 antibody) was used as a positive therapeutic control antibody, and antibody HB121 (ATCC) was used as an IgG2a isotype negative control.

[0047] Fig. 6 shows that anti-CD98 antibodies prolong significantly time to progression of treated RAMOS tumors. Tumor doubling time of previous tumor regrowth data (Figs. 4A-C) was calculated and used for further prediction of time to progression (TTP). TTP was then extrapolated for each animal within the treatment groups, until 2000mm^3 would have been reached and graphed as a Kaplan-Meier curve.

[0048] Fig. 7 shows the inhibition of in vivo tumor growth in a lymphoma xenograft by the anti-CD98 monoclonal antibody 18-2A as compared to rituxan and a negative control IgG2a. Arrows indicate administration of antibody treatment.

[0049] Fig. 8 shows the inhibition of in vivo tumor growth in an acute myeloid leukemia xenograft by the anti-CD98 monoclonal antibodies 18-2A and 8-34B as compared to a negative control IgG2a. Arrows indicate administration of antibody treatment.

[0050] Fig. 9 shows the inhibition of in vivo tumor growth in a colorectal cancer xenograft by the anti-CD98 monoclonal antibody 18-2A as compared to erbitux and a negative control IgG2a (first study) and to DC101 + CTX (cyclophosphamide) and a negative control IgG2a (second study). DC101 is a rat anti-mouse VEGFR2/KDR IgG_1 mAb (ATCC No. HB-11534) and serves as a positive control. Arrows indicate administration of antibody treatment.

[0051] Fig. 10 shows the inhibition of in vivo tumor growth in a non-small cell lung carcinoma xenograft by the anti-CD98 monoclonal antibody 18-2A as compared to Erbitux (anti-EGFR) and a negative control IgG2a. Arrows indicate administration of antibody treatment.

[0052] Fig. 11 shows the effect of anti-CD98 monoclonal antibodies on in vivo tumor growth of a lymphoma xenograft in mouse strains with different immunodeficient backgrounds: (A) NSG mice; (B) NOD.SCID mice, and (C) SCID mice.

[0053] Fig. 12 shows a comparison of the effect of chimeric anti-CD98 monoclonal antibodies (18-2A-ch7.1 and 8-34B-ch) as compared to their parent murine monoclonal antibodies (18-2A and 8-34B) on in vivo tumor growth of a lymphoma xenograft.

[0054] Fig. 13 illustrates the regions of the mouse CD98 sequence (SEQ ID NO: 96) that were substituted into the human CD98 sequence (SEQ ID NO: 1) to form the 13 mouse-human CD98 chimera constructs used to map the epitope on human CD98 bound by humanized monoclonal antibody IGN523.

[0055] Fig. 14 shows the binding of humanized monoclonal antibody IGN523 and a control antibody to each of the 13 mouse-human CD98 chimera constructs as determined by FACS analysis.

[0056] Fig. 15 shows the sequence of the region of human CD98 within which IGN523 binds, as identified using the mouse-human CD98 chimera constructs, and the location of this sequence within the three-dimensional structure of CD98. Amino acids T358-G368 (underlined) are buried in the crystal structure and are unlikely to be part of the binding interface. Non-conserved residues between the human and mouse sequences are shown in bold.

[0057] Fig. 16 shows the binding of IGN523 to four constructs made by introducing nonhomologous residues from mouse CD98 into the targeted loop region of the human sequence. Construct 4.1 consists of mutations: I371L, D374Q, A375G and Deletion of A376. Construct 4.2 consists of mutations: M383A, and E384K. Construct 4.3 consists of mutations: D391N, F395I, P396F and D397H. Construct 4.4 consists of mutations: G400R, A401P and A404L. Binding was detected by FACS analysis of CHO cells transfected with the respective constructs.

[0058] Fig. 17 shows the binding of IGN523 to single mutation constructs of hydrophobic residues in the targeted loop region. Each indicated hydrophobic residue was substituted with a highly charged amino acid as shown. Binding was detected by FACS analysis of CHO cells transfected with the respective constructs.

[0059] Fig. 18 shows the binding of IGN523 to constructs containing multiple mutations of residues in the targeted loop region, as detected by FACS analysis of CHO cells transfected with the respective constructs. M1 containts mutations D374Q, D397H, G400R and A401P. M2 contains mutations D374E and A375E. M3 contains mutations D397S and I398T.

[0060] Fig. 19 shows the results of a variable-length peptide screen for epitope mapping of humanized monoclonal antibody IGN523. ELISA results for each peptide are shown as a horizontal line. Start and end points of the lines indicate which residues are included in the peptide. The Y-value of the line shows the ELISA result obtained for that peptide. The results indicated dominant binding for $_{395}$FPDIPGA$_{401}$ and secondary binding for $_{379}$PGQP$_{382}$ (shaded regions).

[0061] Fig. 20 shows the results of a best-binding single-positions alanine-replacement peptide set. Each residue was replaced by A (or G if the orginal amino acid was A). The height at which

the replacement letter is plotted in the graph is the obtained ELISA value for that mutated peptide. The central line and shaded interval indicate the reference ELISA value.

[0062] Fig. 21 shows heat maps representing the data obtained from CLIPS conformational matrix structures that combined two partial sequences of human CD98 (SEQ ID NOS 45-59 shown on the X axis, and SEQ ID NOS 60-74 shown on the Y axis).

[0063] Fig. 22 shows the results of a mutagenesis screen of strongly-binding peptides from the matrix analyses shown in Fig. 21. SEQ1 shows the sequence of the peptide and DIF1 indicates where the mutation is located in the peptide. Grey fields indicate peptides having non-mutated sequences. The last column shows the difference in ELISA value between wild-type and mutated peptide. High values indicate that the mutation has a strong negative effect on binding.

[0064] Fig. 23 shows the location in the sequence and on the surface of human CD98 of the amino acid residues determined to be important for binding of humanized monoclonal antibody IGN523. Fig. 23A shows the location in the sequence of residues determined by the chimera and mutagenesis studies (bold), by Pepscan analysis (gray) or both (shaded). Fig. 23B shows the location of the residues determined by the chimera and mutagenesis studies (dark gray). Fig. 23C shows the location of residues determined by Pepscan analysis (light gray). Fig. 23D shows the overlap of both sets of residues (black).

[0065] Fig. 24 shows the inhibition of in vivo tumor growth in a RAMOS (RA.1) Burkitt lymphoma xenograft by the humanized monoclonal antibody IGN523 as compared to Rituximab and a negative control IgG. Antibodies were dosed interperitoneally at 10 mg/kg on days 11, 17 and 25. Arrows indicate administration of antibody treatment.

[0066] Fig. 25 shows the inhibition of in vivo tumor growth in a DAU Burkitt lymphoma xenograft by the humanized monoclonal antibody IGN523 as compared to rituxan and a negative control IgG. Antibodies were dosed interperitoneally at 10 mg/kg on days 20 and 26. Arrows indicate administration of antibody treatment.

[0067] Fig. 26A shows the inhibition of in vivo tumor growth in a IGN-LNG-12 lung tumor xenograft by the humanized monoclonal antibody IGN523 as compared to carboplatin and a negative control IgG. IGN523 and carboplatin were dosed interperitoneally on days 17, 24 and 31 at 10 mg/kg or 75 mg/kg, respectively. Arrows indicate administration of treatment. Fig. 26B shows body weight measurements corresponding to mice in Fig. 26A treated with the indicated reagents. Carboplatin was dosed at its maximum tolerated dose, which induced body weight loss in NOD-SCID mice.

[0068] Fig. 27 shows the inhibition of in vivo tumor growth in a KG-1 acute myeloid leukemia xenograft by the humanized monoclonal antibody IGN523 as compared to rituxan and a negative control IgG. Antibodies were dosed interperitoneally at 15 mg/kg on days 21, 28 and 34. Arrows indicate administration of antibody treatment.

[0069] Fig. 28 shows dose dependent inhibition of in vivo tumor growth in a lung tumor xenograft by the humanized monoclonal antibody IGN523. The antibody was dosed intraperitoneally at the indicated doses on days 12 and 19.Arrows indicate administration of antibody treatment.

[0070] Fig. 29 shows staining of human and cynomolgus monkey frozen tissue sections by humanized monoclonal antibody IGN523. Cryosections of human and cynomolgus monkey kidney, cerebrum, and placenta were stained with 10 μg/mL of IGN523. Modifications of the methods of Tuson, Fung, and Hierck for immunohistochemistry were used to eliminate the requirement for labeling of IGN523 and to preclude nonspecific reactivity between the secondary labeled anti-human IgG and IgG endogenous to the tissues to be examined (Fung 1992, Hierck 1994, Tuson 1990). Sections were cut at approximately 5 μm. All slides were initially assessed for the adequacy of tissue elements and staining, then evaluated and subjectively graded by the Study Pathologist for intensity of staining. Representative images are shown at 40x magnification with the exception of human cerebrum (20x).

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

General Techniques

[0071] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Current Protocols in Molecular Biology (F. M. Ausubel, et al. eds., (2003)); Therapeutic Monoclonal Antibodies: From Bench to Clinic, Z. An, ed, Wiley, Hoboken N.J. (2009); Monoclonal Antibodies: Methods and Protocols, M. Albitar, ed., Humana Press, Totawa, N.J. (2010); and Antibody Engineering, 2nd Ed., Vols 1 and 2, Kontermann and Dubel, eds., Springer-Verlag, Heidelberg, 2010.

DEFINITIONS AND ABBREVIATIONS

Definitions

[0072] For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth conflicts with any document incorporated herein by reference, the definition set forth below shall control.

[0073] The term "CD98", as used herein, refers to any native CD98 from any vertebrate source, including mammals such as primates (e.g. humans, cynomolgus monkey (cyno)), dogs, and rodents (e.g., mice and rats), unless otherwise indicated. The amino acid and encoding nucleic acid sequences of human CD98 are provided below as SEQ ID NO:1 and SEQ ID NO:2, respectively.

[0074] MSQDTEVDMKEVELNELEPEKQPMNAASGAAMSLAGAEKNGLVKIKVAEDEAEAAA
AAKFTGLSKEELLKVAGSPGWVRTRWALLLLFWLGWLGMLAGAVVIIVRAPRCRELPAQKWWHTGALYRI
GDLQAFQGHGAGNLAGLKGRLDYLSSLKVKGLVLGPIHKNQKDDVAQTDLLQIDPNFGSKEDFDSLLQSA
KKKSIRVILDLTPNYRGENSWFSTQVDTVATKVKDALEFWLQAGVDGFQVRDIENLKDASSFLAEWQNIT

KGFSEDRLLIAGTNSSDLQQILSLLESNKDLLLTSSYLSDSGSTGEHTKSLVTQYLNATGNRWCSWSLSQ
ARLLTSFLPAQLLRLYQLMLFTLPGTPVFSYGDEIGLDAAALPGQPMEAPVMLWDESSFPDIPGAVSANM
TVKGQSEDPGSLLSLFRRLSDQRSKERSLLHGDFHAFSAGPGLFSYIRHWDQNERFLVVLNFGDVGLSAG
LQASDLPASASLPAKADLLLSTQPGREEGSPLELERLKLEPHEGLLLRFPYAA (SEQ ID NO:1)

[0075] ATGAGCCAGGACACCGAGGTGGATATGAAGGAGGTGGAGCTGAATGAGTTAGAGCC
CGAGAAGCAGCCGATGAACGCGGCGTCTGGGGCGGCCATGTCCCTGGCGGGAGCCGAGAAGAATGGTCTG
GTGAAGATCAAGGTGGCGGAAGACGAGGCGGAGGCGGCAGCCGCGGCTAAGTTCACGGGCCTGTCCAAGG
AGGAGCTGCTGAAGGTGGCAGGCAGCCCCGGCTGGGTACGCACCCGCTGGGCACTGCTGCTGCTCTTCTG
GCTCGGCTGGCTCGGCATGCTTGCTGGTGCCGTGGTCATAATCGTGCGAGCGCCGCGTTGTCGCGAGCTA
CCGGCGCAGAAGTGGTGGCACACGGGCGCCCTCTACCGCATCGGCGACCTTCAGGCCTTCCAGGGCCACG
GCGCGGGCAACCTGGCGGGTCTGAAGGGGCGTCTCGATTACCTGAGCTCTCTGAAGGTGAAGGGCCTTGT
GCTGGGTCCAATTCACAAGAACCAGAAGGATGATGTCGCTCAGACTGACTTGCTGCAGATCGACCCCAAT
TTTGGCTCCAAGGAAGATTTTGACAGTCTCTTGCAATCGGCTAAAAAAAAAGAGCATCCGTGTCATTCTGG
ACCTTACTCCCAACTACCGGGGTGAGAACTCGTGGTTCTCCACTCAGGTTGACACTGTGGCCACCAAGGT
GAAGGATGCTCTGGAGTTTTGGCTGCAAGCTGGCGTGGATGGGTTCCAGGTTCGGGACATAGAGAATCTG
AAGGATGCATCCTCATTCTTGGCTGAGTGGCAAAATATCACCAAGGGCTTCAGTGAAGACAGGCTCTTGA
TTGCGGGGACTAACTCCTCCGACCTTCAGCAGATCCTGAGCCTACTCGAATCCAACAAAGACTTGCTGTT
GACTAGCTCATACCTGTCTGATTCTGGTTCTACTGGGGAGCATACAAAATCCCTAGTCACACAGTATTTG
AATGCCACTGGCAATCGCTGGTGCAGCTGGAGTTTGTCTCAGGCAAGGCTCCTGACTTCCTTCTTGCCGG
CTCAACTTCTCCGACTCTACCAGCTGATGCTCTTCACCCTGCCAGGGACCCCTGTTTTCAGCTACGGGGA
TGAGATTGGCCTGGATGCAGCTGCCCTTCCTGGACAGCCTATGGAGGCTCCAGTCATGCTGTGGGATGAG
TCCAGCTTCCCTGACATCCCAGGGGCTGTAAGTGCCAACATGACTGTGAAGGGCCAGAGTGAAGACCCTG
GCTCCCTCCTTTCCTTGTTCCGGCGGCTGAGTGACCAGCGGAGTAAGGAGCGCTCCCTACTGCATGGGGA
CTTCCACGCGTTCTCCGCTGGGCCTGGACTCTTCTCCTATATCCGCCACTGGGACCAGAATGAGCGTTTT
CTGGTAGTGCTTAACTTTGGGGATGTGGGCCTCTCGGCTGGACTGCAGGCCTCCGACCTGCCTGCCAGCG
CCAGCCTGCCAGCCAAGGCTGACCTCCTGCTCAGCACCCAGCCAGGCCGTGAGGAGGGCTCCCCTCTTGA
GCTGGAACGCCTGAAACTGGAGCCTCACGAAGGGCTGCTGCTCCGCTTCCCCTACGCGGCCTGA (SEQ ID NO:2)

[0076] The term "CD98" encompasses "full-length," unprocessed CD98 as well as any form of CD98 that results from processing in the cell. The term also encompasses naturally occurring variants or mutations of CD98, e.g., splice variants, allelic variants, SNP variants and isoforms. The CD98 polypeptides described herein may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods. A "native sequence CD98 polypeptide" comprises a polypeptide having the same amino acid sequence as the corresponding CD98 polypeptide derived from nature. Such native sequence CD98 polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence CD98 polypeptide" specifically encompasses naturally-occurring truncated or secreted forms of the specific CD98

polypeptide (*e.g.*, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide.

[0077] The term "antibody" is used in the broadest sense and specifically covers, for example, single anti-CD98 monoclonal antibodies (including agonist, antagonist, neutralizing antibodies, full length or intact monoclonal antibodies), anti-CD98 antibody compositions with polyepitopic specificity, polyclonal antibodies, multivalent antibodies, multispecific antibodies (e.g., bispecific antibodies so long as they exhibit the desired biological activity), formed from at least two intact antibodies, single chain anti-CD98 antibodies, and fragments of anti-CD98 antibodies, as defined below. The term "immunoglobulin" (Ig) is used interchangeable with antibody herein. An antibody can be human, humanized and/or affinity matured.

[0078] An "antigen" is a predetermined antigen to which an antibody can selectively bind. The target antigen may be a polypeptide, carbohydrate, nucleic acid, lipid, hapten or other naturally occurring or synthetic compound. Preferably, the target antigen is a polypeptide.

[0079] An antibody "which binds" an antigen of interest is one that binds the antigen with sufficient affinity such that the antibody is useful as a therapeutic agent in targeting a cell or tissue expressing the antigen, and does not significantly cross-react with other proteins. In such embodiments, the extent of binding of the antibody to a "non-target" protein will be less than about 10% of the binding of the antibody to its particular target protein as determined by fluorescence activated cell sorting (FACS) analysis or radioimmunoprecipitation (RIA). With regard to the binding of an antibody to a target molecule, the term "specific binding" or "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide target means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target, for example, an excess of non-labeled target. In this case, specific binding is indicated if the binding of the labeled target to a probe is competitively inhibited by excess unlabeled target. The term "specific binding" or "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide target as used herein can be exhibited, for example, by a molecule having a Kd for the target of at least about 10^{-4} M, alternatively at least about 10^{-5} M, alternatively at least about 10^{-6} M, alternatively at least about 10^{-7} M, alternatively at least about 10^{-8} M, alternatively at least about 10^{-9} M, alternatively at least about 10^{-10} M, alternatively at least about 10^{-11} M, alternatively at least about 10^{-12} M, or greater. In one embodiment, the term "specific binding" refers to binding where a molecule binds to a particular polypeptide or epitope on a particular polypeptide without substantially binding to any other polypeptide or polypeptide epitope.

[0080] The term "anti-CD98 antibody" or "an antibody that binds to CD98" refers to an antibody that is capable of binding CD98 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting CD98. Preferably, the extent of binding of an anti-CD98

antibody to an unrelated, non-CD98 protein is less than about 10% of the binding of the antibody to CD98 as measured, e.g., by fluorescence activated cell sorting (FACS) analysis or a radioimmunoassay (RIA). An antibody that "specifically binds to" or is "specific for" CD98 is defined as above. In certain embodiments, an antibody that binds to CD98 has a dissociation constant (Kd) of ≤ 1 μM, ≤ 100 nM, $\leq$ 10 nM, ≤ 1 nM, or ≤ 0.1 nM. In certain embodiments, anti-CD98 antibody binds to an epitope of CD98 that is conserved among CD98 from different species.

[0081] An "isolated antibody" is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment include, but are not limited to, materials that would interfere with therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method (Lowry et al., J. Bio. Chem. 193: 265-275, 1951) and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0082] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to a H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ε isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain (C_L) at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_H1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, e.g., Basic and Clinical Immunology, 8th edition, Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

[0083] The "variable region" or "variable domain" or "V domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain may be referred to as "VH." The variable domain of the light chain may be referred to as "VL." The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable domains. Instead, the V regions consist of

relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC).

[0084] An "intact" antibody is one comprising an antigen-binding site as well as a C_L and at least heavy chain constant domains, C_H1, C_H2 and C_H3. The constant domains may be native sequence constant domains (*e.g.* human native sequence constant domains) or amino acid sequence variant thereof. Preferably, the intact antibody has one or more effector functions.

[0085] "Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include, without limitation, Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies and di-diabodies (see, e.g. Holliger, P. et al (1993) Proc. Natl. Acad. Sci. 90:6444-8; Lu, D. et al. (2005) J. Biol. Chem. 280:19665-72; Hudson et al., Nat. Med. 9:129-134 (2003); WO 93/11161; and U.S. Patent Nos. 5,837,242 and 6,492,123); single-chain antibody molecules (see, e.g. U.S. Patent Nos. 4,946,778; 5,260,203; 5,482,858 and 5,476,786); dual variable domain antibodies (see, e.g. U.S. Patent No. 7,612,181); single variable domain antibodies (SdAbs) (see, e.g. Woolven et al., Immunogenetics 50: 98-101, 1999; Streltsov et al., Proc Natl Acad Sci USA. 101:12444-12449, 2004); and multispecific antibodies formed from antibody fragments.

[0086] A "functional fragment" of a therapeutic antibody will exhibit at least one if not some or all of the biological functions attributed to the intact antibody, the function comprising at least specific binding to the target antigen.

[0087] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies useful in the present invention may be prepared by the hybridoma methodology first described by Kohler et al., Nature, 256:495 (1975), or may be made using recombinant DNA methods in bacterial, eukaryotic animal or plant cells (see, e.g., U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., Nature, 352:624-628 (1991) and Marks et al., J. Mol. Biol., 222:581-597 (1991), for example.

[0088] The monoclonal antibodies herein include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see U.S. Patent No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)).

[0089] "Humanized" forms of non-human (*e.g.*, rodent) antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired antibody specificity, affinity, and capability. In some instances, corresponding non-human residues replace framework region (FR) residues of the human immunoglobulin. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also the following review articles and patents and references cited therein: Vaswani and Hamilton, *Ann. Allergy, Asthma and Immunol.*, 1:105-115 (1998); Harris, *Biochem. Soc. Transactions*, 23:1035-1038 (1995); Almagro and Fransson, Front. Biosci. 13: 1619-1633 (2008); U.S. Patent Nos. 5,585,089; 5,693,762; 6,180,370; and 6,054,297).

[0090] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries (Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991) and yeast display libraries (Chao et al., Nature Protocols 1: 755-768 (2006)). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.*, 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been

disabled, *e.g.*, mice (see, *e.g.*, Jakobovits, A., Curr. Opin. Biotechnol. 1995, 6(5):561-6; Brüggemann and Taussing, Curr. Opin. Biotechnol. 1997, 8(4):455-8; and U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al., Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0091] The term "hypervariable region", "HVR", or "HV", when used herein refers to the regions of an antibody variable domain that are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). A number of hypervariable region delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" hypervariable regions are based on an analysis of the available complex crystal structures. The residues from each of these hypervariable regions are noted below.

Loop	Kabat	AbM	Chothia	Contact	
L1	L24-L34	L24-L34	L26-L32	L30-L36	
L2	L50-L56	L50-L56	L50-L52	L46-L55	
L3	L89-L97	L89-L97	L91-L96	L89-L96	
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B	(Kabat Numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35	(Chothia Numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58	
H3	H95-H102	H95-H102	H96-H101	H93-H101	

[0092] Hypervariable regions may comprise "extended hypervariable regions" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 or 26-35A (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat et al., supra, for each of these definitions. As used herein, the terms "HVR" and "CDR" are used interchangeably.

[0093] "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues herein defined.

[0094] The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat", and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991). Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0095] The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g, Kabat *et al.*, *Sequences of Immunological Interest.* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat *et al., supra*). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody. Unless stated otherwise herein, references to residue numbers in the variable domain of antibodies means residue numbering by the Kabat numbering system. Unless stated otherwise herein, references to residue numbers in the constant domain of antibodies means residue numbering by the EU numbering system.

[0096] An "affinity matured" antibody is one with one or more alterations in one or more HVRs thereof which result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. For review, see Hudson and Souriau, Nature Medicine 9 :129-134 (2003); Hoogenboom, Nature Biotechnol. 23 : 1105-1116 (2005); Quiroz and Sinclair, Revista Ingeneria Biomedia 4 : 39-51 (2010).

[0097] A "blocking" antibody or an "antagonist" antibody is one which inhibits or reduces biological activity of the antigen it binds. Preferred blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen.

[0098] An "agonist antibody", as used herein, is an antibody which mimics at least one of the functional activities of a polypeptide of interest.

[0099] "Binding affinity" generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant

(Kd). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative embodiments are described in the following.

[0100] "Or better" when used herein to refer to binding affinity refers to a stronger binding between a molecule (e.g. antibody) and its binding partner, and is represented by a smaller numerical Kd value. For example, an antibody which has an affinity for an antigen of ".6 nM or better", the antibody's affinity for the antigen is <.6 nM, i.e. .59 nM, .58 nM, .57 nM etc. or any value less than .6 nM.

[0101] In one embodiment, the "Kd" or "Kd value" according to this invention is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay that measures solution binding affinity of Fabs for antigen by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (Chen, et al., (1999) *J. Mol Biol* 293:865-881). According to another embodiment the Kd or Kd value is measured by using surface plasmon resonance assays using, for example, a BIAcore™-2000 or a BIAcore™-3000 (BIAcore, Inc., Piscataway, NJ).

[0102] An "on-rate" or "rate of association" or "association rate" or "k_{on}" according to this invention can also be determined with the same surface plasmon resonance technique described above using, for example, a BIAcore™-2000 or a BIAcore™-3000 (BIAcore, Inc., Piscataway, NJ).

[0103] The phrase "substantially similar," or "substantially the same", as used herein, denotes a sufficiently high degree of similarity between two numeric values (generally one associated with an antibody of the invention and the other associated with a reference antibody) such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values). The difference between the two values is preferably less than about 50%, preferably less than about 40%, preferably less than about 30%, preferably less than about 20%, preferably less than about 10% as a function of the value for the reference antibody.

[0104] The phrase "substantially reduced," or "substantially different", as used herein, denotes a sufficiently high degree of difference between two numeric values (generally one associated with an antibody of the invention and the other associated with a reference antibody) such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values, HAMA response). The difference between said two values is preferably greater than about 10%, preferably greater than about 20%, preferably greater than about 30%, preferably greater than about 40%, preferably greater than about 50% as a function of the value for the reference antibody.

[0105] An antibody that "inhibits the growth of tumor cells expressing a CD98 polypeptide" or a "growth inhibitory" antibody is one which results in measurable growth inhibition of cancer cells expressing or overexpressing the appropriate CD98 polypeptide. The CD98 polypeptide may be a transmembrane polypeptide expressed on the surface of a cancer cell or may be a polypeptide that is produced and secreted by a cancer cell. Preferred growth inhibitory anti-CD98 antibodies inhibit growth of CD98-expressing tumor cells by greater than 20%, preferably from about 20% to about 50%, and even more preferably, by greater than 50% (e.g., from about 50% to about 100%) as compared to the appropriate control, the control typically being tumor cells not treated with the antibody being tested. In one embodiment, growth inhibition can be measured at an antibody concentration of about 0.1 to 30 μg/ml or about 0.5 nM to 200 nM in cell culture, where the growth inhibition is determined 1-10 days after exposure of the tumor cells to the antibody. Growth inhibition of tumor cells *in vivo* can be determined in various ways such as is described below. The antibody is growth inhibitory in vivo if administration of the anti-CD98 antibody at about 1 μg/kg to about 100 mg/kg body weight results in reduction in tumor size or tumor cell proliferation within about 5 days to 3 months from the first administration of the antibody, preferably within about 5 to 30 days.

[0106] An antibody that "induces apoptosis" is one that induces programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is usually one that overexpresses a CD98 polypeptide. Preferably the cell is a tumor cell. Various methods are available for evaluating the cellular events associated with apoptosis. For example, phosphatidyl serine (PS) translocation can be measured by annexin binding; DNA fragmentation can be evaluated through DNA laddering; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the antibody which induces apoptosis is one which results in about 2 to 50 fold, preferably about 5 to 50 fold, and most preferably about 10 to 50 fold, induction of annexin binding relative to untreated cell in an annexin binding assay.

[0107] An antibody that "induces cell death" is one that causes a viable cell to become nonviable. The cell is of a cell type that specifically expresses or overexpresses a CD98 polypeptide. The cell may be cancerous or a normal cell of the particular cell type. The CD98 polypeptide may be a transmembrane polypeptide expressed on the surface of a cancer cell or may be a polypeptide that is produced and secreted by a cancer cell. Cell death *in vitro* may be determined in the absence of complement and immune effector cells to distinguish cell death induced by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). Thus, the assay for cell death may be performed using heat inactivated serum (i.e., in the absence of complement) and in the absence of immune effector cells. To determine whether the antibody is able to induce cell death, loss of membrane integrity as evaluated by uptake of propidium iodide (PI), trypan blue (see Moore et al. Cytotechnology 17:1-11 (1995)) or 7AAD can be assessed relative to untreated cells. Preferred cell death-inducing antibodies are those which induce PI uptake in the PI uptake assay in BT474 cells.

[0108] Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor); and B cell activation.

[0109] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue.

[0110] A "functional Fc region" possesses an "effector function" of a native sequence Fc region. Exemplary "effector functions" include C1q binding; CDC; Fc receptor binding; ADCC; phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.*, an antibody variable domain) and can be assessed using various assays as disclosed, for example, in definitions herein

[0111] A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.

[0112] A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, preferably one or more amino acid substitution(s). Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% homology therewith, more preferably at least about 95% homology therewith.

[0113] Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g., Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind

specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are absolutely required for such killing. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. (USA) 95:652-656 (1998).

[0114] "Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one that binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof (see review M. in Daëron, Annu. Rev. Immunol. 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol. 9:457-492 (1991); Capel et al., Immunomethods 4:25-34 (1994); and de Haas et al., J. Lab. Clin. Med. 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)). Antibody variants with improved or diminished binding to FcRs are described, for example, in WO 2000/42072, and U.S. Patent Nos. 7,183,387; 7,332,581; and 7.335,742. See also, *e.g.*, Shields *et al. J. Biol. Chem.* 9(2):6591-6604 (2001).

[0115] "Complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed. Polypeptide variants with altered Fc region amino acid sequences (polypeptides with a variant Fc region) and increased or decreased C1q binding capability are described, *e.g.*, in US Patent No. 6,194,551 B1 and WO 1999/51642. See also, *e.g.*, Idusogie *et al. J. Immunol.* 164: 4178-4184 (2000).

[0116] The CD98 polypeptide "extracellular domain" or "ECD" refers to a form of the CD98 polypeptide that is essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a CD98 polypeptide ECD will have less than 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than 0.5% of such domains. The transmembrane domain of CD98 comprises amino acid residues 76-103 (Parmacek et al., Nucleic Acids Res. 17: 1915-1931, 1989). The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified. Optionally, therefore, an extracellular domain of a CD98

polypeptide may comprise amino acids from about 98-108 to 529 of the sequence of CD98 as disclosed in Parmacek et al, supra..

[0117] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0118] A "modification" of an amino acid residue/position, as used herein, refers to a change of a primary amino acid sequence as compared to a starting amino acid sequence, wherein the change results from a sequence alteration involving said amino acid residue/positions. For example, typical modifications include substitution of the residue with another amino acid (e.g., a conservative or non-conservative substitution), insertion of one or more (generally fewer than 5 or 3) amino acids adjacent to said residue/position, and deletion of said residue/position.

[0119] An "epitope" is the site on the surface of an antigen molecule to which a single antibody molecule binds. Generally an antigen has several or many different epitopes and reacts with many different antibodies. The term specifically includes linear epitopes and conformational epitopes.

[0120] An antibody binds "essentially the same epitope" as a reference antibody, when the two antibodies recognize identical or sterically overlapping epitopes. The most widely used and rapid methods for determining whether two epitopes bind to identical or sterically overlapping epitopes are competition assays, which can be configured in all number of different formats, using either labeled antigen or labeled antibody. Usually, the antigen is immobilized on a 96-well plate, and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured using radioactive or enzyme labels.

[0121] "Epitope mapping" is the process of identifying the binding sites, or epitopes, of antibodies on their target antigens. Antibody epitopes may be linear epitopes or conformational epitopes. Linear epitopes are formed by a continuous sequence of amino acids in a protein. Conformational epitopes are formed of amino acids that are discontinuous in the protein sequence, but which are brought together upon folding of the protein into its three-dimensional structure.

[0122] "Epitope binning", as defined herein, is the process of grouping antibodies based on the epitopes they recognize. More particularly, epitope binning comprises methods and systems for discriminating the epitope recognition properties of different antibodies, combined with computational processes for clustering antibodies based on their epitope recognition properties and identifying antibodies having distinct binding specificities.

[0123] A "disorder" is any condition or disease that would benefit from treatment with an substance/molecule or method of the invention. This includes chronic and acute disorders including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include cancerous conditions such as bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, as well as metastases of these cancers.

[0124] The terms "cell proliferative disorder" and "proliferative disorder" refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer.

[0125] "Tumor," as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer," "cancerous," "cell proliferative disorder," "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

[0126] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, oral cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, multiple myeloma and B-cell lymphoma, brain cancer, as well as head and neck cancer, and associated metastases.

[0127] A "CD98-expressing cell" is a cell that expresses endogenous or transfected CD98 on the cell surface. A "CD98-expressing cancer" is a cancer comprising cells that have CD98 protein present on the cell surface. A "CD98-expressing cancer" produces sufficient levels of CD98 on the surface of cells thereof, such that an anti-CD98 antibody can bind thereto and have a therapeutic effect with respect to the cancer. A cancer that "overexpresses" CD98 is one that has significantly higher levels of CD98 at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. CD98 overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the CD98 protein present on the surface of a cell (e.g. via an immunohistochemistry assay; FACS analysis). Alternatively, or additionally, one may measure levels of CD98-encoding nucleic acid or mRNA in the cell, e.g. via fluorescent in situ hybridization; (FISH; see WO98/45479 published October, 1998), Southern blotting, Northern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). Aside from the above assays, various in vivo assays are available to

the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, e.g. a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, e.g. by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody. A CD98-expressing cancer includes, but is not limited to, bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

[0128] As used herein, "treatment" (and variations such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis in the case of cancer, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or disorder or to slow the progression of a disease or disorder.

[0129] The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician. For cancer therapy, efficacy can be measured, for example, by assessing the time to disease progression (TTP) and/or by determining the response rate (RR). Other endpoints for measuring efficacy include, for example, overall survival (OS), disease-free survival (DFS) and recurrence-free (or relapse-free) survival (RFS). Metastasis can be determined by staging tests and by bone scan and tests for calcium level and other enzymes to determine spread to the bone. CT scans can also be done to look for spread to the pelvis and lymph nodes in the area. Chest X-rays and measurement of liver enzyme levels by known methods are used to look for metastasis to the lungs and liver, respectively. Other routine methods for monitoring the disease include transrectal ultrasonography (TRUS) and transrectal needle biopsy (TRNB).

[0130] An "individual" is a vertebrate. In certain embodiments, the vertebrate is a mammal. Mammals include, but are not limited to, farm animals (such as cows), sport animals, pets (such as cats, dogs, and horses), primates, mice and rats. In certain embodiments, a mammal is a human.

[0131] An "effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A "therapeutically effective amount" of a substance/molecule of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule, to elicit a desired response in the individual. A therapeutically effective amount encompasses an amount in which any toxic or detrimental effects of the substance/molecule are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount would be less than the therapeutically effective amount. In the case of cancer, the therapeutically

effective amount of the drug may, for example, reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. See preceding definition of "treating". To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic.

[0132] "Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

[0133] Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

[0134] "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0135] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulation may be sterile.

[0136] A "sterile" formulation is aseptic of free from all living microorganisms and their spores. An "effective amount" of an antibody as disclosed herein is an amount sufficient to carry out a specifically stated purpose. An "effective amount" may be determined empirically and in a routine manner, in relation to the stated purpose.

[0137] The term "therapeutically effective amount" refers to an amount of an antibody or other drug effective to "treat" a disease or disorder in a subject or mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. See the definition herein of "treating". To the extent the drug may prevent growth and/or kill existing cancer

cells, it may be cytostatic and/or cytotoxic. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0138] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu), chemotherapeutic agents e.g. methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents, enzymes and fragments thereof such as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

[0139] A "toxin" is any substance capable of having a detrimental effect on the growth or proliferation of a cell.

[0140] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer, regardless of mechanism of action. Chemotherapeutic agents include compounds used in "targeted therapy" and conventional chemotherapy. Examples of chemotherapeutic agents include, but are not limited to, alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine,

ADRIAMYCIN®, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Ill.), and TAXOTERE® doxetaxel (Rhone-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine (XELODA®); pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovovin. Additional chemotherapeutic agents include cytotoxic agents useful as antibody drug conjugates, such as maytansinoids (DM1 and DM4, for example) and auristatins (MMAE and MMAF, for example).

[0141] Also included in the definition of "chemotherapeutic agent" are: (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX®; tamoxifen citrate), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® (toremifine citrate); (ii) aromatase inhibitors that inhibit the enzyme

aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® (megestrol acetate), AROMASIN® (exemestane; Pfizer), formestanie, fadrozole, RIVISOR® (vorozole), FEMARA® (letrozole; Novartis), and ARIMIDEX® (anastrozole; AstraZeneca); (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) protein kinase inhibitors such as MEK inhibitors (WO 2007/044515); (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, for example, PKC-alpha, Raf and H-Ras, such as oblimersen (GENASENSE®, Genta Inc.); (vii) ribozymes such as VEGF expression inhibitors (e.g., ANGIOZYME®) and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines, for example, ALLOVECTIN®, LEUVECTIN®, and VAXID®; PROLEUKIN® rIL-2; topoisomerase 1 inhibitors such as LURTOTECAN®; ABARELIX® rmRH; (ix) anti-angiogenic agents such as bevacizumab (AVASTIN®, Genentech); and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0142] The term "prodrug" as used in this application refers to a precursor or derivative form of a compound of the invention that may be less cytotoxic to cells compared to the parent compound or drug and is capable of being enzymatically or hydrolytically activated or converted into the more active parent form. See, e.g., Wilman, "Prodrugs in Cancer Chemotherapy" Biochemical Society Transactions, 14, pp. 375-382, 615th Meeting Belfast (1986) and Stella et al., "Prodrugs: A Chemical Approach to Targeted Drug Delivery," *Directed Drug Delivery*, Borchardt et al., (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β-lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs, optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, compounds of the invention and chemotherapeutic agents such as described above.

[0143] A “small molecule” is defined herein to have a molecular weight below about 500 Daltons.

[0144] An "isolated nucleic acid" is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other genome DNA sequences as well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native sequence. The term embraces a nucleic acid sequence that has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule.

[0145] "Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. "Oligonucleotide," as used herein, generally refers to short, generally single-stranded, generally synthetic polynucleotides that are generally, but not necessarily, less than about 200 nucleotides in length. The terms "oligonucleotide" and "polynucleotide" are not mutually exclusive. The description above for polynucleotides is equally and fully applicable to oligonucleotides.

[0146] The cell that produces an anti-CD98 antibody of the invention will include the parent hybridoma cell e.g., the hybridomas that are deposited with the ATCC, as well as bacterial and eukaryotic host cells into which nucleic acid encoding the antibodies have been introduced. Suitable host cells are disclosed below.

[0147] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

COMPOSITIONS AND METHODS OF MAKING THE SAME

[0148] Antibodies that bind to CD98 are provided. Immunoconjugates comprising anti-CD98 antibodies are provided. Antibodies and immunoconjugates of the invention are useful, e.g., for the diagnosis or treatment of disorders associated with altered expression, e.g., increased expression, of CD98. In certain embodiments, antibodies or immunoconjugates of the invention are useful for the diagnosis or treatment of a cell proliferative disorder, such as cancer.

Anti-CD98 Antibodies

[0149] In one embodiment, the present invention provides anti-CD98 antibodies that may find use herein as therapeutic agents. Exemplary antibodies include polyclonal, monoclonal, humanized, human, bispecific, and heteroconjugate antibodies, as well as variants thereof having improved affinity or other properties.

1. Polyclonal Antibodies

[0150] The antibodies of the invention may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the CD98 polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are

not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation. The mammal can then be bled, and the serum assayed for CD98 antibody titer. If desired, the mammal can be boosted until the antibody titer increases or plateaus.

2. Monoclonal Antibodies

[0151] The antibodies of the invention may alternatively be monoclonal antibodies. Monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567).

[0152] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as described above to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. After immunization, lymphocytes are isolated and then fused with a myeloma cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)).

[0153] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium which medium preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells (also referred to as fusion partner). For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the selective culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[0154] Preferred fusion partner myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a selective medium that selects against the unfused parental cells. Preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 and derivatives e.g., X63-Ag8-653 cells available from the American Type Culture Collection, Manassas, Virginia, USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); and Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0155] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis described in Munson et al., Anal. Biochem., 107:220 (1980).

[0156] Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal e.g,, by i.p. injection of the cells into mice.

[0157] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, affinity chromatography (e.g., using protein A or protein G-Sepharose) or ion-exchange chromatography, hydroxylapatite chromatography, gel electrophoresis, dialysis, etc.

[0158] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., Curr. Opinion in Immunol., 5:256-262 (1993) and Plückthun, Immunol. Revs. 130:151-188 (1992).

[0159] In a further embodiment, monoclonal antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in, e.g, Antibody Phage Display: Methods and Protocols, P.M. O'Brien and R. Aitken, eds, Humana Press, Totawa N.J., 2002. In principle, synthetic antibody clones are selected by screening phage libraries containing phage that display various fragments of antibody variable region (Fv) fused to phage coat protein. Such phage libraries are screened for against the desired antigen. Clones expressing Fv fragments capable of binding to the desired antigen are adsorbed to the antigen and thus separated from the non-binding clones in the library. The binding clones are then eluted from the antigen, and can be further enriched by additional cycles of antigen adsorption/elution.

[0160] Variable domains can be displayed functionally on phage, either as single-chain Fv (scFv) fragments, in which VH and VL are covalently linked through a short, flexible peptide, or as Fab fragments, in which they are each fused to a constant domain and interact non-covalently, as described in Winter et al., Ann. Rev. Immunol., 12: 433-455 (1994).

[0161] Repertoires of VH and VL genes can be separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be searched for antigen-binding clones as described in Winter et al., supra. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned to provide a single source of human antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., EMBO J, 12: 725-

734 (1993). Finally, naive libraries can also be made synthetically by cloning the unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro as described by Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992).

[0162] Screening of the libraries can be accomplished by various techniques known in the art. For example, CD98 can be used to coat the wells of adsorption plates, expressed on host cells affixed to adsorption plates or used in cell sorting, or conjugated to biotin for capture with streptavidin-coated beads, or used in any other method for panning display libraries. The selection of antibodies with slow dissociation kinetics (and good binding affinities) can be promoted by use of long washes and monovalent phage display as described in Bass et al., Proteins, 8: 309-314 (1990) and in WO 92/09690, and a low coating density of antigen as described in Marks et al., Biotechnol., 10: 779-783 (1992).

[0163] Any of the anti-CD98 antibodies of the invention can be obtained by designing a suitable antigen screening procedure to select for the phage clone of interest followed by construction of a full length anti-CD98 antibody clone using the Fv sequences from the phage clone of interest and suitable constant region (Fc) sequences described in Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3.

3. Antibody Fragments

[0164] The present invention encompasses antibody fragments. In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved access to solid tumors. For a review of certain antibody fragments, see Hudson et al. (2003) Nat. Med. 9:129-134.

[0165] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992); and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from E. coli or yeast cells, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form $F(ab')_2$ fragments (Carter et al., Bio/Technology 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Fab and $F(ab')_2$ fragment with increased in vivo half-life comprising salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. Fv and scFv are the only species with intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during in vivo use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv. See Antibody Engineering, ed. Borrebaeck, supra. The antibody fragment

may also be a "linear antibody", e.g., as described for example, in the references cited before. Such linear antibodies may be monospecific or multi-specific, such as bispecific.

[0166] The smallest antibody-derived binding structures are the separate variable domains (V domains) also termed single variable domain antibodies (SdAbs). Certain types of organisms, the camelids and cartilaginous fish, possess high affinity single V-like domains mounted on an Fc equivalent domain structure as part of their immune system. (Woolven et al., Immunogenetics 50: 98-101, 1999; Streltsov et al., Proc Natl Acad Sci USA. 101:12444-12449, 2004). The V-like domains (called VhH in camelids and V-NAR in sharks) typically display long surface loops, which allow penetration of cavities of target antigens. They also stabilize isolated VH domains by masking hydrophobic surface patches. These VhH and V-NAR domains have been used to engineer sdAbs. Human V domain variants have been designed using selection from phage libraries and other approaches that have resulted in stable, high binding VL- and VH-derived domains.

4. Humanized Antibodies

[0167] The invention encompasses humanized antibodies. Various methods for humanizing non-human antibodies are known in the art. For example, a humanized antibody can have one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.* (1986) *Nature* 321:522-525; Riechmann *et al.* (1988) *Nature* 332:323-327; Verhoeyen *et al.* (1988) *Science* 239:1534-1536), by substituting hypervariable region sequences for the corresponding sequences of a human antibody.

[0168] In some cases, the humanized antibodies are constructed by CDR grafting, in which the amino acid sequences of the six complementarity determining regions (CDRs) of the parent rodent antibody are grafted onto a human antibody framework. Padlan et al. (FASEB J. 9:133-139, 1995) determined that only about one third of the residues in the CDRs actually contact the antigen, and termed these the "specificity determining residues," or SDRs. In the technique of SDR grafting, only the SDR residues are grafted onto the human antibody framework (Kashmiri et al., Methods 36: 25-34, 2005).

[0169] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies can be important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework for the humanized antibody (Sims *et al.* (1993) *J. Immunol.* 151:2296; Chothia *et al.* (1987) *J. Mol. Biol.* 196:901. Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, 89:4285; Presta *et al.* (1993) *J. Immunol.*, 151:2623. In some cases, the framework is derived from the consensus sequences of the most abundant human subclasses, $V_L\kappa$

subgroup I ($V_LκI$) and V_H subgroup III (V_HIII). In another method, human germline genes are used at the source of the framework regions.

[0170] In an alternative paradigm based on comparison of CDRs, called Superhumanization, FR homology is irrelevant. The method consists of comparison of the non-human sequence with the functional human germline gene repertoire. Those genes encoding the same or closely related canonical structures to the murine sequences are then selected. Next, within the genes sharing the canonical structures with the non-human antibody, those with highest homology within the CDRs are chosen as FR donors. Finally, the non-human CDRs are grafted onto these FRs. (Tan et al., J. Immunol. 169: 1119-1125, 2002).

[0171] It is further generally desirable that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to one method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. These include, for example, WAM (Whitelegg and Rees, Protein Eng. 13: 819-824, 2000), Modeller (Sali and Blundell, J. Mol. Biol. 234: 779-815, 1993), and Swiss PDB Viewer (Guex and Peitsch, Electrophoresis 18: 2714-2713, 1997). Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

[0172] Another method for antibody humanization is based on a metric of antibody humanness termed Human String Content (HSC). This method compares the mouse sequence with the repertoire of human germline genes and the differences are scored as HSC. The target sequence is then humanized by maximizing its HSC rather than using a global identity measure to generate multiple diverse humanized variants. (Lazar et al., Mol. Immunol. 44: 1986-1998, 2007).

[0173] In contrast to the methods described above, empirical methods may be used to generate and select humanized antibodies. These methods are based upon the generation of large libraries of humanized variants and selection of the best clones using enrichment technologies or high throughput screening techniques. Antibody variants may be isolated from phage, ribosome and yeast display libraries as well as by bacterial colony screening. (Hoogenboom, Nat. Biotechnol. 23: 1105-1116, 2005; Dufner et al., Trends Biotechnol. 24: 523-529, 2006; Feldhaus et al., Nat. Biotechnol. 21: 163-70, 2003; Schlapschy et al., Protein Eng. Des. Sel. 17: 847-60, 2004).

[0174] In the FR library approach, a collection of residue variants are introduced at specific positions in the FR followed by selection of the library to select the FR that best supports the grafted

CDR. The residues to be substituted may include some or all of the "Vernier" residues identified as potentially contributing to CDR structure (Foote and Winter, J. Mol. Biol. 224: 487-499, 1992), or from the more limited set of target residues identied by Baca et al. (J. Biol. Chem. 272: 10678-10684, 1997).

[0175] In FR shuffling, whole FRs are combined with the non-human CDRs instead of creating combinatorial libraries of selected residue variants. (Dall'Acqua et al., Methods 36: 43-60, 2005). The libraries may be screened for binding in a two-step selection process, first humanizing VL, followed by VH. Alternatively, a one-step FR shuffling process may be used. Such a process has been shown to be more efficient than the two-step screening, as the resulting antibodies exhibited improved biochemical and physico-chemical properties including enhanced expression, increased affinity and thermal stability (Damschroder et al, Mol. Immunol. 44: 3049-60, 2007)

[0176] The "humaneering" method is based on experimental identification of essential minimum specificity determinants (MSDs) and is based on sequential replacement of non-human fragments into libraries of human FRs and assessment of binding. It begins with regions of the CDR-3 of non-human VH and VL chains and progressively replaces other regions of the non-human antibody into the human FRs, including the CDR-1 and CDR-2 of both VH and VL. This methodology typically results in epitope retention and identification of antibodies from multiple sub-classes with distinct human V-segment CDRs. Humaneering allows for isolation of antibodies that are 91-96 % homologous to human germline gene antibodies. (Alfenito, Cambridge Healthtech Institute's Third Annual PEGS, The Protein Engineering Summit, 2007).

5. Human Antibodies

[0177] Human anti-CD98 antibodies of the invention can be constructed by combining Fv clone variable domain sequence(s) selected from human-derived phage display libraries with known human constant domain sequences(s) as described above. Alternatively, human monoclonal anti-CD98 antibodies of the invention can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor J. Immunol., 133: 3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol., 147: 86 (1991).

[0178] It is also possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. Transgenic mice that express human antibody repertoires have been used to generate high-affinity human sequence monoclonal antibodies against a wide variety of potential drug targets. See, e.g., Jakobovits, A., Curr. Opin. Biotechnol. 1995, 6(5):561-6; Brüggemann and Taussing, Curr. Opin. Biotechnol. 1997, 8(4):455-8; U.S. Pat. Nos. 6,075,181 and 6,150,584; and Lonberg et al., Nature Biotechnol. 23: 1117-1125, 2005).

[0179] Alternatively, the human antibody may be prepared via immortalization of human B lymphocytes producing an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or may have been immunized *in vitro).* See, *e.g.*, Cole *et al.,* Monoclonal

Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner *et al.*, J. Immunol., 147 (1):86-95 (1991); and US Pat No. 5,750,373.

[0180] Gene shuffling can also be used to derive human antibodies from non-human, e.g. rodent, antibodies, where the human antibody has similar affinities and specificities to the starting non-human antibody. According to this method, which is also called "epitope imprinting" or "guided selection", either the heavy or light chain variable region of a non-human antibody fragment obtained by phage display techniques as described herein is replaced with a repertoire of human V domain genes, creating a population of non-human chain/human chain scFv or Fab chimeras. Selection with antigen results in isolation of a non-human chain/human chain chimeric scFv or Fab wherein the human chain restores the antigen binding site destroyed upon removal of the corresponding non-human chain in the primary phage display clone, i.e. the epitope guides (imprints) the choice of the human chain partner. When the process is repeated in order to replace the remaining non-human chain, a human antibody is obtained (see PCT WO 93/06213; and Osbourn et al, Methods., 36, 61-68, 2005). Unlike traditional humanization of non-human antibodies by CDR grafting, this technique provides completely human antibodies, which have no FR or CDR residues of non-human origin. Examples of guided selection to humanize mouse antibodies towards cell surface antigens include the folate-binding protein present on ovarian cancer cells (Figini et al., Cancer Res., 58, 991-996, 1998) and CD147, which is highly expressed on hepatocellular carcinoma (Bao et al., Cancer Biol. Ther., 4, 1374-1380, 2005).

[0181] A potential disadvantage of the guided selection approach is that shuffling of one antibody chain while keeping the other constant could result in epitope drift. In order to maintain the epitope recognized by the non-human antibody, CDR retention can be applied (Klimka et al., Br. J. Cancer., 83, 252-260, 2000; Beiboer et al., J. Mol. Biol., 296, 833-49, 2000) In this method, the non-human CDR-H3 is commonly retained, as this CDR is at the center of the antigen-binding site and has proven to be the most important region of the antibody for antigen recognition. In some instances, however, CDR-H3 and CDR-L3, as well as CDR-H3, CDR-L3 and CDR-L2 of the non-human antibody may be retained.

6. Bispecific Antibodies

[0182] Bispecific antibodies are monoclonal antibodies that have binding specificities for at least two different antigens. In certain embodiments, bispecific antibodies are human or humanized antibodies. In certain embodiments, one of the binding specificities is for CD98 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of CD98. Bispecific antibodies may also be used to localize cytotoxic agents to cells that express CD98. These antibodies possess a CD98-binding arm and an arm which binds a cytotoxic agent, such as, e.g., saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. $F(ab')_2$ bispecific antibodies).

[0183] Methods for making bispecific antibodies are known in the art, such as, for example, by co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have

different specificities (Milstein and Cuello, Nature, 305: 537 (1983)). For further details of generating bispecific antibodies see, for example, Bispecific Antibodies, Kontermann, ed., Springer-Verlag, Hiedelberg (2011).

7. Multivalent Antibodies

[0184] A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g. tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. In certain embodiments, the dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. In certain embodiments, a multivalent antibody comprises (or consists of) three to about eight antigen binding sites. In one such embodiment, a multivalent antibody comprises (or consists of) four antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (for example, two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VD1-(X1)n -VD2-(X2)n -Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein may further comprise at least two (for example, four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain.

8. Effector Function Engineering

[0185] It may be desirable to modify the antibody of the invention with respect to effector function, e.g., so as to enhance antigen-dependent cell-mediated cyotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. See, e.g. Lazar et al., Proc. Natl. Acad. Sci. USA 2006, 103(11):4005-4010; Presta, L.G., Curr. Opin. Immunol. 2008, 20(4):460-70; and U.S. Patent Nos. 7,183,387; 7,332,581; and 7.335,742.

[0186] Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., J. Exp Med. 176:1191-1195 (1992) and Shopes, B. J. Immunol. 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff et al., Cancer Research 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual

Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., Anti-Cancer Drug Design 3:219-230 (1989). To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Patent 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

9. Alternative Binding Agents

[0187] The invention encompasses non-immunoglobulin binding agents that specifically bind to the same epitope as an anti-CD98 antibody disclosed herein. In some embodiments, a binding agent is identified an agent that displaces or is displaced by an anti-CD98 antibody of the invention in a competive binding assay. These alternative binding agents may include, for example, any of the engineered protein scaffolds known in the art. Such scaffolds include, for example, anticalins, which are based upon the lipocalin scaffold, a protein structure characterized by a rigid beta-barrel that supports four hypervariable loops which form the ligand binding site. Novel binding specificities are engineered by targeted random mutagenesis in the loop regions, in combination with functional display and guided selection (Skerra (2008) FEBS J. 275: 2677-2683). Other suitable scaffolds may include, for example, adnectins, or monobodies, based on the tenth extracellular domain of human fibronectin III (Koide and Koide (2007) Methods Mol. Biol. 352: 95-109); affibodies, based on the Z domain of staphylococcal protein A (Nygren et al. (2008) FEBS J. 275: 2668-2676)); DARPins, based on ankyrin repeat proteins (Stumpp et al. (2008) Drug. Discov. Today 13: 695-701); fynomers, based on the SH3 domain of the human Fyn protein kinase Grabulovski et al. (2007) J. Biol. Chem. 282: 3196-3204); affitins, based on Sac7d from Sulfolobus acidolarius (Krehenbrink et al. (2008) J. Mol. Biol. 383: 1058-1068); affilins, based on human y-B-crystallin (Ebersbach et al. (2007) J. Mol. Biol. 372: 172-185); avimers, based on the A domains of membrane receptor proteins (Silverman et al. (2005) Biotechnol. 23: 1556-1561); cysteine-rich knottin peptides (Kolmar (2008) FEBS J. 275: 2684-2690); and engineered Kunitz-type inhibitors (Nixon and Wood (2006) Curr. Opin. Drug. Discov. Dev. 9: 261-268) For a review, see Gebauer and Skerra (2009) Curr. Opin. Chem. Biol. 13: 245-255.

Antibody Variants

[0188] In some embodiments, amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody, including but not limited to specificity, thermostability, expression level, effector functions, glycosylation, reduced immunogenicity or solubility. In addition to the anti-CD98 antibodies described herein, it is contemplated that anti-CD98 antibody variants can be prepared. Anti-CD98 antibody variants can be prepared by introducing appropriate nucleotide changes into the encoding DNA, and/or by synthesis of the desired antibody or polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the anti-CD98

antibody, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

[0189] Variations may be a substitution, deletion or insertion of one or more codons encoding the antibody or polypeptide that results in a change in the amino acid sequence as compared with the native sequence antibody or polypeptide. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, i.e., conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

[0190] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for antibody-directed enzyme prodrug therapy) or a polypeptide which increases the serum half-life of the antibody.

[0191] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Amino acids may be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in Biochemistry, 2nd Ed., pp. 73-75, Worth Publishers, New York (1975)):

(1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)

(3) acidic: Asp (D), Glu (E)

(4) basic: Lys (K), Arg (R), His(H)

[0192] Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0193] Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, into the remaining (non-conserved) sites.

[0194] The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis (Carter et al., Nucl. Acids Res., 13:4331 (1986); Zoller et al., Nucl. Acids Res., 10:6487 (1987)), cassette mutagenesis (Wells et al., Gene, 34:315 (1985)), restriction selection mutagenesis (Wells et al., Philos. Trans. R. Soc. London SerA, 317:415 (1986)) or other known techniques can be performed on the cloned DNA to produce the anti-CD98 antibody variant DNA.

[0195] Any cysteine residue not involved in maintaining the proper conformation of the anti-CD98 antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the anti-CD98 antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment). Cysteine-engineered antibodies, which can be used to generate antibody-drug conjugates, are described, for example, in WO 2006/034488.

[0196] In an embodiment, an anti-CD98 antibody molecule of the invention is a "de-immunized" antibody. A "de-immunized" anti-CD98 antibody is an antibody derived from a humanized or chimeric anti-CD98 antibody, that has one or more alterations in its amino acid sequence resulting in a reduction of immunogenicity of the antibody, compared to the respective original non-de-immunized antibody. One of the procedures for generating such antibody mutants involves the identification and removal of T-cell epitopes of the antibody molecule. In a first step, the immunogenicity of the antibody molecule can be determined by several methods, e.g. by in vitro determination of T-cell epitopes or in silico prediction of such epitopes, as known in the art. Once the critical residues for T-cell epitope function have been identified, mutations can be made to remove immunogenicity and retain antibody activity. For review, see, e.g., Jones et al., Methods in Molecular Biology 525: 405-423, 2009.

In vitro affinity maturation

[0197] In an embodiment, antibody variants having an improved property such as affinity, stability, or expression level as compared to a parent antibody is in vitro affinity maturation. Like the natural prototype, in vitro affinity maturation is based on the principles of mutation and selection. Libraries of antibodies are displayed as Fab, scFv or V domain fragments either on the surface of an organism (e.g., phage, bacteria or yeast) or in association (covalently or non-covalently) with their encoding mRNA or DNA. Affinity selection of the displayed antibodies allows isolation of organisms or complexes carrying the genetic information encoding the antibodies. Two or three rounds of mutation and selection using display methods such as phage display usually results in antibody fragments with affinities in the low nanomolar range. Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen.

[0198] Phage display is the most widepread method for display and selection of antibodies. The antibodies are displayed on the surface of Fd or M13 bacteriophages as fusions to the bacteriophage coat protein. Selection involves exposure to antigen to allow phage-displayed antibodies to bind their targets, a process referred to as "panning." Phage bound to antigen are recovered and infected in bacteria

to produce phage for further rounds of selection. For review, see Hoogenboom, Methods. Mol. Biol. 178: 1-37, 2002; Bradbury and Marks, J. Immuno. Methods 290: 29-49, 2004).

[0199] In the yeast display system (Boder et al., Nat. Biotech. 15: 553–57, 1997; Chao et al., Nat. Protocols 1:755-768, 2006), the antibody is displayed as single-chain variable fusions (scFv) in which the heavy and light chains are connected by a flexible linker. The scFv is fused to the adhesion subunit of the yeast agglutinin protein Aga2p, which attaches to the yeast cell wall through disulfide bonds to Aga1p. Display of a protein via Aga2p projects the protein away from the cell surface, minimizing potential interactions with other molecules on the yeast cell wall. Magnetic separation and flow cytometry are used to screen the library to select for antibodies with improved affinity or stability. Binding to a soluble antigen of interest is determined by labeling of yeast with biotinylated antigen and a secondary reagent such as streptavidin conjugated to a fluorophore. Variations in surface expression of the antibody can be measured through immunofluorescence labeling of either the hemagglutinin or c-Myc epitope tag flanking the scFv. Expression has been shown to correlate with the stability of the displayed protein, and thus antibodies can be selected for improved stability as well as affinity (Shusta et al., J. Mol. Biol. 292: 949-956, 1999). An additional advantage of yeast display is that displayed proteins are folded in the endoplasmic reticulum of the eukaryotic yeast cells, taking advantage of endoplasmic reticulum chaperones and quality-control machinery. Once maturation is complete, antibody affinity can be conveniently 'titrated' while displayed on the surface of the yeast, eliminating the need for expression and purification of each clone. A theoretical limitation of yeast surface display is the potentially smaller functional library size than that of other display methods; however, a recent approach uses the yeast cells' mating system to create combinatorial diversity estimated to be 10^{14} in size (US Patent Publication 2003/0186,374; Blaise et al., Gene 342: 211–218, 2004).

[0200] In ribosome display, antibody-ribosome-mRNA (ARM) complexes are generated for selection in a cell-free system. The DNA library coding for a particular library of antibodies is genetically fused to a spacer sequence lacking a stop codon. This spacer sequence, when translated, is still attached to the peptidyl tRNA and occupies the ribosomal tunnel, and thus allows the protein of interest to protrude out of the ribosome and fold. The resulting complex of mRNA, ribosome, and protein can bind to surface-bound ligand, allowing simultaneous isolation of the antibody and its encoding mRNA through affinity capture with the ligand. The ribosome-bound mRNA is then reversed transcribed back into cDNA, which can then undergo mutagenesis and be used in the next round of selection. (Fukuda et al., Nucleic Acids Res. 34, e127, 2006). In mRNA display, a covalent bond between antibody and mRNA is established using puromycin as an adaptor molecule (Wilson et al., Proc. Natl. Acad. Sci. USA 98, 3750–3755, 2001).

[0201] As these methods are performed entirely in vitro, they provide two main advantages over other selection technologies. First, the diversity of the library is not limited by the transformation efficiency of bacterial cells, but only by the number of ribosomes and different mRNA molecules present in the test tube. Second, random mutations can be introduced easily after each selection round, for

example, by non-proofreading polymerases, as no library must be transformed after any diversification step.

[0202] Diversity may be introduced into the CDRs or the whole V genes of the antibody libraries in a targeted manner or via random introduction. The former approach includes sequentially targeting all the CDRs of an antibody via a high or low level of mutagenesis or targeting isolated hot spots of somatic hypermutations (Ho, et al., J. Biol. Chem. 280: 607–617, 2005) or residues suspected of affecting affinity on experimental basis or structural reasons. Random mutations can be introduced throughout the whole V gene using E. coli mutator strains, error-prone replication with DNA polymerases (Hawkins et al., J. Mol. Biol. 226: 889-896, 1992) or RNA replicases. Diversity may also be introduced by replacement of regions that are naturally diverse via DNA shuffling or similar techniques ((Lu et al., J. Biol. Chem 278: 43496-43507, 2003; US Pat. No. 5,565,332; US Pat. No. 6,989,250). Alternative techniques target hypervariable loops extending into framework-region residues (Bond et al., J. Mol. Biol. 348: 699-709, 2005) employ loop deletions and insertions in CDRs or use hybridization-based diversification (US Patent Publication No. 2004/0005709). Additional methods of generating diversity in CDRs are disclosed in US Pat. No. 7,985,840.

[0203] Screening of the libraries can be accomplished by various techniques known in the art. For example, CD98 can be immobilized onto solid supports, columns, pins or cellulose/poly(vinylidene fluoride) membranes/ other filters, expressed on host cells affixed to adsorption plates or used in cell sorting, or conjugated to biotin for capture with streptavidin-coated beads, or used in any other method for panning display libraries.

[0204] For review of in vitro affinity maturation methods, see Hoogenboom, Nature Biotechnology 23: 1105-1116, 2005 and Quiroz and Sinclair, Revista Ingeneria Biomedia 4: 39-51, 2010 and references therein.

Modifications of Anti-CD98 Antibodies

[0205] Covalent modifications of anti-CD98 antibodies are included within the scope of this invention. Covalent modifications include reacting targeted amino acid residues of an anti-CD98 antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the anti-CD98 antibody. Other modifications include deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[0206] Other types of covalent modification of the anti-CD98 antibody included within the scope of this invention include altering the native glycosylation pattern of the antibody or polypeptide (Beck et al., Curr. Pharm. Biotechnol. 9: 482-501, 2008; Walsh, Drug Discov. Today 15: 773-780, 2010), and linking the antibody to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol

(PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

[0207] The anti-CD98 antibody of the present invention may also be modified to form chimeric molecules comprising an anti-CD98 antibody fused to another, heterologous polypeptide or amino acid sequence, e.g., an epitope tag (Terpe, Appl. Microbiol. Biotechnol. 60: 523-533, 2003) or the Fc region of an IgG molecule (Aruffo, "Immunoglobulin fusion proteins" in Antibody Fusion Proteins, S.M. Chamow and A. Ashkenazi, eds., Wiley-Liss, New York, 1999, pp. 221-242).

Preparation of Anti-CD98 Antibodies

[0208] Anti-CD98 antibodies may be produced by culturing cells transformed or transfected with a vector containing anti-CD98 antibody-encoding nucleic acid. Polynucleotide sequences encoding polypeptide components of the antibody of the invention can be obtained using standard recombinant techniques. Desired polynucleotide sequences may be isolated and sequenced from antibody producing cells such as hybridoma cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in host cells. Many vectors that are available and known in the art can be used for the purpose of the present invention. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Host cells suitable for expressing antibodies of the invention include prokaryotes such as Archaebacteria and Eubacteria, including Gram-negative or Gram-positive organisms, eukaryotic microbes such as filamentous fungi or yeast, invertebrate cells such as insect or plant cells, and vertebrate cells such as mammalian host cell lines. Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. Antibodies produced by the host cells are purified using standard protein purification methods as known in the art.

[0209] Methods for antibody production including vector construction, expression and purification are further described in Plückthun et al., (1996) in Antibody Engineering: Producing antibodies in Escherichia coli: From PCR to fermentation (McCafferty, J., Hoogenboom, H. R., and Chiswell, D. J., eds), 1 Ed., pp. 203-252, IRL Press, Oxford; Kwong, K. & Rader, C. E. coli expression and purification of Fab antibody fragments. Current protocols in protein science editorial board John E Coligan et al., Chapter 6, Unit 6.10 (2009); Tachibana and Takekoshi, "Production of Antibody Fab Fragments in Escherischia coli," in Antibody Expression and Production, M. Al-Rubeai, Ed., Springer, New York, 2011; Therapeutic Monoclonal Antibodies: From Bench to Clinic (ed Z. An), John Wiley & Sons, Inc., Hoboken, NJ, USA.

[0210] It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare anti-CD98 antibodies. For instance, the appropriate amino acid sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques (see, e.g.,

Stewart et al., Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, J. Am. Chem. Soc., 85:2149-2154 (1963)). *In vitro* protein synthesis may be performed using manual techniques or by automation. Various portions of the anti-CD98 antibody may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the desired anti-CD98 antibody. Alternatively, antibodies may be purified from cells or bodily fluids, such as milk, of a transgenic animal engineered to express the antibody, as disclosed, for example, in US Pat. No. 5,545,807 and US Pat. No. 5,827,690.

Immunoconjugates

[0211] The invention also provides immunoconjugates (interchangably referred to as "antibody drug conjugates," or "ADCs") comprising any one of the anti-CD98 antibodies of the invention covalently bound by a synthetic linker to one or more cytotoxic agents. ADCs combine the high specificity of monoclonal antibodies with the pharmacological potency of cytotoxic molecules, allowing specific targeting of cytotoxic agents to tumor cells and avoiding the nonspecific toxicity of most anti-cancer drugs. For review, see, e.g. Carter and Senter, Cancer J. 14: 154-169 (2008); Ducry and Stump, Bioconjugate Chem. 21:5-13 (2010); Beck et al., Discov. Med. 10: 329-339 (2010).

[0212] Cytotoxic agents for use in the immunoconjugates of the invention may include chemotherapeutic agents, drugs or growth inhibitory agents as described above, toxins (e.g., an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof) or radioisotopes. In some embodiments, the immunoconjugate comprises a DNA binder (e.g., calicheamycin) or a tubulin depolymerization agent (e.g., a maytansinoid or an auristatin). The present invention further contemplates an immunoconjugate formed between an antibody and a compound with nucleolytic activity (e.g., a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

[0213] Enzymatically active toxins and fragments thereof that can be used in the immunoconjugates of the invention include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. See, e.g., WO 93/21232.

[0214] A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the conjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc^{99m} or I^{123}, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. The radioisotopes may be incorporated in the conjugate in known ways as described, e.g., in Reilly, "The radiochemistry of monoclonal antibodies and peptides," in Monoclonal Antibody and Peptide-Targeted Radiotherapy of Cancer, R.M. Reilly, ed., Wiley, Hoboken N.J., 2010.

[0215] The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell, but non-cleavable linkers are also contemplated herein. Linkers for use in the immunoconjugates of the invention include without limitation acid labile linkers (e.g., hydrazone linkers), disulfide-containing linkers, peptidase-sensitive linkers (e.g, peptide linkers such as citrulline-valine or phenylalanine-lysine), photolabile linkers, dimethyl linkers (Chari et al., Cancer Research 52:127-131 (1992); U.S. Patent No. 5,208,020), thioether linkers, or hydrophilic linkers designed to evade multidrug transporter-mediated resistance (Kovtun et al., Cancer Res. 70: 2528-2537, 2010).

[0216] Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate)). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). The invention further contemplates that conjugates of antibodies and cytotoxic agents may be prepared using any suitable methods as disclosed in the art, e.g., in Bioconjugate Techniques, 2nd Ed., G.T. Hermanson, ed., Elsevier, San Francisco, 2008.

[0217] Conventional antibody-drug conjugation strategies have been based on random conjugation chemistries involving the ε-amino group of Lys residues or the thiol group of Cys residues, which results in heterogenous conjugates. Recently developed techniques allow site-specific conjugation to antibodies, resulting in homogeneous drug loading and avoiding ADC subpopulations with altered antigen-binding or pharmacokinetics. These include engineering of "thiomabs" comprising cysteine substitutions at positions on the heavy and light chains that provide reactive thiol groups and do not disrupt immunoglobulin folding and assembly or alter antigen binding (Junutula et al., J. Immunol. Meth. 332: 41-52 (2008); Junutula et al., Nat. Biotechnol. 26: 925-932, 2008). In another method, selenocysteine is cotranslationally inserted into an antibody sequence by recoding the stop codon UGA from termination to selenocysteine insertion, allowing site specific covalent conjugation at the nucleophilic selenol group of selenocysteine in the presence of the other natural amino acids (Hofer et al., Proc. Natl. Acad. Sci. USA 105: 12451-12456 (2008); Hofer et al, Biochemistry 48(50): 12047-12057, 2009).

Pharmaceutical Formulations

[0218] The antibodies or antibody-drug conjugates (ADC) of the invention may be administered by any route appropriate to the condition to be treated. The antibody or ADC will typically be administered parenterally, i.e., infusion, subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural.

[0219] For treating cancers, in one embodiment, the antibody or antibody-drug conjugate is administered via intravenous infusion. The dosage administered via infusion is in the range of about 1 $\mu g/m^2$ to about 10,000 $\mu g/m^2$ per dose, generally one dose per week for a total of one, two, three or four doses. Alternatively, the dosage range is of about 1 $\mu g/m^2$ to about 1000 $\mu g/m^2$, about 1 $\mu g/m^2$ to about 800 $\mu g/m^2$, about 1 $\mu g/m^2$ to about 600 $\mu g/m^2$, about 1 $\mu g/m^2$ to about 400 $\mu g/m^2$, about 10 $\mu g/m^2$ to

about 500 μg/m^2, about 10 μg/m^2 to about 300 μg/m^2, about 10 μg/m^2 to about 200 μg/m^2, and about 1 μg/m^2 to about 200 μg/m^2. The dose may be administered once per day, once per week, multiple times per week, but less than once per day, multiple times per month but less than once per day, multiple times per month but less than once per week, once per month or intermittently to relieve or alleviate symptoms of the disease. Administration may continue at any of the disclosed intervals until remission of the tumor or symptoms of the cancer being treated. Administration may continue after remission or relief of symptoms is achieved where such remission or relief is prolonged by such continued administration.

[0220] In one aspect, the invention further provides pharmaceutical formulations comprising at least one anti-CD98 antibody of the invention and/or at least one immunoconjugate thereof and/or at least one anti-CD98 antibody-drug conjugate of the invention. In some embodiments, a pharmaceutical formulation comprises 1) an anti-CD98 antibody and/or an anti-CD98 antibody-drug conjugate and/or an immunoconjugate thereof, and 2) a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical formulation comprises 1) an anti-CD98 antibody and/or an immunoconjugate thereof, and optionally, 2) at least one additional therapeutic agent.

[0221] Pharmaceutical formulations comprising an antibody or immunoconjugate of the invention or the antibody-drug conjugate of the invention are prepared for storage by mixing the antibody or antibody-drug conjugate having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)) in the form of aqueous solutions or lyophilized or other dried formulations. The formulations herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, in addition to an anti-CD98 antibody, it may be desirable to include in the one formulation, an additional antibody, e.g., a second anti-CD98 antibody which binds a different epitope on the CD98 polypeptide, or an antibody to some other target such as a growth factor that affects the growth of the particular cancer. Alternatively, or additionally, the composition may further comprise a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0222] The antibodies or immunoconjugates of the invention may be formulated in any suitable form for delivery to a target cell/tissue, e.g, as microcapsules or macroemulsions (Remington's Pharmaceutical Sciences, 16th edition, Osol, A. Ed. (1980); Park et al., Molecules 10: 146-161 (2005); Malik et al., Curr. Drug. Deliv. 4: 141-151 (2007)); as sustained release formulations (Putney and Burke, Nature Biotechnol. 16: 153-157, (1998)) or in liposomes (Maclean et al., Int. J. Oncol. 11: 235-332 (1997); Kontermann, Curr. Opin. Mol. Ther. 8: 39-45 (2006)).

Therapeutic methods

[0223] An antibody or immunoconjugate of the invention may be used in, for example, in vitro, ex vivo, and in vivo therapeutic methods. In one aspect, the invention provides methods for

inhibiting cell growth or proliferation, either in vivo or in vitro, the method comprising exposing a cell to an anti-CD98 antibody or immunoconjugate thereof under conditions permissive for binding of the immunoconjugate to CD98. "Inhibiting cell growth or proliferation" means decreasing a cell's growth or proliferation by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, and includes inducing cell death. In certain embodiments, the cell is a tumor cell. In certain embodiments, the cell is a bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate tumor cell, or a sarcoma, melanoma, glioma, lymphoma or leukemia cell.

[0224] In one aspect, an antibody or immunoconjugate of the invention is used to treat or prevent a cell proliferative disorder, such as cancer. In certain embodiments, the cell proliferative disorder is associated with increased expression and/or activity of CD98. For example, in certain embodiments, the cell proliferative disorder is associated with increased expression of CD98 on the surface of a cancer cell. Examples of cell proliferative disorders to be treated by the antibodies or immunoconjugates of the invention include, but are not limited to, bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancers, or sarcomas, melanomas, gliomas, lymphomas or leukemias, or metatases of any of these cancers.

[0225] In one aspect, the invention provides methods for treating a cell proliferative disorder comprising administering to an individual an effective amount of an anti-CD98 antibody or immunoconjugate thereof. In certain embodiments, a method for treating a cell proliferative disorder comprises administering to an individual an effective amount of a pharmaceutical formulation comprising an anti-CD98 antibody or anti-CD98 immunoconjugate and, optionally, at least one additional therapeutic agent, such as those provided below. In one embodiment, an anti-CD98 antibody or immunoconjugate can be used for targeting CD98 on cancer cells by contacting the antibody or immunoconjugate with CD98 to form an antibody or immunoconjugate-antigen complex such that a conjugated cytotoxic agent of the immunoconjugate accesses the interior of the cell. In one embodiment, the bound antibody or immunoconjugate is internalized into the cancer cell expressing CD98.

[0226] An anti-CD98 antibody or immunoconjugate can be administered to a human for therapeutic purposes. Moreover, an anti-CD98 antibody or immunoconjugate can be administered to a non-human mammal expressing CD98 with which the antibody cross-reacts (e.g., a primate, pig, rat, or mouse) for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies or immunoconjugates of the invention (e.g., testing of dosages and time courses of administration).

[0227] Antibodies or immunoconjugates of the invention can be used either alone or in combination with other compositions in a therapy. For instance, an antibody or immunoconjugate of the invention may be co-administered with at least one additional therapeutic agent and/or adjuvant. In certain embodiments, an additional therapeutic agent is a cytotoxic agent, a chemotherapeutic agent, or a growth inhibitory agent. In some embodiments, a chemotherapeutic agent is an agent or a combination of agents such as an alkylating agent (for example, bendamustine hydrochloride, cyclophosphamide or ifosfamide) a nucleoside analog (for example, fludurabine, cytarabine or gemcitabine) a corticosteroid

(for example, prednisone, prednisolone or methylprednisolone), an anti-mitotic agent (for example, paclitaxel, docetaxel or vinorelbine), a vinca alkaloid (for example, vincristine or etoposide), a topoisomerase inhibitor (for example, irinotecan), an antibiotic (for example, anthracycline or adriamycin), a platinum analog (for example, cisplatin or carboplatin), a therapeutic antibody (for example, rituximab) or a combination of agents (for example CHOP or CVP) wherein the combination therapy is useful in the treatment of cancers. In some embodiments, the additional compound is a therapeutic antibody other than an anti-CD98 antibody (for example, rituximab).

[0228] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody or immunoconjugate of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies or immunoconjugates of the invention can also be used in combination with additional therapeutic regimens including without limitation radiation therapy and/or bone marrow and peripheral blood transplants.

[0229] An antibody or immunoconjugate of the invention (and any additional therapeutic agent or adjuvant) can be administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the antibody or immunoconjugate is suitably administered by pulse infusion, particularly with declining doses of the antibody or immunoconjugate. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

[0230] Antibodies or immunoconjugates of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody or immunoconjugate need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody or immunoconjugate present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0231] For the prevention or treatment of disease, the appropriate dosage of an antibody or immunoconjugate of the invention (when used alone or in combination with one or more other additional therapeutic agents, such as chemotherapeutic agents) will depend on the type of disease to be treated, the type of antibody or immunoconjugate, the severity and course of the disease, whether the antibody or immunoconjugate is administered for preventive or therapeutic purposes, previous therapy, the patient's

clinical history and response to the antibody or immunoconjugate, and the discretion of the attending physician. The antibody or immunoconjugate is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 100 mg/kg (e.g. 0.1mg/kg-20mg/kg) of antibody or immunoconjugate can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody or immunoconjugate would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) of antibody or immunoconjugate may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody or immunoconjugate). An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of the antibody. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Diagnostic methods and methods of detection

[0232] In one aspect, anti-CD98 antibodies and immunoconjugates of the invention are useful for detecting the presence of CD98 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue. In certain embodiments, such tissues include normal and/or cancerous tissues that express CD98 at higher levels relative to other tissues, for example, bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

[0233] In one aspect, the invention provides a method of detecting the presence of CD98 in a biological sample. In certain embodiments, the method comprises contacting the biological sample with an anti- CD98 antibody under conditions permissive for binding of the anti- CD98 antibody to CD98, and detecting whether a complex is formed between the anti- CD98 antibody and CD98.

[0234] In one aspect, the invention provides a method of diagnosing a disorder associated with increased expression of CD98. In certain embodiments, the method comprises contacting a test cell with an anti-CD98 antibody; determining the level of expression (either quantitatively or qualitatively) of CD98 by the test cell by detecting binding of the anti-CD98 antibody to CD98; and comparing the level of expression of CD98 by the test cell with the level of expression of CD98 by a control cell (e.g., a normal cell of the same tissue origin as the test cell or a cell that expresses CD98 at levels comparable to such a normal cell), wherein a higher level of expression of CD98 by the test cell as compared to the

control cell indicates the presence of a disorder associated with increased expression of CD98. In certain embodiments, the increased expression corresponds to higher density of CD98 expression on the surface of a tumor cell as compared toa normal cell. In certain embodiments, the test cell is obtained from an individual suspected of having a disorder associated with increased expression of CD98. In certain embodiments, the disorder is a cell proliferative disorder, such as a cancer or a tumor. Exemplary cell proliferative disorders that may be diagnosed using an antibody of the invention include bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

[0235] In certain embodiments, a method of diagnosis or detection, such as those described above, comprises detecting binding of an anti-CD98 antibody to CD98 expressed on the surface of a cell or in a membrane preparation obtained from a cell expressing CD98 on its surface. In certain embodiments, the method comprises contacting a cell with an anti-CD98 antibody under conditions permissive for binding of the anti-CD98 antibody to CD98, and detecting whether a complex is formed between the anti-CD98 antibody and CD98 on the cell surface. An exemplary assay for detecting binding of an anti-CD98 antibody to CD98 expressed CD98 on the surface of a cell is a "FACS" assay.

[0236] Certain other methods can be used to detect binding of anti-CD98 antibodies to CD98. Such methods include, but are not limited to, antigen-binding assays that are well known in the art, such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, fluorescent immunoassays, protein A immunoassays, and immunohistochemistry (IHC).

[0237] In certain embodiments, anti-CD98 antibodies are labeled. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

[0238] In certain embodiments, anti-CD98 antibodies are immobilized on an insoluble matrix. Immobilization entails separating the anti-CD98 antibody from any CD98 that remains free in solution. This conventionally is accomplished by either insolubilizing the anti-CD98 antibody before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich et al.., U.S. 3,720,760), or by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the anti-CD98

antibody after formation of a complex between the anti-CD98 antibody and CD98, e.g., by immunoprecipitation.

[0239] Any of the above embodiments of diagnosis or detection may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-CD98 antibody.

Assays

[0240] Anti-CD98 antibodies and immunoconjugates of the invention may be characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

Activity assays

[0241] In one aspect, assays are provided for identifying anti-CD98 antibodies or immunoconjugates thereof having biological activity. Biological activity may include, e.g., the ability to inhibit cell growth or proliferation (e.g., "cell killing" activity), or the ability to induce cell death, including programmed cell death (apoptosis). Antibodies or immunoconjugates having such biological activity in vivo and/or in vitro are also provided.

[0242] In certain embodiments, an anti-CD98 antibody or immunoconjugate thereof is tested for its ability to inhibit cell growth or proliferation in vitro. Assays for inhibition of cell growth or proliferation are well known in the art. Certain assays for cell proliferation, exemplified by the "cell killing" assays described herein, measure cell viability. One such assay is the CellTiter-Glo™ Luminescent Cell Viability Assay, which is commercially available from Promega (Madison, WI). That assay determines the number of viable cells in culture based on quantitation of ATP present, which is an indication of metabolically active cells. See Crouch et al (1993) J. Immunol. Meth. 160:81-88, US Pat. No. 6602677. The assay may be conducted in 96- or 384-well format, making it amenable to automated high-throughput screening (HTS). See Cree et al (1995) AntiCancer Drugs 6:398-404.

[0243] Another assay for cell proliferation is the "MTT" assay, a colorimetric assay that measures the oxidation of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan by mitochondrial reductase. Like the CellTiter-Glo™ assay, this assay indicates the number of metabolically active cells present in a cell culture. See, e.g., Mosmann (1983) J. Immunol. Meth. 65:55-63, and Zhang et al. (2005) Cancer Res. 65:3877-3882.

[0244] In one aspect, an anti-CD98 antibody is tested for its ability to induce cell death in vitro. Assays for induction of cell death are well known in the art. In some embodiments, such assays measure, e.g., loss of membrane integrity as indicated by uptake of propidium iodide (PI), trypan blue (see Moore et al. (1995) Cytotechnology, 17:1-11), or 7AAD. In an exemplary PI uptake assay, cells are cultured in Dulbecco's Modified Eagle Medium (D-MEM): Ham's F-12 (50:50) supplemented with 10% heat-inactivated FBS (Hyclone) and 2 mM L-glutamine. Thus, the assay is performed in the absence of complement and immune effector cells. Cells are seeded at a density of 3×10^6 per dish in 100 x 20 mm dishes and allowed to attach overnight. The medium is removed and replaced with fresh medium alone or medium containing various concentrations of the antibody or immunoconjugate. The cells are incubated for a 3-day time period. Following treatment, monolayers are washed with PBS and detached by

trypsinization. Cells are then centrifuged at 1200 rpm for 5 minutes at 4 °C, the pellet resuspended in 3 ml cold Ca^{2+} binding buffer (10 mM Hepes, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) and aliquoted into 35 mm strainer-capped 12 x 75 mm tubes (1 ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 μg/ml). Samples are analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Antibodies or immunoconjugates that induce statistically significant levels of cell death as determined by PI uptake are thus identified.

[0245] In one aspect, an anti-CD98 antibody or immunoconjugate is tested for its ability to induce apoptosis (programmed cell death) in vitro. An exemplary assay for antibodies or immunconjugates that induce apoptosis is an annexin binding assay, for example, as in Zhang et al. (BioTechniques 23: 525-531, 1997). Another exemplary assay for antibodies or immunconjugates that induce apoptosis is a histone DNA ELISA colorimetric assay for detecting internucleosomal degradation of genomic DNA. Such an assay can be performed using, e.g., the Cell Death Detection ELISA kit (Roche, Palo Alto, CA).

[0246] Cells for use in any of the above in vitro assays include cells or cell lines that naturally express CD98 or that have been engineered to express CD98. Such cells include tumor cells that overexpress CD98 relative to normal cells of the same tissue origin. Such cells also include cell lines (including tumor cell lines) that express CD98 and cell lines that do not normally express CD98 but have been transfected with nucleic acid encoding CD98.

[0247] In one aspect, an anti-CD98 antibody or immunoconjugate thereof is tested for its ability to inhibit cell growth or proliferation in vivo. In certain embodiments, an anti-CD98 antibody or immunoconjugate thereof is tested for its ability to inhibit tumor growth in vivo. In vivo model systems, such as xenograft models, can be used for such testing. In an exemplary xenograft system, human tumor cells are introduced into a suitably immunocompromised non-human animal, e.g., a SCID mouse. An antibody or immunoconjugate of the invention is administered to the animal. The ability of the antibody or immunoconjugate to inhibit or decrease tumor growth is measured. In certain embodiments of the above xenograft system, the human tumor cells are tumor cells from a human patient. Such cells useful for preparing xenograft models include without limitation cells expressing exogenous CD98, and cells naturally expressing CD98. In certain embodiments, the human tumor cells are introduced into a suitably immunocompromised non-human animal by subcutaneous injection or by transplantation into a suitable site, such as a mammary fat pad.

Binding assays and other assays

[0248] In one aspect, an anti-CD98 antibody is tested for its antigen binding activity. For example, in certain embodiments, an anti-CD98 antibody is tested for its ability to bind to exogenous or endogenous CD98 expressed on the surface of a cell. A FACS assay may be used for such testing.

[0249] A panel of monoclonal antibodies raised against CD98 may be grouped based upon the epitiopes they recognize, a process known as epitope binning. Epitope binning is typically carried out using competition assays, which evaluate an antibody's ability to bind to an antigen in the presence of another antibody. In an exemplary competition assay, immobilized CD98 is incubated in a solution

comprising a first labeled antibody that binds to CD98 and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to CD98. The second antibody may be present in a hybridoma supernatant. As a control, immobilized CD98 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to CD98, excess unbound antibody is removed, and the amount of label associated with immobilized CD98 is measured. If the amount of label associated with immobilized CD98 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to CD98. In certain embodiments, immobilized CD98 is present on the surface of a cell or in a membrane preparation obtained from a cell expressing CD98 on its surface.

[0250] High-throughput methods of epitope binning are also known in the art. See, for example, Jia et al., J. Immunol. Methods 2004, 288(1-2):91-98, describing a method of multiplexed competitive antibody binning for the characterization of monoclonal antibodies; and Miller et al., J. Immunol. Methods 2011, 365(1-2):118-25, describing epitope binning of murine monoclonal antibodies by a multiplexed pairing assay.

[0251] *Epitope mapping*

[0252] Epitope mapping is the process of identifying the binding sites, or epitopes, of an antibody on its target protein antigen. Antibody epitopes may be linear epitopes or conformational epitopes. Linear epitopes are formed by a continuous sequence of amino acids in a protein. Conformational epitopes are formed of amino acids that are discontinuous in the protein sequence, but which are brought together upon folding of the protein into its three-dimensional structure.

[0253] A variety of methods are known in the art for mapping antibody epitopes on target protein antigens. These include mutagenesis methods, peptide scanning methods, display methods, methods involving and mass spectroscopy, and structural determination.

[0254] The site directed mutagenesis method involves targeted site-directed mutagenesis where critical amino acids are identified by systematically introducing substitutions along the protein sequence and then determining the effects of each substitution on antibody binding. This may be done by "alanine scanning mutagenesis," as described by Cunningham and Wells (1989) Science 244: 1081-1085, or some other form of point mutagenesis of amino acid residues in human CD98. Mutagenesis studies, however, may also reveal amino acid residues that are crucial to the overall three-dimensional structure of CD98 but that are not directly involved in antibody-antigen contacts, and thus other methods may be necessary to confirm a functional epitope determined using this method.

[0255] Shotgun mutagenesis mapping utilizes a comprehensive plasmid-mutation library for the target gene, with each clone in the library bearing a unique amino acid mutation and the entire library covering every amino acid in the target protein. The clones that constitute the mutation library are individually arranged in microplates, expressed within living mammalian cells, and tested for immunoreactivity with antibodies of interest. Amino acids critical for antibody epitopes are identified by a loss of reactivity and are then mapped onto a protein structure to visualize epitopes. By automating the

analysis, new epitope maps can be derived within days to weeks. Because it uses the native structure of proteins within mammalian cells, the technique allows both linear and conformational epitope structures to be mapped on complex proteins. (Paes et al., J. Am. Chem. Soc. 131(20): 6952-6954 (2009); Banik and Doranz, Genetic Engineering and Biotechnology News 3(2): 25-28 (2010)).

[0256] The epitope bound by an anti-CD98 antibody may also be determined using peptide scanning methods. In peptide scanning, libraries of short peptide sequences from overlapping segments of the target protein, CD98 are tested for their ability to bind antibodies of interest. The peptides are synthesized and screened for binding, e.g. using ELISA or BIACORE, or on a chip, by any of the multiple methods for solid-phase screening (Reineke et al, Curr. Opin. Biotechnol. 12: 59-64, 2001) as in the "pepscan" methodology (WO 84/03564; WO 93/09872). Such peptide screening methods may not be capable of detecting some discontinuous functional epitopes, i.e. functional epitopes that involve amino acid residues that are not contiguous along the primary sequence of the CD98 polypeptide chain.

[0257] A recently developed technology termed CLIPS (chemical linkage of peptides onto scaffolds) may be used to map conformational epitopes. The loose ends of the peptides are affixed onto synthetic scaffolds, so that the scaffolded peptide may be able to adopt the same spatial structure as the corresponding sequence in the intact protein. CLIPS technology is used to fix linear peptides into cyclic structures ('single-loop' format), and to bring together different parts of a protein binding site ('double-loop', 'triple-loop', etc. format), so as to create conformational epitopes that may be assayed for antibody binding. (US Pat. No. 7,972,993).

[0258] The epitopes bound by antibodies of the invention may also be mapped using display techniques, including, for example, phage display, microbial display, and ribosome/mRNA display as described above. In these methods, libraries of peptide fragments are displayed on the surface of the phage or cell. Epitopes are then mapped by screening mAbs against these fragments using selective binding assays. A number of computational tools have been developed which allow the prediction of conformational epitopes based upon linear affinity-selected peptides obtained using phage display. (Mayrose et al., Bioinformatics 23: 3244-3246 , 2007). Methods are also available for the detection of conformational epitopes by phage display. Microbial display systems may also be used to express properly folded antigenic fragments on the cell surface for identification of conformational epitopes (Cochran et al., J. Immunol. Meth. 287: 147-158, 2004; Rockberg et al., Nature Methods 5: 1039-1045, 2008).

[0259] Methods involving proteolysis and mass spectroscopy may also be used to determine antibody epitopes (Baerga-Ortiz et al., Protein Sci. 2002 June; 11(6): 1300–1308). In limited proteolysis, the antigen is cleaved by different proteases, in the presence and in the absence of the antibody, and the fragments are identified by mass spectrometry. The epitope is the region of the antigen that becomes protected from proteolysis upon binding of the antibody (Suckau et al., Proc. Natl. Acad. Sci. USA 87: :9848-9852, 1990). Additional proteolysis based methods include, for example, selective chemical modification (Fiedler et al., Bioconjugate Chemistry 1998, 9(2): 236-234, 1998), epitope excision (Van de Water et al., Clin. Immunol. Immunopathol. 1997, 85(3): 229-235, 1997), and the recently developed

method of hydrogen-deuterium (H/D) exchange (Flanagan, N., Genetic Engineering and Biotechnology News 3(2): 25-28, 2010).

[0260] The epitope bound by antibodies of the present invention may also be determined by structural methods, such as X-ray crystal structure determination (e.g., WO 2005/044853), molecular modeling and nuclear magnetic resonance (NMR) spectroscopy, including NMR determination of the H-D exchange rates of labile amide hydrogens in IL-23R when free and when bound in a complex with an antibody of interest (Zinn-Justin et al. (1992) Biochemistry 31:11335-11347; Zinn-Justin et al. (1993) Biochemistry 32:6884-6891).

[0261] Additional antibodies binding to the same epitope as an antibody of the present invention may be obtained, for example, by screening of antibodies raised against CD98 for binding to the epitope, by immunization of an animal with a peptide comprising a fragment of human CD98 comprising the epitope sequence, or by selection of antibodies using phage display for binding to the epitope sequence. Antibodies that bind to the same functional epitope might be expected to exhibit similar biological activities, such as blocking a biological activity of CD98, and such activities can be confirmed by functional assays of the antibodies.

Additional Activity Assays

[0262] In one embodiment, an anti-CD98 antibody of the invention is an antagonist antibody that inhibits a biological activity of CD98. The anti-CD98 antibodies of the invention may be assayed to determine if they inhibit a biological activity of CD98, for example, binding to light chains. In order to determine whether CD98 antibodies of the invention inhibit binding to light chains, the ability of the CD98 antibodies to inhibit amino acid uptake in cancer cell lines is conducted in accordance with the method described in Kim et al., Biochim. Biophys. Acta 1565: 112-122, 2002.

[0263] In one aspect, purified anti-CD98 antibodies can be further characterized by a series of assays including, but not limited to, N-terminal sequencing, amino acid analysis, non-denaturing size exclusion high pressure liquid chromatography (HPLC), mass spectrometry, ion exchange chromatography and papain digestion.

[0264] In one embodiment, the invention contemplates an altered antibody that possesses some but not all effector functions, which make it a desirable candidate for many applications in which the half life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In certain embodiments, the Fc activities of the antibody are measured to ensure that only the desired properties are maintained. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. An example of an in vitro assay to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 or 5,821,337. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. PNAS (USA) 95:652-

656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed. FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art.

[0265] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literatures cited herein are expressly incorporated in their entirety by reference.

EXAMPLES

[0266] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

EXAMPLE 1: IDENTIFICATION OF CD98 ON THE SURFACE OF ACUTE MYELOGENOUS LEUKEMIA (AML) TUMOR CELLS

[0267] A total of 16 primary AML samples were obtained from Fred Hutchinson Cancer Research Center (FHCRC). Eleven samples from healthy donors were analyzed. To monitor the quality of individual AML samples, hematoxylin and eosin staining of AML blasts were performed. Only samples containing at least 75% tumor cells were analyzed. Additionally, analysis was performed on 23 primary chronic lymphocytic leukemia (CLL) samples obtained from Billings Clinic or the University of Florida, and 27 primary colorectal carcinomas (CRC) with 22 normal adjacent colon control samples obtained from The Cooperative Human Tissue Network (CHTN) or the National Disease Research Interchange (NDRI). Sample handling was optimized so as to maximally maintain cell viability during sample isolation. Optimal labeling times for AML, CLL and CRC samples were determined to allow for efficient labeling without compromise of cellular integrity.

[0268] Surface tagged antigen profiling (sTAg) was used to identify and quantitatively profile the repertoire of surface proteins on cells in sixteen core AML samples, five bone marrow mononuclear cell (BMMC) control and six peripheral blood mononuclear cell (PBMC) control samples, 20 core CLL samples, 27 CRC samples, and 22 normal adjacent colon samples. The extracellular domains of proteins associated with the AML tumor cell membranes of intact primary tumor cells were chemically tagged and then chromatographically enriched for tagged proteins using a solid-phase affinity resin. Eluted proteins were stored at -80°C prior to mass spectrometry analysis as described below.

[0269] Proteins enriched by the sTAg method were identified and quantified using high-resolution, shotgun liquid chromatography tandem mass spectrometry (MS). A hybrid ThermoFisher LTQ-Orbitrap Velos mass spectrometer, which combines the sensitivity of a linear ion trap with the high-resolution and mass accuracy afforded by the revolutionary orbitrap mass analyzer (Olsen et al., Mol. Cell Proteomics 8:2759-2769, 2009) coupled to a nanoflow liquid chromatography apparatus was employed for shotgun-based, bottoms-up proteomics to determine the identities and quantitative

abundance measurements of proteins in the AML cell surface enrichment fractions (Yates et al., Annu. Rev. Biomed. Eng. 11: 49-79, 2009). Tryptic digests from enriched surface proteins were separated by hydrophobicity via online, nanoflow liquid chromatography as peptide masses and fragmentation patterns were recorded dynamically by the mass spectrometer. To determine peptide and protein identities, the raw MS data was processed using the SEQUEST algorithm executed on a fast-processing Sorcerer 2 platform (Lundgren et al., Curr. Protoc. Bioinformatics, Chapter 13: Unit 13.3, 2009), to determine best-fit matches between experimental fragmentation patterns and those determined *in-silico* from the human proteome. Resulting matches were statistically validated using the PeptideProphet (Keller et al., Anal. Chem. 74: 5383-5392, 2002) and ProteinProphet (Nesvizhskii et al., Anal. Chem. 75: 4646-4658, 2003) software tools to ensure the lowest possible false discovery rates (FDR) and thus inclusion of only robustly identified proteins in the candidate pool.

[0270] The relative quantitative levels of identified proteins in the sTAg samples were determined using the spectral counting method (Neilson et al., Proteomics 11: 535-553, 2011). Spectral counting is based on the empirical demonstration that the number of assigned (positively identified) spectra associated with peptides from each protein correlates strongly with that protein's relative abundance in the original mixture (Liu et al.,Anal. Chem. 76:4193-4201, 2004). Spectral counts of identified peptides were obtained from proteomics analytical software platforms including Scaffold (Proteome Software) and ProteoIQ (NuSep) that display, sort and filter the results of SEQUEST-searched mass spectrometry data. Raw spectral counts were transformed to percent Normalized Spectral Abundance Factor (%NASF) values (Zybailov et al., J. Proteome Res. 5: 2339-2347, 2006) to account for differences in protein length and variability in sample size. Selected monoclonal antibodies were used to validate the proteomic measurements using quantitative FACS as an independent, external confirmatory measure of the sTAg mass spectrometry-based proteomic profiling of the primary tumor cell surface expression.

[0271] Using sTAg, the heterodimeric type II transmembrane glycoprotein CD98 having the amino acid sequence of SEQ ID NO.: 1 was identified as being present at high density on the surface of AML, CLL and CRC tumor cells. As shown in Fig. 1, using sTAg CD98 was identified in 7 of 16 primary AML samples with a mean %NSAF of 0.11 and in 20 of 20 primary CLL samples with a mean %NSAF of 0.15. CD98 was identified in 5 out of 5 bone marrow mononuclear cells (BMMC) and 5 out of 6 peripheral blood mononuclear cells (PBMC) samples with mean %NSAFs of 0.05 and 0.06 respectively. Furthermore, CD98 was identified in 11 of 27 primary CRC samples with a mean %NSAF of 0.10 and in only one normal adjacent colon sample with a mean %NSAF less than 0.01. Based on this analysis, CD98 is substantially enriched on a significant portion of patient-derived AML, CLL, and CRC primary tumor specimens relative to relevant normal controls.

EXAMPLE 2: IDENTIFICATION OF CD98 IN TUMORS

[0272] Antibody titration experiments were conducted with anti-CD98 monoclonal antibody 8-34B (see Example 3) and the isotype control antibody HB-121 to establish dilutions that would result in minimal background and maximal detection of signal. Serial dilutions were performed using steam-based antigen retrieval (pH 6.0 citrate buffer) at 1:50, 1:100, 1:200, and 1:400 on formalin-fixed, paraffin-embedded (FFPE) tissues or on fresh frozen tissues. Frozen control cell lines (F244 and F244-P) and formalin fixed control cell lines (F244, RM, and F244-P) were provided by Igenica and prepared by LifeSpan. The dilutions of 8-34B of 1:20 and 1:50 were selected for the study on formalin-fixed, paraffin-embedded tissues, whereas 8-34B at a dilution of 1:400 was selected for the study on fresh frozen tissues.

[0273] The principal detection system consisted of a Vector anti-mouse secondary antibody (BA-2000) and a Vector ABC-AP kit (AK-5000) with a Vector Red substrate kit (SK-5100), which was used to produce a fuchsia-colored deposit. Tissues were also stained with positive control antibodies (to CD31 and vimentin) to ensure that the tissue antigens were preserved and accessible for immunohistochemical analysis. Only tissues that were positive for CD31 and vimentin staining were selected for the remainder of the study.

[0274] Antibody 8-34B, at dilutions of 1:20 and 1:50, showed positive staining within ten out of 15 malignant melanomas and four out of 18 lung carcinomas on formalin-fixed, paraffin-embedded tissues. In addition, at a dilution of 1:400, antibody 8-34B showed positive staining of six of the six frozen lung carcinoma samples and also of two of the two frozen melanoma samples.

[0275] Table 1: Frequency of positive CD98 staining in lung carcinomas and melanomas

Carcinoma	**Frequency**
Non-small cell lung	4/18 (FFPE); 6/6 (Frozen)
Melanoma	10/15 (FFPE); 2/2 (Frozen)

EXAMPLE 3: PREPARATION OF MONOCLONAL ANTIBODIES TO CD98

[0276] Monoclonal antibodies were prepared in accordance with a general method as described in "Antibodies A Laboratory Manual" (Harlow and Lane 1988 CSH Press). Male 129S6/SvEv mice purchased from Taconic Farms were used for immunization. Mice were immunized via subcutaneous injection in the flank with 10^6 human CD98 (huCD98) expressing tumor cells. On day 39 post immunization, mice were boosted intraperitoneally with 5 million huCD98 expressing tumor cells. Spleens were harvested on day 42. Individual splenocytes were prepared and fused with CRL-2016 myeloma cells (ATCC) using a PEG based method as generally described in "Antibodies A Laboratory Manual" (Harlow and Lane 1988 CSH Press) to establish hybridomas.

[0277] Hybridomas were grown in 384 well tissue culture plates and supernatants from individual wells were screened by ELISA for production of antibodies recognizing huCD98. Positive wells were then transferred to 48 well plates, expanded, and supernatants were collected for huCD98 binding confirmation by ELISA. Individual hybridomas producing anti-huCD98 antibodies were

established as confirmed unique clones producing monoclonal anti-huCD98 antibodies by plating single hybridoma cells in wells of 96 well plates. These cells were grown into colonies and the supernatant from these individual colonies was screened by ELISA to confirm monoclonal antibody binding to huCD98. Clonal hybridomas were injected into pristane treated Balb/C mice to produce ascites. Ascites was collected and purified using Gammabind sepharose (GE Healthcare product code 17-0885-01), Protein A IgG binding buffer (Thermo Scientific part number 21001), and IgG elution buffer (Thermo Scientific part number 21004) following the general antibody purification protocol published by Thermo Scientific (Product Instructions #21001).

[0278] The nucleic acid and amino acid sequences for the heavy chain and light chain variable regions of the antibody 8-34B are shown below:

[0279] 8-34B heavy chain variable region

[0280] CAGGTGCAGCTGAAGGAGTCCGGCCCCGGCCTGGTGGCCCCCTCCCAGTCCCT GTCCATCACCTGCACCGTGTCCGGCTTCTCCCTGACCTCCTACGGCGTGCACTGGATCCGCCAG CCCCCCGGCAAGGGCCTGGAGTGGCTGGGCCTGATCTGGGCCGGCGGCTCCATCAACTACAACT CCGCCCTGATGTCCCGCCTGTCCATCTCCAAGGACAACTCCAAGTCCCAGGTGTTCCTGAAGAT GAACTCCCTGGAGACCGAGGACACCGCCATGTACTACTGCGCCCGCAAGGGCCACATGTACTCC TACGCCATGGACTACTGGGGCCAGGGCACCTCCGTGACCGTGTCCTCC (SEQ ID NO:3)

[0281] QVQLKESGPGLVAPSQSLSITCTVSGFSLTSYGVHWIRQPPGKGLEWLGLIWA GGSINYNSALMSRLSISKDNSKSQVFLKMNSLETEDTAMYYCARKGHMYSYAMDYWGQGTSVTV SS (SEQ ID NO:4; CDRs are underlined)

[0282] 8-34B light chain variable region

[0283] GACATCGTGATGACCCAGTCCCCCTCCTCCCTGACCGTGACCGCCGGCGAGAA GGTGACCATGTCCTGCAAGTCCTCCCAGTCCCTGCTGAACTCCGGCAACCAGAAGACCTACCTG ACCTGGTACCAGCAGAAGCCCGGCCAGCCCCCCAAGCTGCTGATCTACTGGGCCTCCACCCGCG AGTCCGGCGTGCCCGACCGCTTCACCGGCTCCGGCTCCGGCACCGAGTTCACCCTGACCATCTC CTCCGTGCAGGCCGAGGACCTGGCCGTGTACTACTGCCAGAACGACTACTCCTACCCCCCCTGG ACCTTCGGCGGCGGCACCAAGCTGGAGATCAAG (SEQ ID NO:5)

[0284] DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKTYLTWYQQKPGQPPKLL IYWASTRESGVPDRFTGSGSGTEFTLTISSVQAEDLAVYYCQNDYSYPPWTFGGGTKLEIK (SEQ ID NO:6; CDRs are underlined)

[0285] The nucleic acid and amino acid sequences for the heavy chain and light chain variable regions of the antibody 18-2A 2.2 are shown below:

[0286] 18-2A 2.2 heavy chain variable region

[0287] CAGGTGCAGCTGCAGCAGTCCGGCGCCGAGCTGGTGAAGCCCGGCGCCTCCGT GAAGCTGTCCTGCAAGGCCTCCGGCTACACCTTCACCTCCTACTACATGTACTGGGTGAAGCAG CGCCCCGGCCAGGGCCTGGAGTGGATCGGCGTGATCAACCCCGGCTCCGGCATCACCAACTACA

ACGAGAAGTTCAAGGGCAAGGCCACCCTGACCGCCGACAAGTCCTCCAACACCGCCTACATGCA
GCTGTCCTCCCTGTCCTCCGACGACTCCGCCGTGTACTTCTGCTCCGGCTCCGCCAACTGGTTC
GCCTACTGGGGCCAGGGCACCCTGGTGACCGTGTCCGCC (SEQ ID NO:7)

[0288] QVQLQQSGAELVKPGASVKLSCKASGYTFTSYYMYWVKQRPGQGLEWIGVINP
GSGITNYNEKFKGKATLTADKSSNTAYMQLSSLSSDDSAVYFCSGSANWFAYWGQGTLVTVSA
(SEQ ID NO:8; CDRs are underlined)

[0289] 18-2A 2.2 light chain variable region

[0290] GACATCGTGATGTCCCAGTCCCCCTCCTCCCTGGCCGTGTCCGTGGGCGAGAA
GGTGACCATGTCCTGCAAGTCCTCCCAGTCCCTGCTGTACTCCTCCAACCAGAAGAACTACCTG
GCCTGGTACCAGCAGAAGCCCGGCCAGTCCCCCAAGCTGCTGATCTACTGGGCCTCCACCCGCG
ACTCCGGCGTGCCCGACCGCTTCACCGGCTCCGGCTCCGGCACCGACTTCACCCTGACCATCTC
CTCCGTGAAGGCCGAGGACCTGGCCGTGTACTACTGCCAGCGCTACTACGGCTACCCCTGGACC
TTCGGCGGCGGCACCAAGCTGGAGATCAAG (SEQ ID NO:9)

[0291] DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLL
IYWASTRDSGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQRYYGYPWTFGGGTKLEIK
(SEQ ID NO:10; CDRs are underlined)

[0292] The nucleic acid and amino acid sequences for the heavy chain and light chain variable regions of the antibody 18-2A 7.1 are shown below:

[0293] 18-2A 7.1 heavy chain variable region

[0294] CAGGTGCAGCTGCAGCAGTCCGGCGCCGAGCTGGTGCGCCCCGGCACCTCCGT
GAAGGTGTCCTGCAAGGCCTCCGGCAACGCCTTCACCAACTACCTGATCGAGTGGATCAAGCAG
CGCCCCGGCCAGGGCCTGGAGTGGATCGGCGTGATCAACCCCGGCTCCGGCATCACCAACTACA
ACGAGAAGTTCAAGGGCAAGGCCACCCTGACCGCCGACAAGTCCTCCAACACCGCCTACATGCA
GCTGTCCTCCCTGTCCTCCGACGACTCCGCCGTGTACTTCTGCTCCGGCTCCGCCAACTGGTTC
GCCTACTGGGGCCAGGGCACCCTGGTGACCGTGTCCGCC (SEQ ID NO:11)

[0295] QVQLQQSGAELVRPGTSVKVSCKASGNAFTNYLIEWIKQRPGQGLEWIGVINP
GSGITNYNEKFKGKATLTADKSSNTAYMQLSSLSSDDSAVYFCSGSANWFAYWGQGTLVTVSA
(SEQ ID NO:12; CDRs are underlined)

[0296] 18-2A 7.1 light chain variable region

[0297] GACATCGTGATGTCCCAGTCCCCCTCCTCCCTGGCCGTGTCCGTGGGCGAGAA
GGTGACCATGTCCTGCAAGTCCTCCCAGTCCCTGCTGTACTCCTCCAACCAGAAGAACTACCTG
GCCTGGTACCAGCAGAAGCCCGGCCAGTCCCCCAAGCTGCTGATCTACTGGGCCTCCACCCGCG
ACTCCGGCGTGCCCGACCGCTTCACCGGCTCCGGCTCCGGCACCGACTTCACCCTGACCATCTC
CTCCGTGAAGGCCGAGGACCTGGCCGTGTACTACTGCCAGCGCTACTACGGCTACCCCTGGACC
TTCGGCGGCGGCACCAAGCTGGAGATCAAG (SEQ ID NO:13)

[0298] DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLL IYWASTRDSGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQRYYGYPWTFGGGTKLEIK (SEQ ID NO:14; CDRs are underlined)

[0299] The nucleic acid and amino acid sequences for the heavy chain and light chain variable regions of the antibody 1-47C are shown below:

[0300] 1-47C heavy chain variable region

[0301] CAGGTGCAGTTGAAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCT GTCCATCACTTGCACTGTCTCTGGGTTTTCATTAACCACCTATGGTGTACACTGGGTTCGCCAG CCTCCAGGAAAGGGTCTGGAGTGGCTGGGAGTGATGTGGACTAATGGAATCACAAATTATAATT CGGCTCTCATGTCCAGACTGAGCATCAGCAAAGACAACTCCAAGAGCCAAGTTTTCTTAAAAAT GAACAGTCTGCAAACTGATGACACAGCCATGTACTACTGTGCCAGAGGAGGACACTACGGTAGT ACCTCCTATGCTATGGACTTCTGGAGTCAAGGA (SEQ ID NO: 30)

[0302] QVQLKESGPGLVAPSQSLSITCTVSGFSLTTYGVHWVRQPPGKGLEWLGVMWT NGITNYNSALMSRLSISKDNSKSQVFLKMNSLQTDDTAMYYCARGGHYGSTSYAMDFWSQG (SEQ ID NO:31; CDRs are underlined)

[0303] 1-47C light chain variable region

[0304] GACATCCAGATGACTCAGTCTCCAGCCTCCCTATCTGCATCTGTGGGAGAAAC TGTCACCATCACATGTCGAGCAAGTGGGAATATTCACAATTATTTAACATGGTATCAGCAGAAA CAGGGAAAATCTCCTCAGCTCCTGGTCTATACTGCAAAAACCTTAGCAGATGGTGTGCCATCAA GGTTCAGTGGCAGTGGATCAGGAACACAATATTCTCTCAAGATCAACAGCCTGCAGCCTGAAGA TTTTGGGAGTTATTACTGTCAACATTTTTGGAATACTCCTTACACGTTCGGAGGGGGGACCAAG CTGGAAATAAAACGGGCTGATGCTGCACCAACTGTATCCATC (SEQ ID NO: 32)

[0305] DIQMTQSPASLSASVGETVTITCRASGNIHNYLTWYQQKQGKSPQLLVYTAKT LADGVPSRFSGSGSGTQYSLKINSLQPEDFGSYYCQHFWNTPYTFGGGTKLEIK (SEQ ID NO: 33; CDRs are underlined)

[0306] The nucleic acid and amino acid sequences for the heavy chain and light chain variable regions of the antibody 1-115A are shown below:

[0307] 1-115A heavy chain variable region

[0308] CAGGTGCAGCTGGAGGAGTCAGGACCTGGCCTGGTGGCGACCTCACAGAGCCT GTCCATCACTTGCACTGTCTCTGGGTTTTCATTAACCAACTGTGGTGTACACTGGGTTCGCCAG CCTCAAGGAAAGGGTCTAGAGTGGCTGGGAGTGATATGGCCTAATGGAATCACAATTTATAATT CGGGTCTCATGTCCAGACTGAGTATCAGCAAAGACAACTCCAAGAGCCAAGTTTTCTTAAAAAA GAACAGTCTGCAAACTGATGACACAGCCATGTACTACTGTGCCAGAGGAGGACATTACGGTAGT AGCTCCTATGCTATGGACTACTGGAGTCAAGGA (SEQ ID NO: 34)

[0309] QVQLEESGPGLVATSQSLSITCTVSGFSLTNCGVHWVRQPQGKGLEWLGVIWP NGITIYNSGLMSRLSISKDNSKSQVFLKKNSLQTDDTAMYYCARGGHYGSSSYAMDYWSQG (SEQ ID NO:35; CDRs are underlined)

[0310] 1-115A light chain variable region

[0311] GACATCCAGATGACTCAGTCTCCAGCCTCCCTATCTGCATCTGTGGGAGAAAC TGTCACCATCACATGTCGAGCAAGTGGGAATATTCACAATTATTTAACATGGTATCAGCAGAAA CCGGGAAAATCTCCTCAACTCCTGGTCTATACTGCAAAAACCTTAGCAGATGGTGTGCCATCAA GGTTCAGTGGCAGTGGATCAGGAACACAATATTCTCTCAAGATCAACAGCCTGCAGCCTGAAGA TTTTGGGAGTTATTACTGTCAACATTTTTGGAATACTCCTTACACATTCGGAGGGGGGACCAAG CTGGAAATAAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTAAGC (SEQ ID NO: 36)

[0312] DIQMTQSPASLSASVGETVTITCRASGNIHNYLTWYQQKPGKSPQLLVYTAKT LADGVPSRFSGSGSGTQYSLKINSLQPEDFGSYYCQHFWNTPYTFGGGTKLEIK (SEQ ID NO:37; CDRs are underlined)

EXAMPLE 4: ISOTYPING AND BINNING OF MONOCLONAL ANTIBODIES

[0313] Individual hybridoma supernatants containing antibodies that recognize huCD98 were assessed for isotype by detection on ELISA with isotype specific secondary antibodies purchased from Jackson Immunologicals (*Goat x IgG1 HRP – Product# 115-035-206, Goat x IgG2a HRP - Product# 115-035-206, Goat x IgG2b HRP – Product# 115-035-207, Goat x IgG3 HRP – Product# 115-035-209).*

[0314] A competition ELISA was performed to establish competitive binding bins. Individual anti-huCD98 isotyped antibody containing hybridoma supernatants were allowed to bind to huCD98 in individual wells of an ELISA plate. After 1 hour, the wells were washed and fixed using 4% paraformaldehyde. Then individual anti-huCD98 isotyped antibody (of a different isotype) containing hybridoma supernatants were allowed to bind to huCD98 in individual wells of an ELISA plate for an hour. After washing, the wells were incubated with a specific secondary antibody (Jackson Immunologicals *Goat x IgG2a HRP - Product# 115-035-206*) and detected with Supersignal ELISA Pico Chemiluminescent substrate (Thermo Scientific – Product# 37069). Individual IgG2a isotype antibodies that were able to bind in the presence of an IgG1 are considered to be in a unique epitope bin from that particular IgG1. Individual IgG2a isotype antibodies that were unable to bind in the presence of an IgG1 are considered to be in the same epitope bin as that particular IgG1. In this way multiple epitope bins were defined for huCD98 binding antibodies, as illustrated in Fig. 2.

EXAMPLE 5: BINDING AFFINITY

[0315] Purified anti-CD98 monoclonal antibodies are tested for affinity by the general method published by Carderelli et al. (2002) *Cancer Immunol Immunother* 51; 15-24. Briefly, CD98 expressing cells are incubated with different amounts of anti-CD98 monoclonal antibodies overnight, then assessed

by FACS with a goat anti-human Fc specific or anti-mouse Fc specific PE-conjugated secondary antibody (Jackson Immunologicals). FACS data is analyzed using Graphpad Prism software and Kds are determined using the Graphpad Prism Kd calculation tool.

EXAMPLE 6: PRODUCTION AND CHARACTERIZATION OF CHIMERIC ANTIBODIES

[0316] Total RNA was extracted from hybridomas producing anti-CD98 monoclonal antibodies using the Qiagen RNeasy Mini kit (Cat No. 74104), followed by the Qiagen OneStep RT-PCR Kit (Cat No. 210210). RT-PCR was performed with primer sets specific for murine heavy and light chain sequences. For each RNA sample, 12 individual heavy chain and 11 light chain RT-PCR reactions were set up using degenerate forward primer mixtures covering the leader sequences of murine variable regions. Reverse primers were located in the constant regions of murine heavy and light chains. The RT-PCR products from the first-round reactions were further amplified in the second-round PCR. 12 individual heavy chain and 11 light chain RT-PCR reactions were set up using semi-nested primer sets specific for antibody variable regions. PCR reactions were run on agarose gels and heavy and light chain PCR products were cut from the gel and cloned into sequencing vectors. 10-20 clones per hybridoma were sequenced to determine the anti-CD98 monoclonal antibody variable region. Heavy chain variable regions were then cloned in-frame into a vector containing human IgG1 heavy chain constant region sequence and light chain variable regions were cloned in-frame into a vector containing human kappa light chain constant region sequence. Chimeric antibodies were generated from transient transfection of HEK293 Freestyle cells and purified using the methodology described in Example 3.

[0317] Purified chimeric antibodies were subjected to binding analysis as described in Example 4 for the mouse monoclonal anti-CD98 antibodies. Fig. 3A shows the results of competitive binding assays for the 8-34B, 18-2A 2.1, 18-2A 2.2 and 18-2A 7.1 chimeric antibodies. Reference antibodies 1-4 are as in Fig. 2, while "isotype" is a control IgG_{2a} antibody that does not bind to CD98. The results shown in Fig. 3A demonstrate that the chimeric antibodies retain the epitope binding specificities of the murine antibodies from which they were derived. The binding affinities of the murine and chimeric anti-CD98 monoclonal antibodies were determined by FACS analysis as described in Example 5 with colon cancer cell line DLD1. The Kd values (ranging from 0.9nM to 4.5 nM) are shown in Fig. 3B, indicating that all these recombinant antibodies retain high-affinity binding to CD98, comparable to their parental murine antibodies. Purified chimeric monoclonal antibodies were also subjected to FACS analysis as described in Example 5 with three AML primary tumor samples and a cell line expressing cynomolgus monkey CD98 (cynCD98). The results shown in Fig. 3C demonstrate that all chimeric antibodies retained the binding to human CD98 on AML cells and cynCD98.

[0318] The binding affinities of the murine and chimeric anti-CD98 monoclonal antibodies were determined by on/off rate determination using a BIACORE system (reviewed in Lipschultz et al., Methods 20: 310-318, 2000). As shown in Table 2, the affinities of the chimeric antibodies were similar to those of the parent murine antibodies. Data for the humanized antibody 8-34B H2 L1 (see Example 7) is also shown.

[0319] Table 2

Antibody	k_a (on-rate) [M-1s-1]	k_d (off-rate) [s-1]	K_d [nM]
18-2A	6.2×10^5	3.5×10^{-5}	0.56
18-2A ch7.1	1.2×10^5	2.6×10^{-5}	0.22
18-2A ch2.2	2.5×10^5	3.5×10^{-4}	1.4
8-34B	8.2×10^4	1.6×10^{-4}	2.0
8 -34B ch	1.7×10^5	1.6×10^{-4}	0.94
8-34B H2 L1	2.5×10^5	3.2×10^{-4}	1.3

EXAMPLE 7: PREPARATION OF HUMANIZED ANTIBODIES

[0320] A humanized form of the murine monoclonal antibody 8-34B was made by grafting the CDRs of the murine heavy chain and light chain variable domains into the human acceptor framework regions as shown in Fig. 4. The humanized 8-34B light chain variable domain L1 (SEQ ID NO:15) was constructed by grafting the CDRs of the murine light chain into the human acceptor sequence (GenBank Accession No. ACJ71709.1). The 8-34B humanized light chain variable domain L2 (SEQ ID NO:16) was constructed by replacing two residues in FR3 of the human acceptor light chain with the corresponding residues of the murine monoclonal antibody (amino acid substitutions S63T andD70E by Kabat numbering; see Fig. 4A). The 8-34B humanized heavy chain H1 (SEQ ID NO:17) was constructed by grafting the CDRs of the murine heavy chain into the human acceptor sequence (GenBank Accession No. I37782). The 8-34B humanized heavy chain variable domain H2 (SEQ ID NO:18) was constructed by replacing one residue in FR2 and two residues in FR3 of the human acceptor heavy chain with the corresponding residues of the murine monoclonal antibody, resulting in the amino acid substitutions I48L, V71K and F78V by Kabat numbering (see Fig. 4B). The 8-34B humanized heavy chain variable domain H3 (SEQ ID NO:19) added two additional substitutions back to the murine residue in FR3, V67L and T73N, as shown in Fig. 4B.

[0321] The nucleic acid and amino acid sequences for the H2 heavy chain and the L1 light chain variable regions of the humanized antibody 8-34B are shown below:

[0322] Humanized 8-34B heavy chain variable region H2

[0323] CAGGTGCAGCTGCAGGAGTCCGGCCCCGGCCTGGTGAAGCCCTCCGAGACCCT
GTCCCTGACCTGCACCGTGTCCGGCTTCTCCCTGACCTCCTACGGCGTGCACTGGATCCGCCAG
CCCCCCGGCAAGGGCCTGGAGTGGCTGGGCCTGATCTGGGCCGGCGGCTCCATCAACTACAACT
CCGCCCTGATGTCCCGCGTGACCATCTCCAAGGACACCTCCAAGAACCAGGTGTCCCTGAAGCT

GTCCTCCGTGACCGCCGCCGACACCGCCGTGTACTACTGCGCCCGCAAGGGCCACATGTACTCC
TACGCCATGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGTCCTCC (SEQ ID NO: 25)

[0324] QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVHWIRQPPGKGLEWLGLIWA
GGSINYNSALMSRVTISKDTSKNQVSLKLSSVTAADTAVYYCARKGHMYSYAMDYWGQGTLVTV
SS (SEQ ID NO: 18; CDRs are underlined)

[0325] Humanized 8-34B light chain variable region L1

[0326] GACATCGTGATGACCCAGTCCCCCGACTCCCTGGCCGTGTCCCTGGGCGAGCG
CGCCACCATCAACTGCAAGTCCTCCCAGTCCCTGCTGAACTCCGGCAACCAGAAGACCTACCTG
ACCTGGTACCAGCAGAAGCCCGGCCAGCCCCCCAAGCTGCTGATCTACTGGGCCTCCACCCGCG
AGTCCGGCGTGCCCGACCGCTTCTCCGGCTCCGGCTCCGGCACCGACTTCACCCTGACCATCTC
CTCCCTGCAGGCCGAGGACGTGGCCGTGTACTACTGCCAGAACGACTACTCCTACCCCCCCTGG
ACCTTCGGCCAGGGCACCAAGGTGGAGATCAAG (SEQ ID NO: 24)

[0327] DIVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKTYLTWYQQKPGQPPKLL
IYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYSYPPWTFGQGTKVEIK
(SEQ ID NO: 15; CDRs are underlined)

[0328] A humanized form of the murine monoclonal antibody 18-2A was made by grafting the CDRs of the murine heavy chain and light chain variable domains from the chimeric antibody 18-2A 7.1 into the human acceptor framework regions. The humanized 18-2A 7.1 light chain variable domain L1 (SEQ ID NO:20) was constructed by grafting the CDRs of the murine light chain into the human acceptor sequence (GenBank Accession No. ACJ71709.1). The 18-2A 7.1 light chain variable domain L2 (SEQ ID NO:21) was constructed by replacing certain human framework residues with the corresponding residues from the murine monoclonal antibody. The 18-2A 7.1 humanized heavy chain variable domain H1 (SEQ ID NO: 22) was constructed by grafting the CDRs of the murine heavy chain into the human acceptor sequence. The 18-2A 7.1 humanized heavy chain variable domain H2 (SEQ ID NO: 23) was constructed by replacing certain human framework residues with the corresponding residues from the murine monoclonal antibody.

[0329] The nucleic acid and amino acid sequences for the heavy chain and light chain variable regions of the humanized antibody 18-2A 7.1 are shown below:

[0330] Humanized 18-2A 7.1 heavy chain variable region H1

[0331] CAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGAAGAAGCCCGGCTCCTCCGT
GAAGGTGTCCTGCAAGGCCTCCGGCAACGCCTTCACCAACTACCTGATCGAGTGGGTGCGCCAG
GCCCCCGGCCAGGGCCTGGAGTGGATGGGCGTGATCAACCCCGGCTCCGGCATCACCAACTACA
ACGAGAAGTTCAAGGGCAAGGCCACCATCACCGCCGACAAGTCCACCTCCACCGCCTACATGGA
GCTGTCCTCCCTGCGCTCCGAGGACACCGCCGTGTACTACTGCTCCGGCTCCGCCAACTGGTTC
GCCTACTGGGGCCAGGGCACCCTGGTGACCGTGTCCTCC (SEQ ID NO: 26)

[0332] QVQLVQSGAEVKKPGSSVKVSCKASGNAFTNYLIEWVRQAPGQGLEWMGVINP GSGITNYNEKFKGKATITADKSTSTAYMELSSLRSEDTAVYYCSGSANWFAYWGQGTLVTVSS

[0333] (SEQ ID NO: 22; CDRs are underlined)

[0334] Humanized 18-2A 7.1 light chain variable region L1

[0335] GACATCGTGATGACCCAGTCCCCCGACTCCCTGGCCGTGTCCCTGGGCGAGCG CGCCACCATCAACTGCAAGTCCTCCCAGTCCCTGCTGTACTCCTCCAACCAGAAGAACTACCTG GCCTGGTACCAGCAGAAGCCCGGCCAGCCCCCCAAGCTGCTGATCTACTGGGCCTCCACCCGCG ACTCCGGCGTGCCCGACCGCTTCTCCGGCTCCGGCTCCGGCACCGACTTCACCCTGACCATCTC CTCCCTGCAGGCCGAGGACGTGGCCGTGTACTACTGCCAGCGCTACTACGGCTACCCCTGGACC TTCGGCGGCGGCACCAAGGTGGAGATCAAG (SEQ ID NO: 27)

[0336] DIVMTQSPDSLAVSLGERATINCKSSQSLLYSSNQKNYLAWYQQKPGQPPKLL IYWASTRDSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQRYYGYPWTFGGGTKVEIK (SEQ ID NO: 20; CDRs are underlined)

[0337] Humanized 18-2A 7.1 heavy chain variable region H2

[0338] CAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGAAGAAGCCCGGCTCCTCCGT GAAGGTGTCCTGCAAGGCCTCCGGCAACGCCTTCACCAACTACCTGATCGAGTGGATCCGCCAG GCCCCCGGCCAGGGCCTGGAGTGGATCGGCGTGATCAACCCCGGCTCCGGCATCACCAACTACA ACGAGAAGTTCAAGGGCAAGGCCACCCTGACCGCCGACAAGTCCACCTCCACCGCCTACATGGA GCTGTCCTCCCTGCGCTCCGAGGACACCGCCGTGTACTACTGCTCCGGCTCCGCCAACTGGTTC GCCTACTGGGGCCAGGGCACCCTGGTGACCGTGTCCTCC (SEQ ID NO: 28)

[0339] QVQLVQSGAEVKKPGSSVKVSCKASGNAFTNYLIEWIRQAPGQGLEWIGVINP GSGITNYNEKFKGKATLTADKSTSTAYMELSSLRSEDTAVYYCSGSANWFAYWGQGTLVTVSS (SEQ ID NO: 23; CDRs are underlined)

[0340] Humanized 18-2A 7.1 light chain variable region L2

[0341] GACATCGTGATGACCCAGTCCCCCGACTCCCTGGCCGTGTCCCTGGGCGAGCG CGCCACCATCAACTGCAAGTCCTCCCAGTCCCTGCTGTACTCCTCCAACCAGAAGAACTACCTG GCCTGGTACCAGCAGAAGCCCGGCCAGCCCCCCAAGCTGCTGATCTACTGGGCCTCCACCCGCG ACTCCGGCGTGCCCGACCGCTTCACCGGCTCCGGCTCCGGCACCGACTTCACCCTGACCATCTC CTCCCTGCAGGCCGAGGACGTGGCCGTGTACTACTGCCAGCGCTACTACGGCTACCCCTGGACC TTCGGCGGCGGCACCAAGGTGGAGATCAAG (SEQ ID NO: 29)

[0342] DIVMTQSPDSLAVSLGERATINCKSSQSLLYSSNQKNYLAWYQQKPGQPPKLL IYWASTRDSGVPDRFTGSGSGTDFTLTISSLQAEDVAVYYCQRYYGYPWTFGGGTKVEIK (SEQ ID NO: 21; CDRs are underlined)

EXAMPLE 8: ANTI-CD-98 MONOCLONAL ANTIBODY-MEDIATED INHIBITION OF TUMORS IN VIVO

Antibody efficacy on tumor growth and metastasis formation is studied, e.g., in mouse subcutaneous or orthotopic cancer xenograft models. The antibodies can be unconjugated, or can be conjugated to a therapeutic agent, as appreciated in the art.

Monoclonal antibodies are raised against CD-98 as described in Example 1, and purified and characterized as described above. Chimeric or humanized antibodies as described above may also be used. A therapeutic monoclonal antibody or a cocktail comprising a mixture of individual monoclonal antibodies is prepared and used for the treatment of mice receiving subcutaneous or orthotopic injections of tumor xenografts.

Subcutaneous tumors are generated by injection of 1 x 10^7 cancer cells in a mixture of PBS (without magnesium or calcium) and BD Matrigel (BD Biosciences) at a 1:1 ratio in the right flank of female SCID or *nu* $^{-/-}$ mice. The injected total volume per mouse is 200ml with 50% being Matrigel (BD Biosciences). Mice are randomized once tumors reach a size between 65-200mm^3. Antibodies are administered weekly, and body weights and tumors are measured once and twice weekly, respectively. Tumor volume is calculated as described (van der Horst et al. (2009) Neoplasia 11: 355-364). As a negative control, mice are injected with either purified mouse IgG or PBS; or a purified monoclonal antibody that recognizes an antigen other than CD98.

EXAMPLE 9: EFFECT OF CD-98 MONOCLONAL ANTIBODIES ON THE GROWTH OF B-CELL LYMPHOMA XENOGRAFTS IN MICE

[0343] The Ramos (B-cell lymphoma) cell line was obtained from ATCC and cultured according to the suppliers' protocols. Animals were obtained from Charles River Laboratories.

[0344] 4-6 week-old immunodeficient SCID female mice on a CB.17 background were subcutaneously injected on the right flank with 1x10^7 viable cells in a mixture of PBS (without magnesium or calcium) and BD Matrigel (BD Biosciences) at a 1:1 ratio. The injected total volume per mouse was 200µl with 50% being Matrigel (BD Biosciences). Once the tumor reached a size between 65-200mm^3 mice were randomized. Antibodies were administered weekly, and body weights and tumors were measured once and twice weekly, respectively. Tumor volume was calculated as described (van der Horst et al. (2009) Neoplasia 11: 355-364). All the experiments were performed on groups of at least 7 animals per experimental point. Animal experiments were performed in accordance with protocols approved by the Igenica Inc. Institutional Review Board - Animal Care and Use Committee.

[0345] Statistical significance between treatment and control groups was calculated using the Graphpad Prism software package and applying Student's two-tailed t-test. A p-value of less than 0.05 was considered significant. Doubling time and time to progression analysis was calculated as described in Daniel et al. (2007) Blood 110:4037-4046.

[0346] Rituximab (anti-CD20 antibody) was used as a positive therapeutic control antibody. Antibody HB121 was an IgG2a negative control. Rituximab and the anti-CD98 antibodies 8-93A and 18-3A are IgG1 antibodies, whereas all other anti-CD98 antibodies are IgG2a antibodies.

[0347] Treatment with anti-CD98 antibodies was shown to induce strong tumor growth inhibitiion in established B cell lymphoma (Ramos) tumors. Notably, anti-CD98 antibody treatment was superior to rituximab (see Fig. 5A, Fig. 5B) in inducing tumor growth inhibition.

[0348] Anti-CD98 antibodies were also shown to significantly prolong time to progression of treated RAMOS tumors. The tumor doubling time of the tumor regrowth data from Figs. 5A-C was calculated as described above and used for further prediction of time to progression (TTP). TTP was then extrapolated for each animal within the treatment groups, until 2000mm^3 would have been reached and graphed as a Kaplan-Meier curve, as shown in Figs. 6A-C. As shown in Figs. 6A-C, various anti-CD98 antibodies are superior to rituximab in prolonging time to progression in Ramos tumors.

[0349] The starting tumor volumes of established Ramos tumors were increased in order to assess the potential maximum therapeutic efficacy of anti-CD98 antibodies. Table 3 shows the therapeutic efficacy of anti-CD98 antibodies in Ramos xenograft tumor models with increasing tumor volume starting size. As can be seen, anti-CD98 antibodies retain tumor growth inhibition (TGI) even at increasing tumor volumes.

Table 3

Antibody	[V_0=75.6mm^3]		[V_0=144.3mm^3]		[V_0=249.6mm^3]	
	TGI [%]	p-Value	TGI [%]	p-Value	TGI [%]	p-Value
18-2A	-96.74	0.000127	-93.23	0.000009	-76.08%	0.000022
8-34B	-95.13	0.000121	-86.3	0.000004	-	-
8-101A	-	-	-86.73	0.000009	-65.26%	0.000046
1-115A	-	-	-85.8	0.000006	-51.29%	0.000665
8-213A	-	-	-85.2	0.000015	-	-
8-32A	-	-	-83.96	0.000022	-	-
8-300B	-	-	-	-	-63.52%	0.000105
18-4A	-	-	-	-	-62.14%	0.000072
8-361A6	-	-	-	-	-60.59%	0.000141
8-25C	-	-	-	-	-57.94%	0.000140
456-83A	-	-	-	-	-57.08%	0.000151
8-162A4	-	-	-	-	-55.89%	0.000236
1-47C	-	-	-	-	-54.23%	0.000874
456-26A	-	-	-	-	-53.07%	0.000277
8-319A2	-	-	-	-	-52.55%	0.000307
8-120A	-	-	-	-	-51.97%	0.000576

Rituximab	-87.65	0.005564	-	-	-	-
8-93A	-68.76	0.016095	-	-	-	-
18-3A	-48.42	0.066085	-	-	-	-

EXAMPLE 10: EFFECT OF ANTI-CD-98 MONOCLONAL ANTIBODIES ON THE INHIBITION OF TUMOR GROWTH IN VIVO

[0350] The effects of the anti-CD98 monoclonal antibodies 8-34B and 18-2A were tested in several xenograft models , using the protocol described in Example 7.

[0351] The DLD-1 (colorectal carcinoma), A549 (non small cell lung carcinoma), Ramos (B-cell lymphoma), and OCI-AML-3 (acute myeloid leukemia) cell lines were obtained from ATCC and cultured according to the suppliers' protocols. Animals were obtained from Charles River Laboratories. 4-6 week-old immunodeficient NOD.SCID female mice on a CB.17 background were used for the sarcoma tumor model, 4-6 week-old immunodeficient SCID female mice on a CB.17 background were used for the Ramos and DLD-1 tumor models, and 4-6 week-old immunodeficient *nu* $^{-/-}$ female mice were used for the A549 and the OCI-AML-3 tumor model. Either rituximab (an IgG1 anti-CD20 antibody) or Erbitux (an IgG1 anti-EGFR antibody) was used as a positive control antibody, and an IgG_{2a} antibody to an irrelevant antigen was used as a negative control. DC101 is a rat anti-mouse VEGFR2/KDR IgG_1 mAb (ATCC No. HB-11534) and serves as a positive control. Injections, antibody treatment and statistical calculations were performed as described in Example 9.

[0352] As shown in Figs. 6-9, the anti-CD98 monoclonal antibody 18-2A is a potent inhibitor of tumor growth in the colorectal cancer (DLD-1), non-small cell lung cancer (A549), Burkitts lymphoma (Ramos) andAML (OCI-AML-3) xenografts. The effect of 18-2A compared favorably to that of Rituxan (Fig. 7) and Erbitux (Fig. 9 and Fig. 10).

[0353] The anti-CD98 monoclonal antibody 8-34B inhibited tumor growth in the Ramos and AML (OCI-AML-3) xenografts, as shown in Fig. 7 and Fig. 8.

EXAMPLE 11: ANTI-CD98 MONOCLONAL ANTIBODIES IN MOUSE STRAINS WITH DIFFERENT IMMUNODEFICIENT BACKGROUNDS

[0354] Three anti-CD98 monoclonal antibodies, 8-300B, 8-34B and 18-2A, were tested in the RAMOS xenograft model as described in Example 9, using different mouse strains. An IgG_{2a} antibody was used as a negative control. The three immunodeficient mouse strains ranged from less to highly immunocompromised. SCID mice lack functional B and T cells, but retain natural killer (NK) cell function and some complement function. NOD.SCID mice lack complement function and have only partial NK function, while NSG (NOD/SCID/gamma) mice lack NK function.

[0355] As shown in Fig. 11, the tumor-growth inhibitory effect of anti-CD98 antibodies in the RAMOS xenograft model increases when assessed in more immuno-competent mouse strains, indicating that the in vivo anti-tumor activity of the anti-CD98 antibodies is due to a combination of immunoeffector function and inhibition of CD98 activity. The results in the NSG mice, which lack all

ADCC and CDC function, suggests that anti-CD98 antibodies may also inhibit the biological function of CD98 in the RAMOS xenograft, and that CD98 may be critical for RAMOS tumor growth and/or maintenance in vivo.

EXAMPLE 12: IN VIVO EFFICACY OF MURINE AND CHIMERIC ANTI-CD98 MONCLONAL ANTIBODIES

[0356] The in vivo efficacy of the murine anti-CD98 antibodies 8-34B and 18-2A were compared to that of the chimeric antibodies 8-34B-ch and 18-2A-ch7.1 in the RAMOS xenograft model, as described in Example 9. The antibodies were tested at 0.5 mg/kg dose (~30 nm). The results shown in Fig. 12 confirm that the chimeric anti-CD98 antibodies inhibit *in vivo* tumor growth at an effectiveness similar to their parental murine counterparts.

EXAMPLE 13: EPITOPE MAPPING OF HUMANIZED MONOCLONAL ANTIBODY IGN523

Materials and methods

[0357] *Reagents.* FREESTYLE™ CHO-S cells (Invitrogen) were maintained in FREESTYLE™ CHO expression medium (Invitrogen) supplemented with GlutaMAX™. Control antibodies 4F2 and MEM108 were obtained from Santa Cruz Biotechnology and Thermo Scientific, respectively. Antibodies were labeled with Alexa Fluor 647 using the appropriate Zenon Antibody Labeling Kit (Invitrogen).

[0358] *Plasmid constructions.* Full length CD98, CD98 point mutants and mouse and human CD98 chimeras were constructed by gene synthesis (GeneWiz) and cloned into the pCDNA3.1 vector (Invitrogen). CD98 ECD, CD98 ECD point mutants and CD98 ECD chimeras were constructed by gene synthesis and cloned into the pDisplay vector (Invitrogen). All constructs were confirmed by DNA sequencing.

[0359] *Fluorescence-activated cell sorter (FACS) analysis of IGN523 binding to CD98.* CD98 chimeras, CD98 point mutation constructs and human wild type control constructs were transfected by electroporation with a Nucleofector 4D unit (Lonza). Constructs were mixed with the transfection solution and then transiently transfected into FREESTYLE™ CHO-S cells. Transfected CHO-S cells were harvested 24 hours after transfection. Cells were quantitated and then stained with IGN523, MEM108 or 4F2 antibodies. Before staining, the antibodies were labeled with Alexa Fluor 647 using the appropriate Zenon Antibody Labeling Kit (Invitrogen). Flow data were acquired on a Miltenyi MACSQuant Analyzer (Miltenyi Biotec) and data analysis was performed using FlowJo software version 9.5.3 (Tree Star, Inc.).

Determining the IGN523 binding region on CD98

[0360] To determine the region of CD98 that is involved in IGN523 binding, regions of approximately 40 contiguous amino acids of human CD98 (SEQ ID NO: 1) were substituted with the corresponding regions of mouse CD98 (SEQ ID NO: 96), and the effects of these substitutions on the overall binding of IGN523were monitored using FACS analysis. Fig. 13 shows the regions of the mouse

sequence that were substituted into the human sequence to form the 13 mouse-human CD98 chimera constructs. Fig. 14 shows the binding of IGN523 and a control antibody to murine CD98 (Mu), human CD98 (Hu) and the 13 mouse-human chimeras. The results demonstrate that the region substituted in chimera construct 10 is required for IGN523 binding to CD98. In contrast, it can be seen that the region responsible for binding of the control antibody is found in the regions substituted in chimeras 11 and 12.

[0361] Fig. 15 shows the sequence of the region of human CD98 within which IGN523 binds, as identified using the mouse-human CD98 chimera constructs, and the location of this sequence within the three-dimensional structure of CD98. The region defined by chimera 10 consists of amino acid residues T358-N405 of human CD98. Amino acids T358-G368 (underlined) are buried in the crystal structure and are unlikely to be part of the binding interface. Non-conserved residues between the human and mouse sequences are shown in bold. The substitution of N at site D391 results in an extra glycosylation site in the mouse sequence as compared to human.

[0362] Fine mapping of IGN523 with single and multiple mutations in CD98

[0363] To further define the IGN523 epitope, single or multiple amino acid changes were introduced in the region defined by mouse-human chimera 10. Four constructs were made, each introducing the nonhomologous residues from a portion of the mouse CD98 sequence into the human CD98 sequence. Construct 4.1 contained mutations I371L, D374Q, A375G and a deletion of A376. Construct 4.2 contained mutations M383A and E384K. Construct 4.3 contained mutations D391N, F395I, P396F and D397H. Construct 4.4 contained mutations G400R, A401P and A404L. FACS analysis of CHO cells transfected with the respective constructs showed that the mutations contained in construct 4.1 prevented IGN523 from binding to CD98 (Fig. 16). The mutations contained in construct 4.4 also affected binding of IGN523 to CD98, to a lesser degree (Fig. 16).

[0364] To determine which hydrophobic residues are involved in the binding interface of IGN523 to human CD98, single mutation constructs of hydrophobic residues in the targeted loop region were created by substituting these hydrophobic residues with highly charged amino acids such as aspartic acid or asparagine. The single mutant constructs were individually transfected into CHO cells and analyzed by FACS to determine binding to IGN523. As shown in Fig. 17, mutation of hydrophobic residues A377 and L378 to a charged amino acid negatively affected IGN523 binding. To a lesser extent, mutation of residues I398 and A401 showed a similar negative effect on binding of IGN523.

[0365] Further constructs containing multiple mutations were made to identify additional important residues for IGN523 binding to CD98. As shown in Fig. 18, the multiple mutations in construct M1 (D374Q, D397H, G400R and A401P) completely prevented IGN523 from binding, indicating the significance of these residues. Construct M2 (D374E and A375E) and M3 (D397S and I398T) also resulted in reduced binding. Additional experiments with constructs comprising a deletion of A376 (not shown) indicated that the presence of this residue appears to be required for correct folding of the epitope loop region.

[0366] Based upon the binding studies using the mouse-human chimeras, mutations of hydrophobic residues, and multiple mutations, the following amino acids were determined to be part of the IGN523 epitope: D374, A377, L378, D397, I398, G400 and A401.

Peptide scanning analysis

[0367] As a complement to these mutational studies, the binding epitope of IGN523 was further analyzed using a peptide scanning analysis (PepScan).

[0368] *Synthesis of peptides*: To reconstruct discontinuous epitopes of the target molecule, a library of structured peptides was synthesized using Pepscan's proprietary Chemically Linked Peptides on Scaffolds (CLIPS) technology (Pepscan). Chemical linkage of peptides onto scaffolds was carried out essentially as follows: The side-chains of multiple cysteines in the peptides were coupled to one or two CLIPS templates. A 0.5 mM solution of the T2 CLIPS template 1,3-bis (bromomethyl) benzene was dissolved in ammonium bicarbonate (20 mM, pH 7.9)/acetonitrile (1:1(v/v). This solution was added onto the peptide arrays, causing the CLIPS template to bind to side-chains of two cysteines as present in the solid-phase bound peptides of the peptide-arrays (455 wells plate with 3 µl wells). The peptide arrays were gently shaken in the solution for 30 to 60 minutes while completely covered in solution. Finally, the peptide arrayswere washed extensively with excess of H_2O and sonicated in disrupt-buffer containing 1 percent SDS/0.1 percent beta-mercaptoethanol in PBS (pH 7.2) at 70°C for 30 minutes, followed by sonication in H_2O for another 45 minutes. See also the methods described in Timmerman *et al.* (2007), *J. Mol. Recognit.* 20:283-99; and Slootstra *et al.* (1996), *Molecular Diversity* 1: 87-96.

[0369] *ELISA screening*: The binding of antibody to each of the synthesized peptides was tested in a PEPSCAN-based ELISA. The peptide arrays were incubated with primary antibody solution (overnight at 4°C). After washing, the peptide arrays were incubated with a 1/1000 dilution of an antibody peroxidase conjugate (SBA, catalog no. 2010-05) for one hour at 25°C. After washing, the peroxidase substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) and 2 µl/ml of 3 percent H_2O_2 were added. After one hour, the color development was measured. The color development was quantified with a charge coupled device (CCD)-camera and an image processing system.

[0370] *Data processing*: The values obtained from the CCD camera range from 0 to 3000 mAU, similar to a standard 96-well plate ELISA-reader. The results were quantified and stored into the Peplab database. Occasionally a well contains an air-bubble resulting in a false-positive value. The cards were manually inspected and any values caused by an air-bubble are scored as 0.

[0371] *Synthesis quality control*: To verify the quality of the synthesized peptides, a separate set of positive and negative control peptides was synthesized in parallel. These were screened with antibody 57.9 (Posthumus *et al.*, J. Virology, 1990, 64:3304-3309).

[0372] The results of a variable-length peptide screen are shown in Fig. 19. ELISA results for each peptide are shown as a horizontal line. Start and end points of the lines indicate which residues are included in the peptide, and the Y-value of the line shows the ELISA result obtained for that peptide. The ELISA results for the peptides show dominant binding for ^{395}FPDIPGA^{401} (SEQ ID NO: 42) and secondary binding for ^{379}PGQP^{382} (SEQ ID NO: 43). A global analysis of 29 single-positions alanine

replacement sets showed strongest binding for ^{394}SFDIPGAVASANMTV^{407} (SEQ ID NO: 44). Fig. 20 shows the results of a best-binding single-positions alanine-replacement peptide set, in which each residue of peptide SEQ ID NO:44 was replaced by alanine, or by glycine if the original residue was alanine. The analysis shows strongest dependency for residues F395, P396, D397 and I398, which appear to form the core of this epitope.

[0373] Next, CLIPS conformational matrix structures were used to to bring together pairs of peptides from the two regions identified in the peptide screen, so as to assay antibody binding to conformational epitopes. Fig. 21 shows heat maps representing the data obtained from CLIPS conformational matrix structures that combined two partial sequences of human CD98 (shown on X axis and Y axis). The results indicated a high dependency on secondary structure. The best binding was observed for peptides that combined ^{395}FPDIPGAVSAN^{405} (SEQ ID NO: 70) and ^{372}GLDAAALPGQP^{382} (SEQ ID NO: 50). These two peptides were used as the basis for a mutagenesis screen as shown in Fig. 22. SEQ1 shows the sequence of the peptide and DIF1 indicates where the mutation is located in the peptide. Grey fields indicate peptides having non-mutated sequences. The last column shows the difference in ELISA value between wild-type and mutated peptide. High values indicate that the mutation has a strong negative effect on binding. The mutagenesis screen identified P379, G380, D397 and I398 as important binding residues.

[0374] Fig. 23 shows the location on the surface of human CD98 of the amino acid residues determined to be important for binding of humanized monoclonal antibody IGN523. Fig. 23A shows the location of the residues identified by the chimera and mutagenesis studies, while Fig. 23B shows the location of residues determined by Pepscan analysis. Fig. 23C shows that both sets of residues substantially overlap, confirming that the highlighted loop region is the binding epitope for IGN523.

EXAMPLE 14: *IN VIVO* ANTI-TUMOR ACTIVITY OF HUMANIZED ANTI-CD98 MONOCLONAL ANTIBODY IGN523

[0375] The effects of the anti-CD98 humanized monoclonal antibody IGN523 were tested in several xenograft models , using the protocol described in Example 8. Injections, antibody treatment and statistical calculations were performed as described in Example 9. IGN523 was compared to the standard of care drug rituximab in the RAMOS (RA.1) and DAU Burkitt lymphoma models (Fig. 24 and Fig. 25). IGN523 was then compared to carboplatin, at its maximum tolerated dose, in the patient-derived NSCLC xenograft model IGN-LNG-12 (Fig. 26). IGN523 was also tested in the AML xenograft model KG-1 (Figure 27). The tumors used were minimally passaged in NOD/SCID mice, without any intervening cell culture, in order to preserve the heterogeneity of the original tumors.

[0376] In all cases IGN523 showed significant tumor growth inhibition. Interestingly, IGN-LNG-12 patient-derived tumors lead to weight-loss in NOD-SCID mice, which correlated with tumor burden (Fig. 26A and 26B). Although carboplatin at its maximal tolerated dose induced a significant tumor growth inhibition, it also displayed an increase in body weight loss. On the other hand, IGN523 treatment exerted a similar anti-tumor effect as carboplatin, but reduced IGN-LNG-12 induced body

weight loss. These data demonstrate that IGN523 treatment is as effective as carboplatin in this NOD-SCID model without inducing body weight loss.

[0377] The data demonstrate significant tumor growth inhibition in Burkitt lymphoma models RAMOS (RA.1) and DAU, in the patient-derived lung tumor xenograft IGN-LNG-12, and in the AML model KG-1. Moreover, the tumor growth inhibition of IGN523 is comparable to the anti-cancer agents carboplatin or rituximab, respectively (Table 4).

Table 1

	TGI [%]			
Treatment	**RAMOS (RA.1)**	**DAU**	**IGN-LNG-12**	**KG-1**
IGN523	92	76	44	53
Rituximab	84	67	N/A	N/A
Carboplatin	N/A	N/A	55	N/A

[0378] The dose response relationship of IGN523 in IGN-LNG-12 tumors was also investigated. IGN-LNG-12 was chosen to determine the dose response to the antibody using a "therapeutic dosing regimen". IGN523 was dosed on days 12 and 19 between 1 mg/kg and 30 mg/kg (Figure 15). A dose of 10 and 30 mg/kg produced the maximum tumor growth reduction of 50–66% relative to the control group in IGN-LNG-12 lung tumors.

[0379] Taken together, *in vivo* efficacy data demonstrate that IGN523 induces significant tumor growth inhibition in various xenograft models, which is at least comparable to that of standard clinical agents.

EXAMPLE 15: RECEPTOR BINDING SPECIFICITY FOR HUMANIZED ANTI-CD98 MONOCLONAL ANTIBODY IGN523

[0380] Table 5 shows the percentage of sequence homology of the extracellular domain (ECD) of CD98 between the indicated species and of the epitope of CD98 to which IGN523 binds. As shown in Table 5, the homology between the human and cynomolgus monkey epitope of CD98 to which IGN523 binds is 96%. Receptor binding specificity studies utilizing various methodologies, such as surface plasmon resonance (SPR, Biacore), bio-layer interferometry (Octet) or ELISA, determined that IGN523 binds with high affinity to human and cynomolgus monkey CD98, but does not bind to other species including the murine, rat, rabbit, dog, and pig homologs of CD98, due to decreasing homology (Table 5). Biacore and Octet data demonstrate that the K_D of IGN523 ranges between 2 and 6 nM for human CD98, and between 8 and 14 nM for cynomolgus monkey CD98. ELISA binding data show that the EC_{50} for IGN523 is 9 ng for human and 39 ng for cynomolgus monkey CD98.

Table 5

Species	% Identity to Human CD98 ECD	% Identity to IGN523 Epitope Region of Human CD98	Biacore K_D (nM)	ELISA EC_{50} (ng)	Octet K_D (nM)
Human	100	100	2 - 6	9	2
Cynomolgus monkey	96	95	13 - 14	39	8
Rabbit	80	74	negative	negative	ND
Dog	78	74	negative	negative	ND
Rat	73	66	negative	negative	ND
Mouse	70	60	negative	negative	ND
Pig	79	63	negative	negative	ND

ND = Not Determined.

[0381] In order to corroborate that cynomolgus monkey is the relevant species for toxicology studies, human and cynomolgus frozen tissue sections of kidney, placenta and cerebrum were stained with IGN523 (Fig. 29). In cryosections of human kidney and placenta (positive control), moderate to intense membrane and cytoplasmic staining of renal tubular epithelial (kidney) and trophoblastic epithelium cells (placenta) was observed, which was similar, if not identical, to the staining intensity in counterpart cynomolgus monkey tissue sections. In the case of human and cynomolgus monkey cerebrum, similar weak-to-moderate cytoplasmic staining of neuropil and cytoplasmic granule epithelium was observed. Taken together, it appears that staining in cynomolgus monkey tissue is comparable to that in human tissues. Based on the affinity and staining data the cynomolgus monkey is considered to be the appropriate species to evaluate the safety of IGN523.

[0382] **EXAMPLE 16: SINGLE-DOSE PHARMACOKINETIC STUDY IN CYNOMOLGUS MONKEYS**

[0383] An exploratory non-GLP single dose intravenous pharmacokinetic study of IGN523 was performed in male and female cynomolgus monkeys at doses of 1, 3, 10, and 100 mg/kg (N = 2 per sex/group). Pronounced dose-dependent kinetics of IGN523 was observed following a single 1-h IV infusion of 1, 3, 10 or 100 mg of IGN523 per kg of body weight in the monkey. Therefore, the basic assumption regarding linearity that is implicit in the application of the non-compartmental analysis methods used does not apply to IGN523 over the entire dose-range studied, and the parameters are displayed for making descriptive comparisons among the dose groups. Although only 2 animals per sex were evaluated at each dose level, there were no apparent differences in PK profiles of male versus female animals. The mean concentration-time profiles of male and female animals were similar within each dose group at doses ranging from 1 to 100 mg of IGN523 per kg of body weight (Table 6).

Table 6: PK Parameters of IGN523

Dose (mg/kg)	$T_{1/2}\lambda$ (hours)		CL, (mL/h/kg)		V_{ss}, (mL/kg)	
	Mean	Range	Mean	Range	Mean	Range
1	4.7	3.7-5.9	4.30	3.54-4.82	32.1	27.4-39.8
3	12.4	10.2-13.5	1.7	1.58-1.88	31.5	28.2-35.1
10	12.0	6.4-15.8	1.03	1.00-1.10	29.3	25.7-31.7
100	60.9	44.9-96.9	0.44	0.34-0.49	41.1	37.8-44

$T_{1/2}\lambda$; terminal half-life, CL; clearance, V_{SS}; Volume of distribution

[0384] Taken together, there was a 10-fold reduction in the "apparent" plasma CL of IGN523 over the dose range of 1 to 100 mg/kg. Mean "apparent" MRT and mean "apparent" $T_{1/2}$ increased 12 to 13-fold over the dose range of 1 to 100 mg/kg. There were no differences in mean "apparent" V_{ss} at doses of 1, 3 or 10 mg/kg, but mean "apparent" V_{ss} in the 100 mg/kg dose cohort was approximately 30 to 40% higher compared to the 1, 3 and 10 mg/kg cohorts. All of these findings suggest that the nonlinear disposition characteristics of IGN523 in the monkey may be due to target-mediated drug disposition (TMDD). TMDD models have previously been used to describe the nonlinear disposition of other monoclonal antibodies. TMDD arises when the antibody has specificity for densely populated cell-surface targets that are abundantly expressed so that the target-antibody interactions represent a quantitatively important clearance pathway at low doses (Mager 2001, Mager 2003, Luu 2012).

EXAMPLE 17: REPEAT DOSE GLP STUDY IN CYNOMOLGUS MONKEY

[0385] The toxicology, PK and immunogenicity of IGN523 are studied in a GLP multi-dose IV administration toxicology study in cynomolgus monkeys. This study provides comprehensive data on clinical endpoints, toxicokinetics, immunogenicity (development of anti-IGN523 antibodies), and histopathology involving a broad list of tissues (including the injection site) and is conducted with formulated material representative of that which will be administered in the clinical trial. In addition, selected safety pharmacology endpoints (neurobehavioral, electrocardiography, respiratory behavior) are also evaluated.

[0386] The dosing regimen is a once per week intravenous 60-min infusion for 8 weeks. The doses employed are 10, 30, and 100 mg/kg of IGN523 once weekly (total of 9 doses) followed by a 4-week treatment-free recovery period (Table 5). The 4-week recovery period is considered sufficient to allow for complete clearance of IGN523 and to assess the reversibility of any potential toxicity. The highest dose is anticipated to approximate a MTD and provide for a significant exposure multiple beyond that anticipated in patients. Immunogenicity (anti-IGN523 antibodies) and toxicokinetics are monitored.

Table 7: Treatment Groups for Proposed Cynomolgus GLP Toxicology Study

Group	Treatment in mg/kg	No. of Males	No. of Females	Treatment Schedule
1	0	5	5	Once/Week for 9 doses
2	10	5	5	Once/Week for 9 doses
3	30	5	5	Once/Week for 9 doses
4	100	5	5	Once/Week for 9 doses

[0387] Table 8 contains a detailed summary of the study design for the multi-dose cynomolgus GLP toxicology study. Selected safety pharmacology endpoints (neurobehavioral, electrocardiography, respiratory behavior) will be evaluated in the context of the GLP repeat dose study in cynomolgus monkeys.

Table 8: Tabular Overview of Cynomolgus GLP Toxicology Study

Eight Week Intermittent (9 Weekly Doses) Intravenous Toxicity and Toxicokinetic Study with IGN523 in Cynomolgus Monkeys with a 4-Week Recovery Period	
No. of Animals:	40 (20M, 20F): 3 animals per sex per Main Group (0, 10, 30, 100 mg/kg) plus 2M and 2F per Recovery Group (all dose groups)
Dose:	0, 10, 30, 100 mg/kg, once weekly
Weight:	2–4 kg, 2 to 4 years of age
Route:	Intravenous 60 min infusion
Recovery:	4 weeks (2M and 2F Control; 2M and 2F 10, 30, 100 mg/kg)
Toxicokinetic (TK) Sampling:	Days 0, 56: pre-dose, 0.083, 1, 3, 6, 12, 24, 48, 72, 96, 120 hr post dose. Days 7, 14, 21, 28, 35, 42, and 49: pre and 0.083 hr post dose. Days 56: pre-dose, 0.083, 1, 3, 6, 12, 24, 48 hr post dose.
Additional (TK) Recovery Samples:	Relative to dosing on Day 56, at approximately 0.083, 1, 3, 6, 12, 24, and 48 hr following dosing. During the recovery period, 3, 4, 5, 7, 14, and 28 days following the final dose.
Immunogenicity: (Anti-drug antibody)	Days 0, 14, 28, 42, 56: pre-dose Days 70, 84 (recovery animals)
Complement Activation Evaluation	Day 56: Approximately 0.25 hr following dosing.
Clinical Pathology:	Samples collected from all animals during pretest and on days 2, 58 and 84 are evaluated for hematology, coagulation (including fibrinogen), serum chemistry (including C-reactive protein) and urinalysis.
Morbidity/Mortality:	Twice daily
Peripheral Blood Phenotyping:	Blood collected and analyzed from all animals once during pretest and on Days 7, 14, 58 and once near the end of the recovery period
Detailed Observations:	Once during pre-dose phase, Pre-dose on Day 1, weekly during the dosing and recovery phase, and on day of scheduled sacrifice including neurobehaviour (e.g tremor, convulsions, hyper-, hypoactivity)
Cageside Observations:	1-2 hours post each dose (time recorded) and once daily during non-dose days and recovery period including neurobehaviour (e.g tremor, convulsions, hyper-, hypoactivity)
Qualitative Food Consumption:	Once daily during dosing phase and recovery phase
Physical Examination:	Once prior to treatment, on the days of dosing, once weekly during the remainder of the study period
Ophthalmic Examination:	Once prior to treatment, during the last week of the treatment period and during the last week of recovery phase
ECGs, Respiratory Rate and Blood pressure measurements:	Once prior to treatment, 2-3 hr post-end infusion on day 0 and day 56, near the end of the recovery period

EXAMPLE 18: HEMOLYTIC POTENTIAL EVALUATION OF IGN523

[0388] The hemolytic potential of IGN523 will be assessed *in vitro* using cynomolgus whole blood. The results of this test will be used to identify any potential effects on hemoglobin. The study

will be performed using non-human primate whole blood on the day of whole blood collection. Table 8 contains a summary of the GLP study design for the hemolytic potential evaluation of IGN523.

Table 2: Outline of Proposed Hemolytic Potential Evaluation GLP Study

Group	Treatment	Formulation	Number of Samples	Blood Volume	Treatment Volume
1	Test Article	1 x Projected Concentration	3	0.5 mL	0.5 mL
2	Test Article	2 x Projected Concentration	3	0.5 mL	0.5 mL
3	Test Article	4 x Projected Concentration	3	0.5 mL	0.5 mL
4	Negative Control	Saline	3	0.5 mL	0.5 mL
5	Positive Control	Distilled Water	3	0.5 mL	0.5 mL

EXAMPLE 19:ACUTE INTRAVENOUS AND PERIVASCULAR IRRITATION

[0389] The irritation and local tissue tolerance study is designed to assess the short-term toxicities of compounds in the immediate area of injection at high concentrations. As part of this study the compound is administered intravenously and perivascularly to identify effects on tissues expected to encounter the initial exposure. The rabbit is the standard animal model for this evaluation.

EXAMPLE 20:TISSUE CROSS-REACTIVITY STUDY OF IGN523

[0390] The tissue cross-reactivity profile of IGN523 is characterized using a full range of human tissues as detailed in the FDA guidance, *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (February, 1997)*. A comprehensive list of tissues to be studied is included in Table 9.

Table 3: Outline of Proposed GLP Tissue Cross-Reactivity Study

1. Adrenal	17. Lymph Node
2. Bladder	18. Ovary
3. Blood Cells	19. Pancreas
4. Bone Marrow	20. Parathyroid
5. Breast	21. Pituitary
6. Cerebellum	22. Placenta (if available)
7. Cerebral Cortex	23. Prostate
8. Colon	24. Skin
9. Endothelium	25. Spinal Cord
10. Eye	26. Spleen
11. Fallopian Tube	27. Striated Muscle
12. Gastrointestinal Tract	28. Testis
13. Heart	29. Thymus
14. Kidney (glomerulus, tubule)	30. Thyroid
15. Liver	31. Ureter
16. Lung	32. Uterus (cervix, endometrium)

EXAMPLE 21: PHASE I CLINICAL STUDY TO EVALUATE THE SAFETY AND PHARMACOKINETICS OF IGN523 IN PATIENTS WITH RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA

[0391] Primary objectives

The primary objectives of this study are

- To evaluate the safety and tolerability of IGN523 administered on a schedule starting with weekly dosing to patients with relapsed or refractory acute myeloid leukemia (AML)
- To determine the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of IGN523 when administered weekly x 4
- To identify a recommended Phase 2 dose (RP2D) of IGN523 on the basis of safety, pharmacokinetic, and pharmacodynamics data

[0392] Secondary objectives

- To assess the incidence of antibody formation to IGN523
- To characterize the pharmacokinetics of IGN523 in patients with relapsed or refractory AML
- To make a preliminary assessment of the anti-leukemic activity of IGN523 in patients with relapsed or refractory AML
- To make a preliminary assessment of biologic markers that might predict IGN523 anti-leukemic activity

[0393] Methodology

Open-label, dose-escalation study of approximately 6 dose cohorts using standard 3+3 design, plus expansion cohort at MTD or RP2D

[0394] Number of Subjects

Dose Escalation: approximately 21-30

Dose Expansion: 20

[0395] Investigational drug

[0396] IGN523 drug product will be supplied as 20 mL of a 10 mg/mL solution in a 25-mL single-use glass vial. The appropriate volume of IGN523 drug product will be diluted to 250 mL and infused intravenously (IV) over 1 hour. In the Phase I dose-escalation study (see Protocol Synopsis in Section 10.2.1), cohorts of patients may be treated at escalating doses up to 30 mg/kg weekly for 8 doses. Continued treatment beyond 8 weeks will be offered to patients with ongoing clinical benefit (i.e., lack of disease progression and no unacceptable toxicity). Intra-patient dose escalation may be permitted under specific conditions (described in Section 10.2.1) in order to maximize the accumulation of data at relevant doses and to minimize treatment of patients at potentially sub-therapeutic doses.

[0397] Diagnosis and Main Criteria for Inclusion

Relapsed or Treatment Refractory AML for which no effective standard therapy exists.

- Must have measurable disease
- Age $\geq$18 years,
- Eastern Cooperative Oncology Group (ECOG) performance status 0-2
- Life expectancy of at least 12 weeks
- Platelet count $\geq$ 25,000/mm^3 (may be maintained by transfusion)
- AST(SGOT)/ALT(SGPT) $\leq$ 2.5x institutional upper limit of normal (ULN)
- Total bilirubin $\leq$ 1.5x institutional ULN
- Creatinine $\leq$ 2x institutional ULN or calculated or measured creatinine clearance $\geq$ 50 mL/min
- For women of childbearing potential and men, agreement to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation.
- Ability to understand and the willingness to sign a written informed consent document

[0398] Exclusion Criteria

- Use of monoclonal antibody therapy within 4 weeks, or chemotherapy or radiotherapy within 2 weeks, before Cycle 1 Day 1 (hydroxyurea given to control peripheral blast counts up to 72 hours before Cycle 1, Day 1 is allowed)
- Unresolved acute toxicity of NCI CTCAE v4.0 Grade >1 from prior anti-cancer therapy
- Prior allogeneic stem cell transplant currently requiring immunosuppressive therapy
- History of severe allergic or anaphylactic reactions to monoclonal antibody therapy
- Known leptomeningeal or CNS involvement of leukemia
- Uncontrolled intercurrent illness, including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements
- Recent major surgery within 4 weeks prior to Cycle 1, Day 1
- Pregnant or lactating women

[0399] Diagnostic Plan

[0400] Patient AML samples from peripheral blood (or bone marrow) will be analyzed retrospectively after study enrollment for expression of CD98 (and not analyzed as part of patient screening and used as a condition for study eligibility), based upon the following rationale:

- CD98 is differentially overexpressed in the $CD34^+/CD33^+$ and $CD34^+/CD33^-$ subpopulations of the majority (~94%) of AML patients compared to similarly "gated" cells from normal bone marrow samples.
- There is no clinically validated method of detecting and quantitating CD98 expression on patient samples that would be adequate to select patients for participation (or exclude patients from participating) in the proposed Phase I study.
- Qualification of assays and analysis of Phase I results will be used to inform the design of future clinical studies that could require patient selection.

[0401] Test Product, Mode of Administration, Starting Dose

IGN523

Intravenous Infusion

Starting Dose: No greater than 1/6 the human-equivalent dose (HED) of the No Observed Adverse Effect Level (NOAEL) observed in the GLP multi-dose cynomolgus toxicity study

[0402] Cohort Initiation and Duration of Treatment

[0403] The first patient in each new dose cohort will be dosed at least 1 day prior to any other patients in that cohort, to allow for observation of possible severe and/or serious acute (e.g. infusion-related) toxicities that might affect subsequent patient enrollment or dosing decisions.

[0404] Patients will receive weekly intravenous doses of IGN523 for 8 weeks (two 28-day cycles). Dosing beyond 8 weeks will be permitted for patients meeting criteria for ongoing clinical benefit (i.e. lack of disease progression) and acceptable safety for up to 1 year.

[0405] Intra-Patient Dose Escalation

[0406] To maximize the collection of information at relevant doses and to minimize the exposure of patients to potentially sub-optimal doses, intra-patient dose escalation may be permitted under the following conditions:

- Patients must complete at least two 28-day cycles at their originally assigned dose level prior to any dose escalation
- Patients may only escalate their dose to the highest dose level cleared by a completed 3-6 patient dose cohort through at least one 28-day cycle of IGN523 administration
- All intra-patient dose escalation decisions will be based on the investigator's judgment of whether it is felt to be in the best interest of the patient, in coordination with the Medical Monitor

[0407] Definition of DLT

[0408] A DLT will be any of the following adverse events considered by the investigator to be related to IGN523 (and not attributable to another clearly identifiable cause) occurring during Days 1-28 of Cycle 1:

- Grade 3 or 4 non-hematologic toxicity, except for
  - Reversible Grade 3 non-allergic infusion toxicities (including symptoms such as fever, chills/rigors, nausea, vomiting, pruritis, headache, rhinitis, rash, asthenia, and/or hypoxia (in the absence of signs/symptoms of respiratory distress) occurring during or within 24 hours after completing an infusion and resolving within 24 hours with a reduced infusion rate, supportive care, and/or administration or corticosteroids
  - Grade 3 or 4 hyperuricemia, hyperphosphatemia, or hypocalcemia, or Grade 3 hyperkalemia, if transient (i.e. lasting <48 hours) and without manifestations of clinical tumor lysis syndrome (i.e. creatinine $\geq$ 1.5 x ULN, cardiac arrhythmias, sudden death, or seizures)
- Grade 3 or 4 thrombocytopenia (in a patient without pre-existing thrombocytopenia requiring transfusion support) that either results in bleeding, or does not improve to $\geq$ 80% of baseline value within 2 weeks without platelet transfusion
- Grade 3 or 4 neutropenia (in a patient without pre-existing neutropenia requiring growth factor support) that either is associated with a fever (oral or tympanic temperature of 100.4°F/38°C) or does not improve to $\geq$ 80% of baseline value within 2 weeks without growth factor support

[0409] DLT Window

Days 1-28 of Cycle 1

[0410] Dose Escalation Scheme

[0411] Dose escalation may only take place after each individual in a given cohort has reached Day 28. Patients who experience disease progression and withdraw from the study prior to Day 28 without DLT will not be evaluable for DLT and will be replaced. Dose escalation will proceed between cohorts at up to 100% increments (or less if significant AEs are observed) according to the following scheme:

- If 0/3 patients have DLT at a given dose level, 3 patients may be enrolled at the next dose level
- If ≥ 2/3 patients experience a DLT at a given dose level, dose escalation will be stopped, and this dose will be declared to exceed the MTD.
- If 1/3 patients experience DLT at a given dose level, at least 3 more patients will be enrolled at the same dose level. If 0 of these 3 patients experience DLT, proceed to the next dose level (which may be at a <100% dose increment). If 1 or more of this group experience DLT, then dose escalation is stopped, and this dose will be declared to exceed the MTD.
- Once MTD has been exceeded, if the preceding dose escalation increment was ≤ 30%, then a minimum of 6 evaluable patients may be enrolled at the previous dose level to evaluate it as an MTD. If the preceding dose escalation increment was > 30%, then at least one dose level intermediate between the two highest dose levels may be evaluated.
- The highest dose level resulting in DLTs in less than one-third of a minimum of 6 patients will be declared the MTD.

[0412] Dose Escalation Committee

[0413] Agreement to proceed with dose escalation, modify dose escalation scheme, or stop the study will be made by an internal Dose Escalation Committee made up of the Medical Monitor, Drug Safety representative, and additional ad hoc study team members, in consultation with the study investigators at the conclusion of each study cohort. This committee will review all available study data from the current cohort and all available safety data in the previous cohorts before deciding on dose escalation for the subsequent cohort. All study subjects will receive study therapy until disease progression, the development of unacceptable toxicity, noncompliance, or withdrawal of consent by the subject, or by investigator decision.

[0414] Criteria for Evaluation Efficacy

Peripheral blood counts

Bone marrow aspirate and biopsy

Safety

Safety outcome measures are as follows:

Incidence and nature of DLTs,

Incidence and severity of adverse events

[0415] PK Sampling

PK assessment will be done on the following schedule:

Day 1 dose: pre-dose, and 30 minutes, 4 hours, 24 hours, and 48 (or 72 hours) post-dose

Days 8, 15, and 21 doses: pre-dose, and 30 minutes, 4 hours, and 48 (or 72 hours) post-dose

Subsequent doses: pre-dose and 30 minutes post-dose

[0416] Expansion Cohort

[0417] In order to obtain additional safety, tolerability, and pharmacokinetic data, and preliminary evidence of clinical activity, up to an additional 20 patients with relapsed or refractory AML will be enrolled into an expansion cohort at the MTD or RP2D. While the MTD will be determined

primarily by the safety (DLT) data observed during the DLT observation periods of Phase I, the RP2D will also take into account additional safety data beyond the DLT windows, and may also include information gathered during Phase I dose escalation, including PK and target occupancy data.

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WHAT IS CLAIMED IS:

1. An isolated antibody or a functional fragment thereof that specifically binds to human CD98, wherein the antibody or functional fragment binds to an epitope comprising residues A377, D397, I398, G400 and A401 of human CD98.

2. The isolated antibody or functional fragment of claim 1, wherein said epitope further comprises residues D374 and L378 of human CD98.

3. The isolated antibody or functional fragment of claim 2, wherein said epitope further comprises residues P379 and G380 of human CD98.

4. The isolated antibody or functional fragment of claim 3, wherein said epitope further comprises residues F395 and P396 of human CD98.

5. The isolated antibody or functional fragment of claim 3, wherein said epitope further comprises residues Q381, P382 and P399 of human CD98.

6. The isolated antibody or functional fragment of claim 1, wherein said epitope further comprises any one or more additional residues selected from the group consisting of D374, L378, P379, G380, Q381, P382, F395, P396 and P399 of human CD98.

7. An isolated antibody or a functional fragment thereof that specifically binds to human CD98, wherein the antibody binds to an epitope comprising residues P379, G380, D397 and I398 of human CD98.

8. The isolated antibody or functional fragment of claim 7, wherein said epitope further comprises residues F395 and P396 of human CD98.

9. The isolated antibody or functional fragment of claim 8, wherein said epitope further comprises residues Q381, P382, P399, G400 and A401 of human CD98.

10. The isolated antibody or functional fragment of claim 8, wherein said epitope further comprises residues D374, A377 and L378 of human CD98.

11. The isolated antibody or functional fragment of claim 7, wherein said epitope further comprises any one or more additional residues selected from the group consisting of D374, A377, L378, Q381, P382, F395, P396, P399, G400 and A401 of human CD98.

12. The isolated antibody or a functional fragment according to claim 1, wherein the antibody or functional fragment binds to an epitope comprising residues D374, A377, L378, P379, G380, Q381, P382, F395, P396, D397, I398, P399, G400 and A401 of human CD98.

13. An isolated antibody or a functional fragment thereof that specifically binds to human CD98, wherein the antibody or functional fragment binds to an epitope comprised within amino acid residues 369-405 of human CD98.

14. An isolated antibody or a functional fragment thereof that specifically binds to human CD98, wherein the antibody or functional fragment binds to an epitope consisting of amino acid residues 369-405 of human CD98.

15. The antibody or functional fragment of claim 1, wherein the antibody is a monoclonal antibody.

16. The antibody or functional fragment of claim 15, wherein the monoclonal antibody is a humanized, human or chimeric antibody.

17. The antibody or functional fragment of claim 1, wherein the fragment is an Fab, F(ab')2, Fv or Sfv fragment.

18. An isolated antibody or a functional fragment thereof comprising all three heavy chain complementarity determining regions (CDRs) from a heavy chain variable domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8 and SEQ ID NO: 12, and/or all three light chain CDRs from a light chain variable domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 10 and SEQ ID NO: 14.

19. An isolated antibody or a functional fragment thereof comprising all three heavy chain CDRs from a heavy chain variable domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8 and SEQ ID NO: 12, and all three light chain CDRs from a light chain variable domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 10 and SEQ ID NO: 14.

20. The antibody of claim 19, wherein the antibody comprises a heavy chain variable domain sequence selected from the group consisting of SEQ ID NO: 8 and SEQ ID NO: 12.

21. The antibody of claim 19, wherein the antibody comprises a light chain variable domain sequence consisting of SEQ ID NO: 10 and SEQ ID NO: 14.

22. Then antibody of claim 20, wherein the antibody further comprises a light chain variable domain sequence consisting of SEQ ID NO: 10 and SEQ ID NO: 14.

23. The antibody of claim 22, wherein the antibody comprises the heavy chain variable domain sequence of SEQ ID NO: 8 and the light chain variable domain sequence of SEQ ID NO: 10.

24. The antibody of claim 22, wherein the antibody comprises the heavy chain variable domain sequence of SEQ ID NO: 12 and the light chain variable domain sequence of SEQ ID NO: 14.

25. The humanized antibody of claim 16, wherein the antibody comprises a heavy chain variable domain sequence selected from SEQ ID NO:22 and SEQ ID NO:23.

26. The humanized antibody of claim 16, wherein the antibody comprises a light chain variable domain sequence selected from SEQ ID NO:20 and SEQ ID NO:21.

27. The humanized antibody of claim 25, further comprising a light chain variable domain sequence selected from SEQ ID NO:20 and SEQ ID NO:21.

28. The humanized antibody of claim 27,wherein the antibody comprises the light chain variable domain sequence of SEQ ID NO:20 and the heavy chain variable domain sequence of SEQ ID NO:22.

29. The humanized antibody of claim 27,wherein the antibody comprises the light chain variable domain sequence of SEQ ID NO:21 and the heavy chain variable domain sequence of SEQ ID NO:23.

30. The humanized antibody of claim 27,wherein the antibody comprises the light chain variable domain sequence of SEQ ID NO:21 and the heavy chain variable domain sequence of SEQ ID NO:22.

31. An antibody that binds to essentially the same epitope as an antibody of claim 30.

32. A binding agent that binds to essentially the same epitope as an antibody of any one of claims 1-31.

33. The binding agent of claim 32, wherein the binding agent inhibits the growth of a tumor expressing CD98.

34. The binding agent of claim 32, which is an antibody or a functional fragment thereof.

35. The binding agent of claim 32, which is an anticalin, an adnectin, an affibody, a DARPin, a fynomer, an affitin, an affilin, an avimer, a cysteine-rich knottin peptide, or an engineered Kunitz-type inhibitor.

36. A binding agent capable of binding to CD98, wherein the antibody of any one of claims 1-31 displaces the binding agent in a competitive binding assay.

37. A binding agent capable of binding to CD98, wherein the binding agent displaces the antibody of any one of claims 1-31 in a competitive binding assay.

38. The binding agent of claim 36, wherein the binding agent is an antibody, or a functional fragment thereof.

39. The binding agent of claim 37, wherein the binding agent is an antibody, or a functional fragment thereof.

40. The antibody or functional fragment of any one of claims 1-31, 34, and 38-39, wherein the antibody or fragment is conjugated to a cytotoxic agent.

41. The antibody or functional fragment of claim 40, wherein the cytotoxic agent is selected from a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin, or a radioactive isotope.

42. The antibody or functional fragment of any one of claims 1-31, 34, and 38-39, wherein the antibody or fragment is conjugated to a detectable marker.

43. The antibody or functional fragment of claim 40, wherein the detectable marker is selected from a radioisotope, a metal chelator, an enzyme, a fluorescent compound, a bioluminescent compound and a chemiluminescent compound.

44. A transgenic animal that produces the monoclonal antibody of claim 15.

45. A hybridoma that produces the monoclonal antibody of claim 15.

46. A vector comprising a polynucleotide encoding the antibody or fragment thereof of any one of claims 1-31, 34, and 38-39.

47. A pharmaceutical composition that comprises the antibody or functional fragment of any one of claims 1-31, 34, and 38-43, and a pharmaceutically acceptable carrier.

48. A method of inhibiting growth of cancer cells that express CD98, the method comprising exposing the cells to the antibody or functional fragment of any one of claims 1-31, 34, and 38-43.

49. The method of claim 48, wherein the cancer cells are from a cancer selected from bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

50. The method of claim 49, wherein the cancer cells are from acute myeloid leukemia.

51. A method for treating a cancer in a subject comprising administering to the subject the pharmaceutical composition of claim 47.

52. The method of claim 51, wherein the cancer is selected from bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

53. The method of claim 52, wherein the cancer is acute myeloid leukemia.

54. The method of claim 51, wherein the subject has relapsed or refractory acute myeloid leukemia.

55. The method of claim 51, wherein the subject is administered one or more chemotherapeutic compound in combination with the antibody or functional fragment, wherein the chemotherapeutic compound is selected from bendamustine hydrochloride, cyclophosphamide, ifosfamide, fludurabine, cytarabine, gemcitabine, prednisone, prednisolone, methylprednisolone, paclitaxel, docetaxel, vinorelbine, vincristine, etoposide, irinotecan, anthracycline, adriamycin, cisplatin, carboplatin and rituximab.

56. The method of claim 51 wherein the cancer is associated with increased expression of CD98 on the surface of a cell.

57. A method of detecting the presence of CD98 in a biological sample, comprising contacting the biological sample with an antibody of any one of claims 1-31, 34, and 38-43 under

conditions permissive for binding of the antibody to CD98, and detecting whether a complex is formed between the antibody and CD98.

58. The method of claim 57, wherein the biological sample is from a mammal having or suspected of having a cancer selected from bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

59. A method of diagnosing a cancer associated with increased expression of CD98, comprising contacting a test cell with an antibody of any one of claims 1-31, 34, and 38-43; determining the level of expression of CD98 by detecting binding of the antibody to CD98; and comparing the level of expression of CD98 in the test cell with the level of expression of CD98 in a control cell, wherein a higher level of expression of CD98 in the test cell as compared to the control cell indicates the presence of a cancer associated with increased expression of CD98.

60. The method of claim 59 wherein the test cell is from a patient suspected of having a cancer selected from bladder, breast, colon, rectal, gastric, esophageal, lung, laryx, kidney, oral, ovarian, or prostate cancer, or a sarcoma, melanoma, glioma, lymphoma or leukemia, or a metatasis of any of these cancers.

61. The method of claim 60 wherein the method comprises determining the level of expression of CD98 on the surface of the test cell and comparing the level of expression of CD98 on the surface of the test cell with the level of expression of CD98 on the surface of the control cell.

62. The method of claim 61 wherein the test cell is a cancer cell and the control cell is a normal cell of the same tissue type.

63. Use of the antibody or functional fragment of any one of claims 1-31, 34, and 38-43 in the manufacture of a medicament, wherein the medicament is for use in a method of inhibiting growth of cancer cells that express CD98.

64. An antibody or functional fragment of any one of claims 1-31, 34, and 38-43 for use in inhibiting the growth of cancer cells that express CD98.

65. Use of the pharmaceutical composition of claim 47 in the manufacture of a medicament, wherein the medicament is for use in a method of treating cancer in a subject.

66. A pharmaceutical composition that comprises the antibody or functional fragment of any one of claims 1-31, 34, and 38-43, and a pharmaceutically acceptable carrier, for use in treating cancer in a subject.

67. Use of an antibody or functional fragment of any one of claims 1-31, 34, and 38-43 in the manufacture of a medicament, wherein the medicament is for use in a method for detecting the presence of CD98 in a biological sample.

68. An antibody or functional fragment of any one of claims 1-31, 34, and 38-43 for use in a method of detecting the presence of CD98 in a biological sample.

69. Use of an antibody or functional fragment of any one of claims 1-31, 34, and 38-43 in the manufacture of a medicament, wherein the medicament is for use in a method of diagnosing a cancer associated with increased expression of CD98.

70. An antibody or functional fragment of any one of claims 1-31, 34, and 38-43 for use in a method of diagnosing a cancer associated with increased expression of CD98.