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(71) Demandeur/Applicant:
RAVEN BIOTECHNOLOGIES, INC., US

(72) Inventeurs/Inventors:
MATHER, JENNIE P., US;
LOO, DERYK T., US

(74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : ANTIGENE KID31 (CARBOXYPEPTIDASE M) ET ANTICORPS DE LIAISON A CELUI-CI
(54) Title: KID31 AND ANTIBODIES THAT BIND THERETO

(57) Abrégé/Abstract:
The invention provides the identification and characterization of disease and cancer-associated antigen, KID31. The invention also provides a family of monoclonal antibodies that bind to antigen KID31, methods of diagnosing and treating various human cancers and diseases that express KID31.

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(71) Applicant *(for all designated States except US)*: **RAVEN BIOTECHNOLOGIES, INC.** [US/US]; BRITANNIA OYSTER POINT, 1140 Veterans Blvd., South San Francisco, California 94080 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **MATHER, Jennie, P.** [US/US]; 269 La Prenda Drive, Millbrae, California 94030 (US). **LOO, Deryk, T.** [CA/US]; 1509 Pine Knoll Drive, Belmont, California 94402 (US).

(74) Agents: **ZHOU, Jie** et al.; MORRISON & FOERSTER LLP, 755 Page Mill Road, Palo Alto, California 94304-1018 (US).

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KID31 AND ANTIBODIES THAT BIND THERETO

TECHNICAL FIELD

[001] This invention is in the fields of biology and immunotherapy. More specifically, it concerns the discovery that carboxypeptidase M, a known antigen, is associated with a variety of human cancers and related disease. The invention further provides for the diagnosis and/or treatment of a variety of human diseases and cancers associated with carboxypeptidase M using antagonists, modulators and peptides that bind to carboxypeptidase M, including anti-carboxypeptidase M antibodies.

BACKGROUND OF THE INVENTION

[002] Human carboxypeptidase M is a membrane-bound metalloprotease-peptide that specifically cleaves carboxyl terminal arginine or lysine residues from peptides or proteins. Carboxypeptidase M is a glycoprotein with a molecular weight at around 62 kDa and is a member of a family of regulatory metallo-carboxypeptidase proteins.

[003] Carboxypeptidase M is widely expressed on a variety of human tissues and is believed to play a role in the control of peptide hormones and growth factor activity at the cell surface and in the membrane-localized degradation of extracellular proteins (Reverter et al., *J. Mol. Bio. Vol. 338, pp. 257-269 (2003)*). Using carboxypeptidase M-specific antibodies, researchers have identified carboxypeptidase M as a marker for macrophage maturation.

[004] Despite being described as a marker for macrophage maturation, there is little data to show any correlation between carboxypeptidase M expression and cancer. Carboxypeptidase M expression has been reported in a patient with a granulomatous reaction within a conventional clear cell renal cancer using immunohistochemical techniques and an antibody specific for carboxypeptidase M (Kovacs et al., *Pathology Oncology Res., Vol. 10, No, pp. 169-171 (2004)).*

[005] In addition to their known uses in diagnostics, antibodies have been shown to be useful as therapeutic agents. For example, immunotherapy, or the use of antibodies for therapeutic purposes has been used in recent years to treat

cancer. Passive immunotherapy involves the use of monoclonal antibodies in cancer treatments. See for example, *Cancer: Principles and Practice of Oncology*, 6th Edition (2001) Chapt. 20 pp. 495-508. These antibodies can have inherent therapeutic biological activity both by direct inhibition of tumor cell growth or survival and by their ability to recruit the natural cell killing activity of the body's immune system. These agents can be administered alone or in conjunction with radiation or chemotherapeutic agents. Rituximab and Trastuzumab, approved for treatment of non-Hodgkin's lymphoma and breast cancer, respectively, are two examples of such therapeutics. Alternatively, antibodies can be used to make antibody conjugates where the antibody is linked to a toxic agent and directs that agent to the tumor by specifically binding to the tumor. Gemtuzumab ozogamicin is an example of an approved antibody conjugate used for the treatment of leukemia. Monoclonal antibodies that bind to cancer cells and have potential uses for diagnosis and therapy have been disclosed in publications. See, for example, the following patent applications which disclose, *inter alia*, some molecular weights of target proteins: U.S. Patent No. 6,054,561 (200 kD c-erbB-2 (Her2), and other unknown antigens 40-200 KD in size) and U.S. Patent No. 5,656,444 (50 kD and 55 kD oncofetal protein). Example of antibodies in clinical trials and/or approved for treatment of solid tumors include: Trastuzumab (antigen: 180 kD, HER2/neu), Edrecolomab (antigen: 40-50 kD, Ep-CAM), Anti-human milk fat globules (HMFG1) (antigen >200 kD, HMW Mucin), Cetuximab (antigens: 150 kD and 170 kD, EGF receptor), Alemtuzumab (antigen: 21-28 kD, CD52), and Rituximab (antigen: 35 kD, CD20).

[006] The antigen targets of trastuzumab (Her-2 receptor), which is used to treat breast cancer, and cetuximab (EGF receptor), which is in clinical trials for the treatment of several cancers, are present at some detectable level on a large number of normal human adult tissues including skin, colon, lung, ovary, liver, and pancreas. The margin of safety in using these therapeutics is possibly provided by the difference in the level of expression or in access of or activity of the antibody at these sites.

[007] Another type of immunotherapy is active immunotherapy, or vaccination, with an antigen present on a specific cancer(s) or a DNA construct

that directs the expression of the antigen, which then evokes the immune response in the individual, *i.e.*, to induce the individual to actively produce antibodies against their own cancer. Active immunization has not been used as often as passive immunotherapy or immunotoxins.

[008] Several models of disease (including cancer) progression have been suggested. Theories range from causation by a single infective/transforming event to the evolution of an increasingly "disease-like" or 'cancer-like' tissue type leading ultimately to one with fully pathogenic or malignant capability. Some argue that with cancer, for example, a single mutational event is sufficient to cause malignancy, while others argue that subsequent alterations are also necessary. Some others have suggested that increasing mutational load and tumor grade are necessary for both initiation as well as progression of neoplasia via a continuum of mutation-selection events at the cellular level. Some cancer targets are found only in tumor tissues, while others are present in normal tissues and are up regulated and/or over-expressed in tumor tissues. In such situations, some researchers have suggested that the over-expression is linked to the acquisition of malignancy, while others suggest that the over-expression is merely a marker of a trend along a path to an increasing disease state.

[009] An ideal diagnostic and/or therapeutic antibody would be specific for an antigen present on a large number of cancers, but absent or present only at low levels on any normal tissue. The discovery, characterization, and isolation of a novel antigen that is specifically associated with cancer(s) would be useful in many ways. First, the antigen could be used to make monoclonal antibodies against the antigen. An antibody would ideally have biological activity against cancer cells and be able to recruit the immune system's response to foreign antigens. An antibody could be administered as a therapeutic alone or in combination with current treatments or used to prepare immunoconjugates linked to toxic agents. An antibody with the same specificity but with low or no biological activity when administered alone could also be useful in that an antibody could be used to prepare an immunoconjugate with a radio-isotope, a toxin, or a chemotherapeutic agent or liposome containing a chemotherapeutic

agent, with the conjugated form being biologically active by virtue of the antibody directing the toxin to the antigen-containing cells.

[010] One aspect desirable for an ideal diagnostic and/or therapeutic antibody is the discovery and characterization of an antigen that is associated with a variety of cancers. There are few antigens that are expressed on a number of types of cancer (*e.g.*, "pan-cancer" antigen) that have limited expression on non-cancerous cells. The isolation and purification of such an antigen would be useful for making antibodies (*e.g.*, diagnostic or therapeutic) targeting the antigen. An antibody binding to the "pan-cancer" antigen could be able to target a variety of cancers found in different tissues in contrast to an antibody against an antigen associated with only one specific type of cancer. The antigen would also be useful for drug discovery (*e.g.*, small molecules) and for further characterization of cellular regulation, growth, and differentiation.

[011] What is needed are novel targets on the surface of diseased and/or cancer cells that may be used to diagnose and treat such diseases and/or cancers with antibodies and other agents which specifically recognize the cell surface targets. There exists a further need, based on the discoveries disclosed herein, for novel antibodies and other agents which specifically recognize targets on the surface of cells that can modulate, either by reducing or enhancing, the disease-promoting activities of carboxypeptidase M. It is an object of this invention to identify antagonists of human carboxypeptidase M that are capable of inhibiting its disease-associated activities. It is another object to provide novel compounds for use in the assay of carboxypeptidase M, and for use as immunogens or for selecting anti-human carboxypeptidase M antibodies.

[012] As will be described in more detail below, the present inventors have discovered a known antigen, carboxypeptidase M, which we refer to herein as KID31, identified as the antigen target of the novel antagonists, modulators and antibodies provided herein.

SUMMARY OF THE INVENTION

[013] The invention disclosed herein concerns the discoveries that the known antigen, carboxypeptidase M (KID31), is present on a variety of both primary

and metastatic human cancers, and that anti-KID31 antibodies may be used to treat such cancers. The invention provides for KID31 antagonists, modulators, and monoclonal antibodies that bind to KID31, which is expressed on a variety of human cancers.

[014] In another aspect, the invention is a monoclonal antibody anti-KID31 that is produced by the host cell line KIDNEY.5.4B8.1A4 deposited on January 12, 2005 at the American Type Culture Collection with a Patent Deposit Designation of PTA# 6516.

[015] In yet another aspect, the invention is a method of generating monoclonal antibody anti-KID31 reactive with diseased and/or cancerous cells comprising the steps of: (a) immunizing a host mammal with an immunogen; (b) obtaining lymphocytes from the mammal; (c) fusing lymphocytes (b) with a myeloma cell line to produce a hybridoma; (d) culturing the hybridoma of (c) to produce monoclonal antibodies; and (e) screening the antibodies to select only those antibodies which bind to diseased and/or cancerous cells or cell lines but do not bind to non-cancerous or normal cells or cell lines, or bind to normal cells at a lower level or in a different fashion.

[016] In another aspect, the invention is a method of generating an anti-KID31 antibody comprising culturing a host cell encoding such antibody or progeny thereof under conditions that allow production of the antibody, and purifying the anti-KID31 antibody.

[017] In another aspect, the invention provides methods of generating any of the antibodies (or polypeptides) described herein by expressing one or more polynucleotides encoding the antibody (which may be separately expressed as a single light or heavy chain, or both a light and a heavy chain are expressed from one vector) in a suitable cell, generally followed by recovering and/or isolating the antibody or polypeptides of interest.

[018] In another aspect, the invention is an anti-KID31 antibody or a polypeptide (which may or may not be an antibody) that competitively inhibits preferential binding of an anti-KID31 antibody to KID31. In some embodiments, the invention is an antibody or a polypeptide (which may or may not be an

antibody) that binds preferentially to the same or different epitope(s) on KID31 as other anti-KID31 antibodies.

[019] In another aspect, the invention is an KID31 modulator (which may or may not be a polypeptide) that competitively inhibits preferential binding of an anti-KID31 antibody to KID31. In some embodiments, the invention can be a small molecule or chemical compound that binds preferentially to the same or different epitope(s) on KID31 as other anti-KID31 antibodies.

[020] In yet another aspect, the invention is a composition comprising KID31 bound by an antibody specific for an epitope of KID31. In one embodiment, the antibody is anti-KID31. In other embodiments, two or more anti-KID31 antibodies are administered, with such antibodies mapping to two or more different epitopes on KID31. In some embodiments, the anti-KID31 antibody is linked to a therapeutic agent or a detectable label.

[021] In another aspect, the invention is an antibody comprising a fragment or a region of an anti-KID31 antibody. In one embodiment, the fragment is a light chain of the antibody. In another embodiment, the fragment is a heavy chain of the antibody. In yet another embodiment, the fragment contains one or more variable regions from a light chain and/or a heavy chain of the antibody. In yet another embodiment, the fragment contains one or more complementarity determining regions (CDRs) from a light chain and/or a heavy chain of the antibody.

[022] In another aspect, the invention provides polypeptides (which may or may not be antibodies) comprising any of the following: (a) one or more CDRs (or fragments thereof) from the light or heavy chain; (b) three CDRs from the light chain; (c) three CDRs from the heavy chain; (d) three CDRs from the light chain and three CDRs from the heavy chain; (e) the light chain variable region; (f) the heavy chain variable region of the anti-KID31 antibody.

[023] In another aspect, the invention is a humanized antibody. In some embodiments, the humanized antibody comprises one or more CDRs of a non-human anti-KID31 antibody. In some embodiments, the humanized antibody binds to the same or different epitope(s) as other anti-KID31 antibodies. Generally, a humanized antibody of the invention comprises one or more (one,

two, three, four, five, six or fragments thereof) CDRs which are the same and/or derived from the CDR(s) of the original non-human anti-KID31 antibody. In some embodiments, the human antibody binds to the same or different epitope(s) as other anti-KID31 antibodies. In another aspect, the invention is a chimeric antibody comprising variable regions derived from variable regions of a heavy chain and a light chain of a non-human anti-KID31 antibody and constant regions derived from constant regions of a heavy chain and a light chain of a human antibody.

[024] In another aspect, the invention is an isolated polynucleotide that encodes an antibody mu-anti-KID31 that is produced by a host cell with a deposit number of ATCC PTA# 6516, or progeny thereof. This invention encompasses antibody polypeptides having the inherent binding or biological activities of any of the above-specified antibodies. In another aspect, the invention provides polynucleotides encoding any of the antibodies (including antibody fragments) as well as any other polypeptides described herein.

[025] In another aspect, the invention is a pharmaceutical composition comprising any of the polypeptides (including any of the antibodies described herein) or polynucleotides described herein, such as pharmaceutical compositions comprising an anti-KID31 antibody linked to a chemotherapeutic agent, an antibody comprising a fragment of an anti-KID31 antibody, a humanized antibody of a non-human KID31 antibody, a chimeric antibody comprising variable regions derived from variable regions of a non-human anti-KID31 antibody and constant regions derived from a human antibody, or a human antibody with one or more properties of a non-human anti-KID31 antibody, or of the anti-KID31 antibody described herein linked to a chemotherapeutic agent (such as a radioactive moiety), and a pharmaceutically acceptable excipient.

[026] In one aspect, the invention is a composition comprising an anti-KID31 antibody bound to KID31 present on a diseased or cancerous cell. In preferred embodiments, the cancer cell is selected from the group consisting of kidney, ovarian, lung, prostate, pancreatic, colon, and breast cancer cells. In some embodiments, the cancer cell is isolated. In some embodiments, the cancer cell is

in a biological sample. Generally, the biological sample is from an individual, such as a human.

[027] In another aspect, the invention is a method of diagnosing disease in an individual by detecting KID31 on cells from the individual, particularly diseases or disorders associated with inflammatory or autoimmune responses in individuals. In other aspects of the invention, methods are provided for modulating inflammatory or autoimmune responses in individuals. Diseases and conditions resulting from inflammation and autoimmune disorders that may be subject to treatment using the compositions and methods of the invention include, by way of illustration and not of limitation, multiple sclerosis, meningitis, encephalitis, stroke, other cerebral traumas, inflammatory bowel disease including ulcerative colitis and Crohn's disease, myasthenia gravis, lupus, rheumatoid arthritis, asthma, acute juvenile onset diabetes, AIDS dementia, atherosclerosis, nephritis, retinitis, atopic dermatitis, psoriasis, myocardial ischemia and acute leukocyte-mediated lung injury.

[028] Still other indications for therapeutic use of antibodies and other therapeutic agents of the invention include administration to individuals at risk of organ or graft rejection. Over recent years there has been a considerable improvement in the efficiency of surgical techniques for transplanting tissues and organs such as skin, kidney, liver, heart, lung, pancreas and bone marrow. Perhaps the principal outstanding problem is the lack of satisfactory agents for inducing immunotolerance in the recipient to the transplanted allograft or organ. When allogeneic cells or organs are transplanted into a host (i.e., the donor and donee are different individuals from the same species), the host immune system is likely to mount an immune response to foreign antigens in the transplant (host-versus-graft disease) leading to destruction of the transplanted tissue.

[029] In another aspect, the invention is a method for diagnosing whether an individual has cancer, comprising determining whether there is expression of KID31 on selected cells from the individual, wherein the expression of KID31 on said cells is indicative of said cancer. In some embodiments, the expression of KID31 is determined using an anti-KID31 antibody. In some embodiments, the method involves detecting the level of KID31 expression from cells. The term

"detection" as used herein includes qualitative and/or quantitative detection (measuring levels) with or without reference to a control.

[030] In yet another aspect, the invention is a method of diagnosing cancer in an individual by detecting KID31 on or released from cells from the individual, wherein the cancer is selected from the group including but not limited to adrenal gland tumors, AIDS-associated cancers, alveolar soft part sarcoma, astrocytic tumors, bladder cancer (squamous cell carcinoma and transitional cell carcinoma), bone cancer (adamantinoma, aneurismal bone cysts, osteochondroma, osteosarcoma), brain and spinal cord cancers, metastatic brain tumors, breast cancer, carotid body tumors, cervical cancer, chondrosarcoma, dhordoma, chromophobe renal cell carcinoma, clear cell carcinoma, colon cancer, colorectal cancer, cutaneous benign fibrous histiocytomas, desmoplastic small round cell tumors, ependymomas, Ewing's tumors, extraskeletal myxoid chondrosarcoma, fibrogenesis imperfecta ossium, fibrous dysplasia of the bone, gallbladder and bile duct cancers, gestational trophoblastic disease, germ cell tumors, head and neck cancers, islet cell tumors, Kaposi's Sarcoma, kidney cancer (nephroblastoma, papillary renal cell carcinoma), leukemias, lipoma/benign lipomatous tumors, liposarcoma/malignant lipomatous tumors, liver cancer (hepatoblastoma, hepatocellular carcinoma), lymphomas, lung cancer, medulloblastoma, melanoma, meningiomas, multiple endocrine neoplasia, multiple myeloma, myelodysplastic syndrome, neuroblastoma, neuroendocrine tumors, ovarian cancer, pancreatic cancers, papillary thyroid carcinomas, parathyroid tumors, pediatric cancers, peripheral nerve sheath tumors, phaeochromocytoma, pituitary tumors, prostate cancer, posterious unveal melanoma, rare hematologic disorders, renal metastatic cancer, rhabdoid tumor, rhabdomysarcoma, sarcomas, skin cancer, soft-tissue sarcomas, squamous cell cancer, stomach cancer, synovial sarcoma, testicular cancer, thymic carcinoma, thymoma, thyroid metastatic cancer, and uterine cancers (carcinoma of the cervix, endometrial carcinoma, and leiomyoma).

[031] In another aspect, the invention is a method for aiding diagnosis of cancer (such as but not limited to kidney, ovarian, lung, prostate, pancreatic, colon, or breast cancer) in an individual comprising determining the expression of KID31

in a biological sample from the individual. In some embodiments, the expression of KID31 is determined using an anti-KID31 antibody. In some embodiments, the method is detecting the level of KID31 expression from cells. The KID31 released from the cancer may contribute to elevated levels of KID31 or a portion thereof, being detectable in body fluids (e.g., blood, salivary or gut mucinous secretions).

[032] In yet another aspect, the invention is a method of treating cancer by administering an effective amount of an antibody that binds to KID31 sufficient to reduce growth of cancerous cells. In some embodiments, the antibody is an anti-KID31 antibody. In certain embodiments, the cancerous cells are selected from the group including but not limited to adrenal gland tumors, AIDS-associated cancers, alveolar soft part sarcoma, astrocytic tumors, bladder cancer (squamous cell carcinoma and transitional cell carcinoma), bone cancer (adamantinoma, aneurismal bone cysts, osteochondroma, osteosarcoma), brain and spinal cord cancers, metastatic brain tumors, breast cancer, carotid body tumors, cervical cancer, chondrosarcoma, dhordoma, chromophobe renal cell carcinoma, clear cell carcinoma, colon cancer, colorectal cancer, cutaneous benign fibrous histiocytomas, desmoplastic small round cell tumors, ependymomas, Ewing's tumors, extraskeletal myxoid chondrosarcoma, fibrogenesis imperfecta ossium, fibrous dysplasia of the bone, gallbladder and bile duct cancers, gestational trophoblastic disease, germ cell tumors, head and neck cancers, islet cell tumors, Kaposi's Sarcoma, kidney cancer (nephroblastoma, papillary renal cell carcinoma), leukemias, lipoma/benign lipomatous tumors, liposarcoma/malignant lipomatous tumors, liver cancer (hepatoblastoma, hepatocellular carcinoma), lymphomas, lung cancer, medulloblastoma, melanoma, meningiomas, multiple endocrine neoplasia, multiple myeloma, myelodysplastic syndrome, neuroblastoma, neuroendocrine tumors, ovarian cancer, pancreatic cancers, papillary thyroid carcinomas, parathyroid tumors, pediatric cancers, peripheral nerve sheath tumors, phaeochromocytoma, pituitary tumors, prostate cancer, posterious unveal melanoma, rare hematologic disorders, renal metastatic cancer, rhabdoid tumor, rhabdomysarcoma, sarcomas, skin cancer, soft-tissue sarcomas, squamous cell

cancer, stomach cancer, synovial sarcoma, testicular cancer, thymic carcinoma, thymoma, thyroid metastatic cancer, and uterine cancers (carcinoma of the cervix, endometrial carcinoma, and leiomyoma). In certain preferred embodiments, the cancerous cells are selected from the group of solid tumors including but not limited to breast cancer, colon cancer, prostate cancer, lung cancer, sarcoma, renal metastatic cancer, thyroid metastatic cancer, and clear cell carcinoma.

[033] In yet another aspect, the invention is a method of delaying development of metastasis in an individual having cancer comprising administering an effective amount of at least one of a family of antibodies that bind specifically to KID31. In one embodiment, the antibody is an anti-KID31 antibody. In another aspect, the invention is a method of inhibiting growth and/or proliferation of cancer cells *in vitro* or in an individual comprising administering an effective amount of a composition comprising an anti-KID31 antibody associated with (including linked to) a chemotherapeutic agent to the cell culture or sample, or to the individual.

[034] In yet another aspect, the invention is a method of delivering a therapeutic agent to a cancerous cell in an individual by administering to the individual an effective amount of at least one member of a family of antibodies, which bind specifically to KID31. In other embodiments, an anti-KID31 antibody is delivered to an individual in combination with (including linked to) another therapeutic agent.

[035] In some embodiments, the anti-KID31 antibody is a humanized antibody derived from a named antibody herein (generally, but not necessarily, comprising one or more partial or intact CDRs of the antibody). In some embodiments, the anti-KID31 antibody is a human antibody with one or more properties of the named antibody. In some embodiments, the chemotherapeutic agent (such as a toxin or a radioactive molecule) is delivered into the cancer cells (is internalized). In some embodiments, the agent is saporin.

[036] In another aspect, the invention is a method of treating cancer in an individual comprising administering an effective amount of a composition

comprising an anti-KID31 antibody associated with (including linked to) a chemotherapeutic agent to the individual.

[037] The present invention further provides methods for modulating, either by enhancing or reducing, the association of KID31 with a cytoplasmic signaling partner. The association of KID31 with a cytoplasmic signaling partner can be impacted by contacting a KID31 molecule presenting on a cell surface, with an agent that modulates the binding of the signaling partner to KID31. Agents which block or reduce KID31 association with its binding and/or signaling partners can be used to modulate biological and pathological processes which are involved in KID31-mediated inflammation or immune responses. Pathological processes involving this action include tumor-associated cell growth.

[038] Agents can be tested for their ability to block, reduce, enhance or otherwise modulate the association of KID31 with a binding partner, such as an anti-KID31 antibody. Specifically, an agent can be tested for the ability to modulate such an interaction by incubating a peptide comprising the KID31 interaction site (typically in its native conformation as it exists on intact living cells) with a binding partner and a test agent, and determining whether the test agent reduces or enhances the binding of the binding partner to the KID31 peptide.

[039] Agonists, antagonists, and other modulators of KID31 function are expressly included within the scope of this invention. These agonists, antagonists and modulators are polypeptides that comprise one or more of the antigenic determinant sites in KID31, or comprise one or more fragments of such sites, variants of such sites, or peptidomimetics of such sites. These agonistic, antagonistic, and KID31 modulatory compounds are provided in linear or cyclized form, and optionally comprise at least one amino acid residue that is not commonly found in nature or at least one amide isostere. These compounds may be glycosylated. The agonists, antagonists, and other modulators of KID31 function of this invention are desirably used in all of the embodiments and methods described above with reference to antibodies.

[040] Other aspects of this invention relate to the novel antigen identified and referred to herein as KID31. This antigen is suitable for use as an immunogen and for a variety of research, diagnostic and therapeutic purposes.

[041] In certain aspects, the invention is a method for aiding in the diagnosis of disease in an individual comprising the steps of (i) assaying for the presence of KID31 in a blood or tissue sample obtained from an individual; (ii) detecting whether said sample has an increased amount of a KID31 marker relative to a normal (non-diseased) blood or tissue sample; and (iii) correlating an increased amount of said marker to a positive diagnosis or correlating the absence of an increased amount of said marker to a negative diagnosis for disease. In certain embodiments, the marker is detected using an anti-KID31 antibody. In certain embodiments, the method is effected by a technique selected from the group consisting of radionuclide imaging, flow cytometry, and immunohistochemistry.

BRIEF DESCRIPTION OF THE DRAWINGS

[042] Figure 1 shows the graphed results of *in vivo* activity of mu-anti-KID31 on sub-renal tumor model using H322M cell line and Caki-2 cell line.

DETAILED DESCRIPTION OF THE INVENTION

[043] The invention provides a known antigen, carboxypeptidase M, referred to herein as KID31, which is expressed on cancerous cells of various tissue types, including but not limited to lung, kidney, pancreatic, ovarian, and colon cancers. Further, the invention provides monoclonal antibodies and polypeptides that bind to KID31 and methods making and using these antibodies and polypeptides to diagnose and treat various diseases human cancers associated with expression and/or over-expression of KID31.

I. General Techniques

[044] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant

techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, *Molecular Cloning: A Laboratory Manual,* second edition (Sambrook et al., 1989) Cold Spring Harbor Press; *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984*); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook* (J.E. Cellis, ed., 1998) Academic Press; *Animal Cell Culture* (R.I. Freshney, ed., 1987); *Introduction to Cell and Tissue Culture* (J.P. Mather and P.E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; *Methods in Enzymology* (Academic Press, Inc.); *Handbook of Experimental Immunology* (D.M. Weir and C.C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller and M.P. Calos, eds., 1987); *Current Protocols in Molecular Biology* (F.M. Ausubel et al., eds., 1987); *PCR: The Polymerase Chain Reaction,* (Mullis et al., eds., 1994); *Current Protocols in Immunology* (J.E. Coligan et al., eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C.A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: a practical approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal antibodies : a practical approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using antibodies: a laboratory manual* (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J.D. Capra, eds., Harwood Academic Publishers, 1995); and *Cancer: Principles and Practice of Oncology* (V.T. DeVita *et al.,* eds., J.B. Lippincott Company, 1993).

II. Definitions

[045] "KID31" refers to that polypeptide antigen, carboxypeptidase M, with a molecular weight of approximately 60 kDa to 80 kDa against which the antibodies of the present invention are directed. The KID31 is a cell surface protein bound by anti-KID31 antibodies and present on skin and pancreatic ducts and several types of carcinomas. This antigen may have more than one different epitope. It is currently believed that KID31 may be over-expressed in certain cancer cells in comparison to their normal tissue counterparts.

[046] Agonists, antagonists, and other modulators of function are expressly included within the scope of this invention. These agonists, antagonists and modulators are polypeptides that comprise one or more of the antigenic determinant sites in KID31, or comprise one or more fragments of such sites, variants of such sites, or peptidomimetics of such sites. These agonistic, antagonistic, and KID31 modulatory compounds are provided in linear or cyclized form, and optionally comprise at least one amino acid residue that is not commonly found in nature or at least one amide isostere. These compounds may be glycosylated.

[047] More specifically, the terms "KID31 modulator" as used herein are defined as any compound that (1) is capable of disrupting or blocking the interaction between human KID31 and its native ligands or an anti-KID31 antibody; (2) is capable of binding to human KID31 and its native ligands or an anti-KID31 antibody; (3) contains an antigenic site that can be used in the raising of antibodies capable of binding to human KID31 and its native ligands or an anti-KID31 antibody; (4) contains an antigenic site that can be used in the screening of antibodies capable of binding to human KID31 and its native ligands or an anti-KID31 antibody; (5) contains an antigenic site that an be used in the raising of antibodies capable of disrupting or blocking the interaction between human KID31 and its native ligands or an anti-KID31 antibody; (6) contains an antigenic site that can be used in the screening of antibodies capable of disrupting or blocking the interaction between human KID31 and its native ligands or an anti-KID31 antibody. KID31 modulators may be "KID31 agonists" or "KID31 antagonists" depending on whether their activity enhances or inhibits normal KID31 biological activity, respectively.

[048] KID31 agonists, antagonists and modulators include KID31 variants, KID31 peptide antagonists, peptidomimetics, and small molecules, anti-KID31 antibodies and immunoglobulin variants, amino acid variants of human KID31 including amino acid substitution, deletion, and addition variants, or any combination thereof, and chimeric immunoglobulins. The KID31 agonists, antagonists and modulators of this invention are based on the inventors' identification of the KID31 domains involved in the binding of human KID31 to

its native ligands or anti-KID31 antibodies. Thus, the invention provides KID31 agonists, antagonists and modulators with molecular structures that duplicate or mimic one or more of the anti-KID31 binding domains of human KID31.

[049] As used herein, the term "KID31 variant" denotes any amino acid variant of human KID31, including amino acid substitution, deletion, and addition variants, or any combination thereof. The definition encompasses chimeric molecules such as human KID31/non-human chimeras and other hybrid molecules. Also included in the definition is any fragment of a KID31 variant molecule that comprises the variant or hybrid region(s) of the molecule.

[050] An "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as Fab, Fab', $F(ab')_2$, Fv), single chain (ScFv), mutants thereof, naturally occurring variants, fusion proteins comprising an antibody portion with an antigen recognition site of the required specificity, humanized antibodies, chimeric antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity.

[051] A "monoclonal antibody" refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring and non-naturally occurring) that are involved in the selective binding of an antigen. Monoclonal antibodies are highly specific, being directed against a single antigenic site. The term "monoclonal antibody" encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', $F(ab')_2$, Fv), single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity and the ability to bind to an antigen. It is not intended to be limited as regards to the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant

expression, transgenic animals, etc.). The term includes whole immunoglobulins as well as the fragments etc. described above under the definition of "antibody".

[052] "Humanized" antibodies refer to a chimeric molecule, generally prepared using recombinant techniques, having an antigen binding site derived from an immunoglobulin from a non-human species and the remaining immunoglobulin structure of the molecule based upon the structure and/or sequence of a human immunoglobulin. The antigen-binding site may comprise either complete variable domains fused onto constant domains or only the complementarity determining regions (CDRs) grafted onto appropriate framework regions in the variable domains. Antigen binding sites may be wild type or modified by one or more amino acid substitutions. This eliminates the constant region as an immunogen in human individuals, but the possibility of an immune response to the foreign variable region remains (LoBuglio, A. F. *et al.*, (1989) Proc Natl Acad Sci USA 86:4220-4224). Another approach focuses not only on providing human-derived constant regions, but modifying the variable regions as well so as to reshape them as closely as possible to human form. It is known that the variable regions of both heavy and light chains contain three complementarity-determining regions (CDRs) which vary in response to the antigens in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When nonhuman antibodies are prepared with respect to a particular antigen, the variable regions can be "reshaped" or "humanized" by grafting CDRs derived from nonhuman antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K., *et al.*, (1993) Cancer Res 53:851-856. Riechmann, L., *et al.*, (1988) Nature 332:323-327; Verhoeyen, M., *et al.*, (1988) Science 239:1534-1536; Kettleborough, C. A., *et al.*, (1991) Protein Engineering 4:773-3783; Maeda, H., *et al.*, (1991) Human Antibodies Hybridoma 2:124-134; Gorman, S. D., *et al.*, (1991) Proc Natl Acad Sci USA 88:4181-4185; Tempest, P. R., *et al.*, (1991) Bio/Technology 9:266-271; Co, M. S., *et al.*, (1991) Proc Natl Acad Sci USA 88:2869-2873; Carter, P., *et al.*, (1992) Proc Natl Acad Sci USA 89:4285-4289; and Co, M. S. *et al.*, (1992) J Immunol 148:1149-1154. In some embodiments,

humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

[053] An epitope that "specifically binds" or "preferentially binds" (used interchangeably herein) to an antibody or a polypeptide is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to a KID31 epitope is an antibody that binds this KID31 epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other KID31 epitopes or non-KID31 epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

[054] The term "immunologically active" in reference to an epitope being or "remaining immunologically active" refers to the ability of an antibody (*e.g.*, anti-KID31 antibody) to bind to the epitope under different conditions, for example, after the epitope has been subjected to reducing and denaturing conditions.

[055] Different biological functions are associated with anti-KID31 antibodies, including, but not limited to, ability to bind to KID31 (including KID31 on cancer

cells, including but not limited to lung, kidney, pancreatic, ovarian, and colon cancer cells); ability to bind to a portion of KID31 that is exposed on the surface of a living cell *in vitro* or *in vivo*; ability to deliver a chemotherapeutic agent to cancerous cells (such as lung, kidney, pancreatic, ovarian, and colon cancer cells) expressing KID31; ability to deliver a therapeutic agent or detectable marker into cancer cells expressing KID31. As discussed herein, polypeptides (including antibodies) of the invention may have any one or more of these characteristics.

[056] An "anti-KID31 equivalent antibody" or "anti-KID31 equivalent polypeptide" refers to an antibody or a polypeptide having one or more biological functions associated with an anti-KID31 antibody, such as, for example binding specificity.

[057] As used herein, "agent" refers to a biological, pharmaceutical, or chemical compound. Non-limiting examples include simple or complex organic or inorganic molecule, a peptide, a protein, an oligonucleotide, an antibody, an antibody derivative, antibody fragment, a vitamin derivative, a carbohydrate, a toxin, or a chemotherapeutic compound. Various compounds can be synthesized, for example, small molecules and oligomers (*e.g.*, oligopeptides and oligonucleotides), and synthetic organic compounds based on various core structures. In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

[058] Agents that are employed in the methods of this invention can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the association of KID31 with its native binding partners or known antibodies. An example of randomly selected agents is the use of a chemical library or a peptide combinatorial library.

[059] As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a nonrandom basis that takes into account the sequence of the target site and/or its conformation in connection with the agent's action. With respect to anti-KID31 agents, it is currently believed that there are at least

three epitopes on KID31 against which antibodies can be raised and therefore at least three sites of action for agents that block KID31/anti-KID31 interaction. This invention also encompasses agents that act at the sites of interaction between KID31 and its native binding partner, although other ligands and their active KID31-interactive sites are also encompassed within the scope of this invention, whether currently known or later identified. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up the contact sites of the receptor/ligand and/or KID31/anti-KID31 antibody complex. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to an epitope appearing on KID31 as it is exposed on the surface of a living cell in its native environment. Such an agent will reduce or block the association of the anti-KID31 antibody with KID31, or the association of KID31 with its native ligand, as desired, by binding to the anti-KID31 antibody or to the native ligand.

[060] As used herein, the term "labeled", with regard to the antibody, is intended to encompass direct labeling of the antibody by coupling (*i.e.*, physically linking) a detectable substance, such as a radioactive agent or a fluorophore (*e.g.* fluorescein isothiocyanate (FITC) or phycoerythrin (PE)) to the antibody, as well as indirect labeling of the probe or antibody by reactivity with a detectable substance.

[061] As used herein, the term "association", with regard to the antibody, includes covalent and non-covalent attachment or binding to an agent (*e.g.*, chemotherapeutic agent). The antibody can be associated with an agent (*e.g.*, chemotherapeutic agent) by direct binding or indirect binding via attachment to a common platform, such that the antibody directs the localization of the agent to the cancerous cell to which the antibody binds and wherein the antibody and agent do not substantially dissociate under physiological conditions such that the agent is not targeted to the same cancerous cell to which the antibody binds or such that the agent's potency is not decreased.

[062] A "biological sample" encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition encompasses saliva, blood and other liquid samples of biological

origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom, and the progeny thereof, for example, cells obtained from a tissue sample collected from an individual suspected of having cancer, in preferred embodiments from ovary, lung, prostate, pancreas, colon, and breast tissue. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as proteins or polynucleotides, or embedding in a semi-solid or solid matrix for sectioning purposes. The term "biological sample" encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples.

[063] A "host cell" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected *in vivo* with a polynucleotide(s) of this invention.

[064] As used herein, "delaying development of metastasis" means to defer, hinder, slow, retard, stabilize, and/or postpone development of metastasis. This delay can be of varying lengths of time, depending on the history of the cancer and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the metastasis.

[065] An "effective amount" of a pharmaceutical composition, in one embodiment, is an amount sufficient to effect beneficial or desired results including, without limitation, clinical results such as shrinking the size of the tumor (in the cancer context, for example, breast or prostate cancer), retardation of cancerous cell growth, delaying the development of metastasis, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication such as via targeting and/or internalization, delaying the progression of the disease, and/or

prolonging survival of individuals. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to reduce the proliferation of (or destroy) cancerous cells and to reduce and/or delay the development, or growth, of metastases of cancerous cells, either directly or indirectly. In some embodiments, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more chemotherapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typical dosages comprise 0.1-to 100 mg/kg/body weight. The preferred dosages comprise 1-to 100-mg/kg/body weight. The most preferred dosages comprise 10-to 100-mg/kg/body weight.

[066] As used herein, a nucleic acid molecule or agent, antibody, composition or cell, etc., is said to be "isolated" when that nucleic acid molecule, agent, antibody, composition, or cell, etc. is substantially separated from contaminant nucleic acid molecules, antibodies, agents, compositions, or cells, etc. from its original source.

[067] An "individual" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, mice and rats.

[068] The terms "polypeptide", "oligopeptide", "peptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for

example, unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that, because the polypeptides of this invention are based upon an antibody, the polypeptides can occur as single chains or associated chains.

[069] Also encompassed within the scope of the invention are peptidomimetics of the KID31 peptide agonists, antagonists and modulators (including anti-KID31 antibodies) described herein. Such peptidomimetics include peptides wherein at least one amino acid residue is substituted with an amino acid residue that is not commonly found in nature, such as the D isomer of the amino acid or an N-alkylated species of the amino acid. In other embodiments, peptidomimetics are constructed by replacing at least one amide bond (--C(.dbd.O)--NH--) in a KID31 peptide agonist, antagonist or modulators with an amide isostere. Suitable amide isosteres include --CH.sub.2 --NH--, --CH.sub.2 --S--, --CH.sub.2 --S(O).sub.n -- (where n is 1 or 2), --CH.sub.2 --CH.sub.2 --, --CH.dbd.CH-- (E or Z), --C(.dbd.O)--CH.sub.2 --, --CH(CN)--NH--, --C(OH)--CH.sub.2 --, and --O--C(.dbd.O)--NH--. The amide bonds in a KID31 peptide agonist, antagonist or modulator that are suitable candidates for replacement with amide isosteres include bonds that are hydrolyzable by the endogenous esterases or proteases of the intended subject of KID31 peptide agonist, antagonist or modulator treatment.

[070] As used herein, "substantially pure" refers to material that is at least 50% pure (*i.e.*, free from contaminants), more preferably at least 90 % pure, more preferably at least 95% pure, more preferably at least 98% pure, more preferably at least 99% pure, or greater, pure.

[071] "Toxin" refers to any substance, which effects an adverse response within a cell. For example, a toxin directed to a cancerous cell would have an adverse, sometimes deleterious effect, on the cancerous cell. Examples of toxins include, but are not limited to, radioisotopes, calicheamicin, and maytansinoids.

[072] As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including and preferably clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: reducing the proliferation of (or

destroying) cancerous cells or other diseased, reducing metastasis of cancerous cells found in cancers, shrinking the size of the tumor, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, delaying the progression of the disease, and/or prolonging survival of individuals.

III. Methods of making antibodies and polypeptides

[073] Methods of making monoclonal antibodies are known in the art. One method which may be employed is the method of Kohler and Milstein, Nature 256:495-497 (1975) or a modification thereof. Typically, monoclonal antibodies are developed in non-human species, such as mice. In general, a mouse or rat is used for immunization but other animals may also be used. The antibodies are produced by immunizing mice with an immunogenic amount of cells, cell extracts, or protein preparations that contain human KID31. The immunogen can be, but is not limited to, primary cells, cultured cell lines, cancerous cells, nucleic acids, or tissue. In one embodiment, human fetal kidney epithelial cells are used. In another embodiment, human bladder or pancreatic progenitor cells are used. Methods for isolating and culturing human fetal kidney cells are detailed in Example 1. Cells used for immunization, for example, human fetal kidney, bladder cells or human pancreatic progenitor cells, may be cultured for a period of time (at least 24 hours) prior to their use as an immunogen. Cells (*e.g.*, human fetal kidney, bladder cells or human pancreatic progenitor cells) may be used as immunogens by themselves or in combination with a non-denaturing adjuvant, such as Ribi. In general, cells should be kept intact and preferably viable when used as immunogens. Intact cells may allow antigens to be better detected than ruptured cells by the immunized animal. Use of denaturing or harsh adjuvants, *e.g.*, Freud's adjuvant, may rupture the human fetal kidney or other cells and therefore is discouraged. The immunogen may be administered multiple times at periodic intervals such as, bi-weekly, or weekly, or may be administered in such a way as to maintain viability in the animal (*e.g.*, in a tissue recombinant). Example 2 describes methods used to generate anti-KID31

antibodies and may be used to generate other monoclonal antibodies, which bind to KID31.

[074] In one embodiment, monoclonal antibodies, which bind to KID31 are obtained by using host cells that over-express KID31 as an immunogen. Such cells include, by way of example and not by limitation, human fetal kidney cells and human lung cancer cells.

[075] To monitor the antibody response, a small biological sample (*e.g.*, blood) may be obtained from the animal and tested for antibody titer against the immunogen. The spleen and/or several large lymph nodes can be removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of non-specifically adherent cells) by applying a cell suspension to a plate or to a well coated with the antigen. B-cells, expressing membrane-bound immunoglobulin specific for the antigen, will bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, can then be fused with myeloma cells (*e.g.*, X63-Ag8.653 and those from the Salk Institute, Cell Distribution Center, San Diego, CA). Polyethylene glycol (PEG) may be used to fuse spleen or lymphocytes with myeloma cells to form a hybridoma. The hybridoma is then cultured in a selective medium (*e.g.*, hypoxanthine, aminopterin, thymidine medium, otherwise known as "HAT medium"). The resulting hybridomas are then plated by limiting dilution, and are assayed for the production of antibodies that bind specifically to the immunogen (*e.g.*, surface of the human fetal kidney cells, surface of cancer cell lines, Ag-KID31, fetal bladder sections, etc.) using FACS or immunohistochemistry (IHC screening). The selected monoclonal antibody-secreting hybridomas are then cultured either *in vitro* (*e.g.*, in tissue culture bottles or hollow fiber reactors), or *in vivo* (*e.g.*, as ascites in mice). Examples 2 and 3 provides further details about the methods utilized to obtain, purify and screen an anti-KID31 antibody.

[076] As another alternative to the cell fusion technique, EBV immortalized B cells may be used to produce monoclonal antibodies of the subject invention. The hybridomas are expanded and subcloned, if desired, and supernatants are assayed for anti-immunogen activity by conventional assay procedures (*e.g.*,

FACS, IHC, radioimmunoassay, enzyme immunoassay, fluorescence immunoassay, etc.).

[077] In another alternative, monoclonal antibody anti-KID31 and any other equivalent antibodies can be sequenced and produced recombinantly by any means known in the art (*e.g.*, humanization, use of transgenic mice to produce fully human antibodies, phage display technology, etc.). In one embodiment, anti-KID31 monoclonal antibody is sequenced and the polynucleotide sequence is then cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use.

[078] The polynucleotide sequence of monoclonal antibody anti-KID31 and any other equivalent antibodies may be used for genetic manipulation to generate a "humanized" antibody, to improve the affinity, or other characteristics of the antibody. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences. There are four general steps to humanize a monoclonal antibody. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains (2) designing the humanized antibody, i.e., deciding which antibody framework region to use during the humanizing process (3) the actual humanizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patent Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415.

[079] A number of "humanized" antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent or modified rodent V regions and their associated complementarity determining regions (CDRs) fused to human constant domains. See, for example, Winter et al. *Nature* 349:293-299 (1991), Lobuglio et al. *Proc. Nat. Acad. Sci. USA* 86:4220-4224 (1989), Shaw et al. *J Immunol.* 138:4534-4538 (1987), and Brown et al. *Cancer Res.* 47:3577-3583 (1987). Other references describe rodent CDRs grafted into a human supporting

framework region (FR) prior to fusion with an appropriate human antibody constant domain. See, for example, Riechmann et al. *Nature* 332:323-327 (1988), Verhoeyen et al. *Science* 239:1534-1536 (1988), and Jones et al. *Nature* 321:522-525 (1986). Another reference describes rodent CDRs supported by recombinantly veneered rodent framework regions. See, for example, European Patent Publication No. 519,596. These "humanized" molecules are designed to minimize unwanted immunological response toward rodent anti-human antibody molecules, which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty et al., *Nucl. Acids Res.*, 19:2471-2476 (1991) and in U.S. Patent Nos. 6,180,377; 6,054,297; 5,997,867; and 5,866,692.

[080] The invention also encompasses single chain variable region fragments ("scFv") of antibodies of this invention, such as mu-anti-KID31. Single chain variable region fragments are made by linking light and/or heavy chain variable regions by using a short linking peptide. Bird et al. (1988) *Science* 242: 423-426 describes example of linking peptides which bridge approximately 3.5 nm between the carboxy terminus of one variable region and the amino terminus of the other variable region. Linkers of other sequences have been designed and used, Bird et al. (1988). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[081] The invention includes modifications to KID31 agonists, antagonists, modulators and antibodies, including functionally equivalent antibodies and polypeptides that do not significantly affect their properties and variants that

have enhanced or decreased activity. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs. Amino acid residues which can be conservatively substituted for one another include but are not limited to: glycine/alanine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; serine/threonine; lysine/arginine; and phenylalanine/tryosine. These polypeptides also include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Preferably, the amino acid substitutions would be conservative, i.e., the substituted amino acid would possess similar chemical properties as that of the original amino acid. Such conservative substitutions are known in the art, and examples have been provided above. Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the variable region. Changes in the variable region can alter binding affinity and/or specificity. Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay, such as the attachment of radioactive moieties for radioimmunoassay. Modified polypeptides are made using established procedures in the art and can be screened using standard assays known in the art.

[082] The invention also encompasses fusion proteins comprising one or more fragments or regions from the polypeptides and antibodies of this invention. In one embodiment, a fusion polypeptide is provided that comprises at least 10 contiguous amino acids of variable light chain region and at least 10 amino acids of variable heavy chain region. In another embodiment, the fusion polypeptide contains a heterologous immunoglobulin constant region. In another embodiment, the fusion polypeptide contains a light chain variable region and a

heavy chain variable region of an antibody produced from a hybridoma deposited with the ATCC as described herein. For purposes of this invention, an antibody fusion protein contains one or more anti-KID31 polypeptides and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region.

[083] An anti-KID31 polypeptide, and other KID31 agonists, antagonists and modulators can be created by methods known in the art, for example, synthetically or recombinantly. One method of producing KID31 peptide agonists, antagonists and modulators involves chemical synthesis of the polypeptide, followed by treatment under oxidizing conditions appropriate to obtain the native conformation, that is, the correct disulfide bond linkages. This can be accomplished using methodologies well known to those skilled in the art (see Kelley, R. F. & Winkler, M. E. in Genetic Engineering Principles and Methods, Setlow, J. K., ed., Plenum Press, N.Y., vol. 12, pp 1-19 (1990); Stewart, J. M. & Young, J. D. Solid Phase Peptide Synthesis Pierce Chemical Co. Rockford, Ill. (1984); see also U.S. Pat. Nos. 4,105,603; 3,972,859; 3,842,067; and 3,862,925).

[084] Polypeptides of the invention may be conveniently prepared using solid phase peptide synthesis (Merrifield, J. Am. Chem. Soc., 85:2149 (1964); Houghten, Proc. Natl. Acad. Sci. USA 82:5132 (1985)).

[085] In yet another alternative, fully human antibodies may be obtained by using commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (e.g., fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are Xenomouse ™ from Abgenix, Inc. (Fremont, CA) and HuMAb-Mouse® and TC Mouse™ from Medarex, Inc. (Princeton, NJ).

[086] In an alternative, antibodies may be made recombinantly and expressed using any method known in the art. Antibodies may be made recombinantly by first isolating the antibodies made from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (*e.g.*, CHO cells). Another method that may be employed is to express

the antibody sequence in plants (*e.g.*, tobacco) or transgenic milk. Methods for expressing antibodies recombinantly in plants or milk have been disclosed. See, for example, Peeters, et al. (2001) *Vaccine* 19:2756; Lonberg, N. and D. Huszar (1995) *Int.Rev.Immunol* 13:65; and Pollock, et al.(1999) *J Immunol Methods* 231:147. Methods for making derivatives of antibodies, *e.g.*, humanized, single chain, etc. are known in the art. In another alternative, antibodies may be made recombinantly by phage display technology. See, for example, U.S. Patent Nos. 5,565,332; 5,580,717; 5,733,743; 6,265,150; and Winter et al., *Annu. Rev. Immunol.* 12:433-455 (1994).

[087] The antibodies or protein of interest may be subjected to sequencing by Edman degradation, which is well known to those of skill in the art. The peptide information generated from mass spectrometry or Edman degradation can be used to design probes or primers that are used to clone the protein of interest.

[088] An alternative method of cloning the protein of interest is by "panning" using purified KID31 or portions thereof for cells expressing the antibody or protein of interest. The "panning" procedure is conducted by obtaining a cDNA library from tissues or cells that express the antibody or protein of interest, over-expressing the cDNAs in a second cell type, and screening the transfected cells of the second cell type for a specific binding to KID31. Detailed descriptions of the methods used in cloning mammalian genes coding for cell surface proteins by "panning" can be found in the art. See, for example, Aruffo, A. and Seed, B. *Proc. Natl. Acad. Sci. USA*, 84, 8573-8577 (1987) and Stephan, J. et al., *Endocrinology* 140: 5841-5854 (1999).

[089] cDNAs encoding anti-KID31 antibodies, and other KID31 peptide agonists, antagonists and modulators can be obtained by reverse transcribing the mRNAs from a particular cell type according to standard methods in the art. Specifically, mRNA can be isolated using various lytic enzymes or chemical solutions according to the procedures set forth in Sambrook, et al. *supra* or extracted by commercially available nucleic-acid-binding resins following the accompanying instructions provided by manufacturers (*e.g.*, Qiagen, Invitrogen, Promega). The synthesized cDNAs are then introduced into an expression vector to produce the antibody or protein of interest in cells of a second type. It

is implied that an expression vector must be replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, and cosmids.

[090] The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (*e.g.*, where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[091] Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of mammalian host cells include but not limited to COS, HeLa, and CHO cells. Preferably, the host cells express the cDNAs at a level of about 5 fold higher, more preferably 10 fold higher, even more preferably 20 fold higher than that of the corresponding endogenous antibody or protein of interest, if present, in the host cells. Screening the host cells for a specific binding to KID31 is effected by an immunoassay or FACS. A cell over-expressing the antibody or protein of interest can be identified.

[092] Various techniques are also available which may now be employed to produce mutant KID31 peptide agonists, antagonists, and modulators which encodes for additions, deletions, or changes in amino acid sequence of the resultant protein relative to the parent KID31 peptide agonist, antagonist or modulator molecule.

[093] The invention includes polypeptides comprising an amino acid sequence of the antibodies of this invention. The polypeptides of this invention can be made by procedures known in the art. The polypeptides can be produced by proteolytic or other degradation of the antibodies, by recombinant methods (i.e., single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical

synthesis are known in the art and are commercially available. For example, an anti-KID31 polypeptide could be produced by an automated polypeptide synthesizer employing the solid phase method.

IV. Methods for screening polypeptides and monoclonal antibodies

[094] Several methods may be used to screen polypeptides and monoclonal antibodies that bind to KID31. It is understood that "binding" refers to biologically or immunologically relevant binding, *i.e.*, binding which is specific for the unique antigen for which the immunoglobulin molecule is encoded, or to which the polypeptide is directed. It does not refer to non-specific binding that may occur when an immunoglobulin is used at a very high concentration against a non-specific target. In one embodiment, monoclonal antibodies are screened for binding to KID31 using standard screening techniques. In this manner, anti-KID31 monoclonal antibody was obtained. In accordance with the Budapest Treaty, a hybridoma which produces anti-KID31 monoclonal antibodies has been deposited in the American Type Culture Collection (ATCC) 10801 University Blvd., Manassas VA 20110-2209 on March 20, 2003 with a Patent Deposit Designation of PTA# 6516.

[095] Monoclonal antibodies that bind to KID31 are screened for binding to cancerous tissues and non-cancerous cells. In one embodiment, monoclonal antibodies which bind to KID31 and that are also cross reactive to human cancerous cells or tissues, but not to normal cells or tissues to the same degree, are selected. One method that may be employed for screening is immunohistochemistry (IHC). Standard immunohistochemical techniques are known to those of average skill in the art. See, for example, *Animal Cell Culture Methods* (J.P. Mather and D. Barnes, eds., Academic Press, Vol. 57, Ch. 18 and 19, pp. 314-350, 1998). Biological samples (*e.g.*, tissues) may be obtained from biopsies, autopsies, or necropsies. To ascertain if KID31 is present only on cancerous cells, anti-KID31 antibodies may be used to detect the presence of KID31 on tissues from individuals with cancer while other non-cancerous tissues from the individual suffering from cancer or tissues from individuals without cancer are used as a control. The tissue can be embedded in a solid or semi-solid

substance that prevents damage during freezing (*e.g.*, agarose gel or OCT) and then sectioned for staining. Cancers from different organs and at different grades can be used to screen monoclonal antibodies. Examples of tissues that may be used for screening purposes include but are not limited to ovary, breast, lung, prostate, colon, kidney, skin, thyroid, brain, heart, liver, stomach, nerve, blood vessels, bone, upper digestive tract, and pancreas. Examples of different cancer types that may be used for screening purposes include but are not limited to carcinomas, adenocarcinomas, sarcomas, adenosarcomas, lymphomas, and leukemias.

[096] In yet another alternative, cancerous cells lines such as BT474 (ATCC # HTB-20), MCF7 (ATCC# HTB-22), ES-2 (ATCC# CRL-1978), SKOV3 (ATCC # HTB-77), SKMES-1 (ATCC# HTB-58), CA130 (Raven proprietary lung adenocarcinoma cell line), CaLu3 (ATCC# HTB-55), 9926 (Raven proprietary pancreas adenocarcinoma cell line, AsPC-1 (ATCC# CRL-1682), Hs700T (ATCC# HTB-147), Colo205 (ATCC# CCL-222), HT-29 (HTB-38), Cos7 (ATCC# CRL-1651), RL-65 (ATCC# CRL-10345), A204 (ATCC# HTB-82), G292 (ATCC# CRL-1423), HT1080 (ATCC# CCL-121), MG63 (ATCC# CRL-1427), RD (ATCC# CCL-136), RD-ES (ATCC# HTB-166), SKES-1 (ATCC# HTB-86), SKLMS-1 (ATCC# HTB-88), SKUT-1 (ATCC# HTB-114), SW684 (ATCC# HTB-91), SW872 (ATCC# HTB-92), 786-O (ATCC# CRL-1932), A498 (ATCC# HTB-44), Caki-2 (ATCC# HTB-47), 22RV1 (ATCC# CRL-2505), DU145 (ATCC# HTB-81), LNCaP (ATCC# CRL-1740), and normal cells from their respective tissues may be used to screen for monoclonal antibodies which are specific for cancerous tissue. Primary, or low passage, cell cultures derived from normal tissues from different organs, including but not limited to, kidney, ovary, breast, lung, prostate, colon, kidney, skin, thyroid, aortic smooth muscle, and endothelial cells can be used as negative controls. The cancerous or non-cancerous cells can be grown on glass slides or coverslips, or on plastic surfaces, or prepared in a CellArray™, as described in WO 01/43869, and screened for the binding of antibody using IHC as described above for tissues. Alternatively, cells may be removed from the growth surface using non-proteolytic means and spun into a pellet, which is then embedded and treated as tissues for IHC analysis as described above. Cells may be inoculated

into immunodeficient animals, a tumor allowed to grow, and then this tumor may be harvested, embedded, and used as a tissue source for IHC analysis. In another alternative, single cells may be screened by incubating with the primary antibody, a secondary "reporter" antibody linked to a fluorescent molecule and then analyzed using a fluorescent activated cell-sorting (FACS) machine.

[097] Several different detection systems may be utilized to detect binding of antibodies to tissue section. Typically, immunohistochemistry involves the binding of a primary antibody to the tissue and then a secondary antibody reactive against the species from the primary antibody was generated and conjugated to a detectable marker (*e.g.*, horseradish peroxidase, HRP, or diaminobenzedine, DAB). One alternative method that may be used is polyclonal mirror image complementary antibodies or polyMICA. PolyMICA (polyclonal Mirror Image Complementary Antibodies) technique, described by D.C. Mangham and P.G. Isaacson (*Histopathology* (1999) 35(2):129-33), can be used to test binding of primary antibodies (*e.g.*, anti-KID31 antibodies) to normal and cancerous tissue. Several kinds of polyMICA™ Detection kits are commercially available from The Binding Site Limited (P.O. Box 4073 Birmingham B29 6AT England). Product No. HK004.D is a polyMICA™ Detection kit which uses DAB chromagen. Product No. HK004.A is a polyMICA™ Detection kit which uses AEC chromagen. Alternatively, the primary antibody may be directly labeled with the detectable marker.

[098] The first step in IHC screening to select for an appropriate antibody is the binding of primary antibodies raised in mice (*e.g.*, anti-KID31 antibodies) to one or more immunogens (*e.g.*, cells or tissue samples). In one embodiment, the tissue sample is sections of frozen tissue from different organs. The cells or tissue samples can be either cancerous or non-cancerous.

[099] Frozen tissues can be prepared, sectioned, with or without fixation, and IHC performed by any of a number of methods known to one familiar with the art. See, for example, Stephan et al. *Dev. Biol.* 212: 264-277 (1999), and Stephan et al. *Endocrinology* 140: 5841-54 (1999).

V. Methods of characterizing anti-KID31 antibodies

[0100] Several methods can be used to characterize anti-KID31 antibodies. One method is to identify the epitope to which it binds. Epitope mapping is commercially available from various sources, for example, Pepscan Systems (Edelhertweg 15, 8219 PH Lelystad, The Netherlands). Epitope mapping can be used to determine the sequence to which an anti-KID31 antibody binds. The epitope can be a linear epitope, *i.e.*, contained in a single stretch of amino acids, or a conformational epitope formed by a three-dimensional interaction of amino acids that may not necessarily be contained in a single stretch. Peptides of varying lengths (*e.g.*, at least 4-6 amino acids long) can be isolated or synthesized (*e.g.*, recombinantly) and used for binding assays with anti-KID31 antibody. The epitope to which anti-KID31 antibody binds can be determined in a systematic screening by using overlapping peptides derived from the extracellular sequence and determining binding by anti-KID31 antibody.

[0101] Yet another method that can be used to characterize an anti-KID31 antibody is to use competition assays with other antibodies known to bind to the same antigen, *i.e.*, KID31 to determine if anti-KID31 antibodies binds to the same epitope as other antibodies. Examples of commercially available antibodies to KID31 may be available and may be identified using binding and competition assays that are well known in the art.. Anti-KID31 antibodies can be further characterized by the tissues, type of cancer or type of tumor to which they bind.

[0102] Another method of characterizing anti-KID31 antibodies is by the antigen to which it binds. Anti-KID31 antibodies were used in Western blots with cell lysates from various human cancers. As is known to one of skill in the art, Western blotting can involve running cell lysates and/or cell fractions on a denaturing or non-denaturing gel, transferring the proteins to nitrocellulose paper, and then probing the blot with an antibody (*e.g.*, anti-KID31 antibody) to see which proteins are bound by the antibody. Alternatively, proteins on the cell surface can be biotinylated and anti-KID31 antibodies can be used to immunoprecipitate biotinylated KID31. Biotinylated KID31 can then be visualized using HRP-strepavidin. This procedure is detailed further in Example 4. KID31 is associated with various human cancers of different tissues including

but not limited to lung, kidney, pancreatic, ovarian, and colon. Further description of KID31 expression is given in Example 5 and 6.

VI. Methods of diagnosing cancer using anti-KID31 antibodies and KID31 modulators

[0103] Monoclonal antibodies to KID31 made by the methods disclosed herein may be used to identify the presence or absence of cancerous cells in a variety of tissues, including but not limited to, ovary, breast, lung, prostate, colon, kidney, pancreas, skin, thyroid, brain, heart, liver, stomach, nerve, blood vessels, bone, and upper digestive tract, for purposes of diagnosis. Monoclonal antibodies to KID31 made by the methods disclosed herein may also be used to identify the presence or absence of cancerous cells, or the level thereof, which are circulating in blood after their release from a solid tumor. Such circulating antigen may be an intact KID31 antigen, or a fragment thereof that retains the ability to be detected according to the methods taught herein. Such detection may be effected by FACS analysis using standard methods commonly used in the art.

[0104] These uses can involve the formation of a complex between KID31 and an antibody that binds specifically to KID31. Examples of such antibodies include but are not limited to those anti-KID31 monoclonal antibodies produced by the hybridoma deposited in the ATCC with the designation PTA# 6516. The formation of such a complex can be *in vitro* or *in vivo*. Without being bound by theory, monoclonal antibody anti-KID31 can bind to KID31 through the extracellular domain of KID31 and may then be internalized.

[0105] In a preferred embodiment of the diagnostic methods of this invention, the antibody bears a detectable label. Examples of labels that may be used include a radioactive agent or a fluorophore, such as fluoroisothiocyanate or phycoerythrin.

[0106] As with other known antibodies used commercially for diagnostic and therapeutic purposes, the target antigen of this invention is broadly expressed in normal tissue. It is also up regulated in some tumors. Therefore, the particular dosages and routes of delivery of the antibodies of this invention as used for

diagnostic or therapeutic agents will be tailored to the particular tumor or disease state at hand, as well as to the particular individual being treated.

[0107] One method of using the antibodies for diagnosis is *in vivo* tumor imaging by linking the antibody to a radioactive or radioopaque agent, administering the antibody to the individual and using an x-ray or other imaging machine to visualize the localization of the labeled antibody at the surface of cancer cells expressing the antigen. The antibody is administered at a concentration that promotes binding at physiological conditions.

[0108] *In vitro* techniques for detection of KID31 are routine in the art and include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations, immunofluorescence, enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis.

[0109] In aspects of this invention, methods of radioimaging of tumours or neoplasms, or of measuring the effectiveness of a method of treatment with a radiolabelled antibody, comprising the step of administering a radiolabelled, tumour-specific antibody to an individual following the practice of this invention. The radiolabelled antibody may be a monoclonal or polyclonal antibody comprising a radiolabel, preferably selected from the group consisting of Technetium-99m, Indium-111, Iodine-131, Rhenium-186, Rhenium-188, Samarium-153, Lutetium-177, Copper-64, Scandium-47, Yttrium-90. Monoclonal antibodies labelled with therapeutic radionuclides such as Iodine-131, Rhenium-188, Holmium-166, Samarium-153 and Scandium-47, which do not compromise the immunoreactivity of antibodies and are not broken down *in vivo*, are especially preferred. The person skilled in the art will appreciate that other radioactive isotopes are known, and may be suitable for specific applications. The radioimaging may be conducted using Single Photon Emission Computer Tomography (SPECT), Position Emmission Tomography (PET), Computer Tomography (CT) or Magnetic Resonance Imaging (MRI). Correlative imaging, which permits greater anatomical definition of location of metastases located by radioimmunoimaging, is also contemplated.

[0110] In other methods, the cancerous cells are removed and the tissue prepared for immunohistochemistry by methods well known in the art (*e.g.*,

embedding in a freezing compound, freezing and sectioning, with or without fixation; fixation and paraffin embedding with or without various methods of antigen retrieval and counterstaining). The monoclonal antibodies may also be used to identify cancerous cells at different stages of development. The antibodies may also be used to determine which individuals' tumors express the antigen on their surface at a pre-determined level and are thus candidates for immunotherapy using antibodies directed against said antigen. The antibodies may recognize both primary and metastasizing cancers of the kidney, ovary, prostate and pancreas and primary cancers of the lung that express KID31. As used herein, detection may include qualitative and/or quantitative detection and may include comparing the level measured to a normal cell for an increased level of expression of KID31 in cancerous cells.

[0111] The invention also provides methods of aiding diagnosis of cancer (such as ovarian, lung, pancreatic, prostate, colon, or breast cancer) in an individual using any antibody that binds to KID31 and any other methods that can be used determine the level of KID31 expression. As used herein, methods for "aiding diagnosis" means that these methods assist in making a clinical determination regarding the classification, or nature, of cancer, and may or may not be conclusive with respect to the definitive diagnosis. Accordingly, a method of aiding diagnosis of cancer can comprise the step of detecting the level of KID31 in a biological sample from the individual and/or determining the level of KID31 expression in the sample. Antibodies recognizing the antigen or a portion thereof may also be used to create diagnostic immunoassays for detecting antigen released or secreted from living or dying cancer cells in bodily fluids, including but not limited to, blood, saliva, urine, pulmonary fluid, or ascites fluid.

[0112] Not all cells in a particular tumor of interest will express KID31, and cancerous cells in other tissues may express KID31, thus an individual should be screened for the presence or absence of KID31 on cancerous cells to determine the usefulness of immunotherapy in the individual. The anti-KID31 antibodies made by the methods disclosed herein may be used to determine whether an individual diagnosed with cancer may be deemed a candidate for immunotherapy using antibodies directed against KID31. In one embodiment, a

cancerous tumor or a biopsy sample may be tested for expression of KID31, using antibodies directed against KID31. Individuals with cancer cells that express KID31 are suitable candidates for immunotherapy using antibodies directed against KID31. Staining with anti-KID31 antibody may also be used to distinguish cancerous tissues from normal tissues.

[0113] Methods of using anti-KID31 antibodies for diagnostic purposes are useful both before and after any form of anti-cancer treatment, *e.g.*, chemotherapy or radiation therapy, to determine which tumors are most likely to respond to a given treatment, prognosis for individual with cancer, tumor subtype or origin of metastatic disease, and progression of the disease or response to treatment.

[0114] The compositions of this invention are also suitable for diagnosis of disease states other than cancer, using the methods generally described above in application with other diseased (non-cancerous) cells. Disease states suitable for use in the methods of this invention include, but are not limited to, diseases or disorders associated with inflammatory or autoimmune responses in individuals.

The methods described above may be used for modulating inflammatory or autoimmune responses in individuals. Diseases and conditions resulting from inflammation and autoimmune disorders that may be subject to diagnosis and/or treatment using the compositions and methods of the invention include, by way of illustration and not of limitation, multiple sclerosis, meningitis, encephalitis, stroke, other cerebral traumas, inflammatory bowel disease including ulcerative colitis and Crohn's disease, myasthenia gravis, lupus, rheumatoid arthritis, asthma, acute juvenile onset diabetes, AIDS dementia, atherosclerosis, nephritis, retinitis, atopic dermatitis, psoriasis, myocardial ischemia and acute leukocyte-mediated lung injury.

[0115] Still other indications for diagnostic and/or therapeutic use of antibodies and other therapeutic agents of the invention include administration to individuals at risk of organ or graft rejection. Over recent years there has been a considerable improvement in the efficiency of surgical techniques for transplanting tissues and organs such as skin, kidney, liver, heart, lung, pancreas and bone marrow. Perhaps the principal outstanding problem is the lack of satisfactory agents for inducing immunotolerance in the recipient to the

transplanted allograft or organ. When allogeneic cells or organs are transplanted into a host (i.e., the donor and donee are different individuals from the same species), the host immune system is likely to mount an immune response to foreign antigens in the transplant (host-versus-graft disease) leading to destruction of the transplanted tissue.

[0116] Uses described anywhere in this application that recite their use for anti-KID31 antibodies also encompass the use of other KID31 agonists, antagonists and modulators as described herein. In such embodiments, the KID31 agonists, antagonist or other non-antibody modulator is substituted for the KID31 antibody in the steps described, and alterations within the scope of the ordinarily skilled practitioner are made to tailor the method to the substituted KID31 modulatory composition.

VII. Compositions of this invention

[0117] This invention also encompasses compositions, including pharmaceutical compositions, comprising anti-KID31 antibodies, polypeptides derived from anti-KID31 antibodies, polynucleotides comprising sequence encoding anti-KID31 antibodies, and other agents as described herein. As used herein, compositions further comprises one or more antibodies, polypeptides and/or proteins that bind to KID31, KID31 agonists, antagonists, modulators, and/or one or more polynucleotides comprising sequences encoding one or more antibodies, polypeptides and proteins that bind to KID31.

[0118] The invention further provides for conjugates of any KID31 peptide agonist, antagonist or modulator, and additional chemical structures that support the intended function or functions of the particular KID31 peptide agonist, antagonist or modulator. These conjugates include KID31 peptide agonist, antagonist or modulator covalently bound to a macromolecule such as any insoluble, solid support matrix used in the diagnostic, screening or purification procedures discussed herein. Suitable matrix materials include any substance that is chemically inert, has high porosity and has large numbers of functional groups capable of forming covalent linkages with peptide ligands. Examples of matrix materials and procedures for preparation of matrix-ligand conjugates are

described in Dean et al. (eds) Affinity Chromatography: A Practical Approach, IRL Press (1985); Lowe, "An Introduction to Affinity Chromatography", in Work et al. (eds) Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 7, Part II, North-Holland (1979); Porath et al., "Biospecific Affinity Chromatography", in Neurath et al. (eds), The Proteins, 3rd ed., Vol. 1, pp. 95-178 (1975); and Schott, Affinity Chromatography, Dekker (1984).

[0119] Also provided herein are conjugates of KID31 peptide agonist, antagonist or modulator and any reporter moiety used in the diagnostic procedures discussed herein.

[0120] The KID31 peptide agonist, antagonist or modulator agents, polypeptides and proteins of this invention, including anti-KID31 antibodies, are further identified and characterized by any (one or more) of the following criteria: (a) ability to bind to KID31 (including KID31 on cancer cells, including but not limited to ovarian, prostate, pancreatic, lung, colon, or breast cancer cells); (b) ability to competitively inhibits preferential binding of a known anti-KID31 antibody to KID31, including the ability to preferentially bind to the same KID31 epitope to which the original antibody preferentially binds; (c) ability to bind to a portion of KID31 that is exposed on the surface of a living cell *in vitro* or *in vivo;* (d) ability to bind to a portion of KID31 that is exposed on the surface of living cancer cells, such as but not limited to ovarian, prostate, pancreatic, lung, colon, or breast cancer cells; (e) ability to deliver a chemotherapeutic agent or detectable marker to cancerous cells (such as but not limited to ovarian, prostate, pancreatic, lung, colon, or breast cancer cells) expressing KID31; (f) ability to deliver a therapeutic agent into cancerous cells (such as but not limited to ovarian cancer cells) expressing KID31.

[0121] In some embodiments, the antibody of the invention is an antibody that is produced by a host cell with a deposit number of ATCC Nos. PTA# 6516, or progeny thereof. The present invention also encompasses various formulations of antibodies produced by these deposited hybridomas and equivalent antibodies or polypeptide fragments (*e.g.*, Fab, Fab', $F(ab')_2$, Fv, Fc, etc.), chimeric antibodies, single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion, humanized antibodies, and any other modified configuration

of any of these or equivalent antibodies that comprises an antigen (KID31), recognition site of the required specificity. The invention also provides human antibodies displaying one or more of the biological characteristics of an anti-KID31 family member. The equivalent antibodies of the anti-KID31 family (including humanized antibodies and human antibodies), polypeptide fragments, and polypeptides comprising any of these fragments are identified and characterized by any (one or more) of the five criteria described above.

[0122] In some embodiments, the antibodies, polypeptides and proteins of the invention that bind to KID31 are antibodies, polypeptides and proteins that competitively inhibit preferential binding of a herein-specified anti-KID31 antibody to KID31. In some embodiments, the antibodies, the polypeptides and the proteins preferentially bind to the same epitope on KID31 as the antibody mu-anti-KID31 preferentially binds.

[0123] Accordingly, the invention provides any of the following (or compositions, including pharmaceutical compositions, comprising any of the following): (a) an antibody produced by the host cell with a deposit number identified above or its progeny; (b) a humanized form of such an antibody; (c) an antibody comprising one or more of the light chain and/or heavy chain variable regions of such an antibody; (d) a chimeric antibody comprising variable regions homologous or derived from variable regions of a heavy chain and a light chain of such an antibody, and constant regions homologous or derived from constant regions of a heavy chain and a light chain of a human antibody; (e) an antibody comprising one or more of the light chain and/or heavy chain CDRs (at least one, two, three, four, five, or six) of such an antibody; (f) an antibody comprising a heavy and/or a light chain of such an antibody; (g) a human antibody that is equivalent to such an antibody. A humanized form of the antibody may or may not have CDRs identical to that original antibody, or antibody produced by a host cell with a deposit number identified above. Determination of CDR regions is well within the skill of the art. In some embodiments, the invention provides an antibody which comprises at least one CDR that is substantially homologous to at least one CDR, at least two, at least three, at least four, at least 5 CDRs of an antibody produced by one of the above-identified deposited hybridomas (or, in

some embodiments substantially homologous to all 6 CDRs of one of these antibodies, or derived from one of these antibodies), or antibody produced by the host cell with a deposit number identified above. Other embodiments include antibodies that have at least two, three, four, five, or six CDR(s) that are substantially homologous to at least two, three, four, five or six CDRs of an antibody produced from a hybridoma deposited as identified herein, or derived from such an antibody. It is understood that, for purposes of this invention, binding specificity and/or overall activity (which may be in terms of delivering a chemotherapeutic agent to or into cancerous cells to reduce the growth and/or proliferation of cancer cells, to induce apoptotic cell death in the cancer cell, to delay the development of metastasis, and/or treating palliatively) is generally retained, although the extent of activity may vary compared to an antibody produced by a deposited hybridoma (may be greater or lesser). The invention also provides methods of making any of these antibodies. Methods of making antibodies are known in the art and are described herein.

[0124] The invention also provides polypeptides comprising an amino acid sequence of the antibodies of the invention. In some embodiments, the polypeptide comprises one or more of the light chain and/or heavy chain variable regions of the antibody. In some embodiments, the polypeptide comprises one or more of the light chain and/or heavy chain CDRs of the antibody. In some embodiments, the polypeptide comprises three CDRs of the light chain and/or heavy chain of the antibody. In some embodiments, the polypeptide comprises an amino acid sequence of the antibody that has any of the following: at least 5 contiguous amino acids of a sequence of the original antibody, at least 8 contiguous amino acids, at least about 10 contiguous amino acids, at least about 15 contiguous amino acids, at least about 20 contiguous amino acids, at least about 25 contiguous amino acids, at least about 30 contiguous amino acids, wherein at least 3 of the amino acids are from a variable region of the antibody. In one embodiment, the variable region is from a light chain of the original antibody. In another embodiment, the variable region is from a heavy chain of the antibody. In another embodiment, the 5 (or more)

contiguous amino acids are from a complementarity-determining region (CDR) of the antibody.

[0125] In some embodiments of this invention, cells of this invention that express KID31, a portion of KID31, anti-KID31 antibodies or other KID31-binding polypeptides of this invention are administered directly to an individual to modulate their *in vivo* KID31 biological activity.

VIII. Methods of using KID31 modulators and anti-KID31 antibodies for therapeutic purposes

[0126] Monoclonal antibodies to KID31 may be used for therapeutic purposes in individuals with cancer or other diseases. Therapy with anti-KID31 antibodies can involve formation of complexes both *in vitro* and *in vivo* as described above. In one embodiment, monoclonal antibody anti-KID31 can bind to and reduce the proliferation of cancerous cells. It is understood that the antibody is administered at a concentration that promotes binding at physiological (*e.g., in vivo*) conditions. In another embodiment, monoclonal antibodies to KID31 can be used for immunotherapy directed at cancerous cells of different tissues such as lung, kidney, pancreatic, ovarian, and colon and other types of cancer such as sarcoma. In another embodiment, monoclonal antibody anti-KID31 alone can bind to and reduce cell division in the cancer cell. In another embodiment, monoclonal antibody anti-KID31 can bind to cancerous cells and delay the development of metastasis. In yet another embodiment, an individual with cancer is given palliative treatment with anti-KID31 antibody. Palliative treatment of a cancer individual involves treating or lessening the adverse symptoms of the disease, or iatrogenic symptoms resulting from other treatments given for the disease without directly affecting the cancer progression. This includes treatments for easing of pain, nutritional support, sexual problems, psychological distress, depression, fatigue, psychiatric disorders, nausea, vomiting, etc.

[0127] In such situations, the anti-KID31 antibody may be administered with agents that enhance or direct an individual's own immune response, such as an agent that strengthens antibody-dependent cellular cytotoxicity (ADCC).In other

embodiments, at least one fucose residue present in an anti-KID31 antibody is removed from the oligosaccharides of that antibody, a modification to enhance ADCC. In similar embodiments, fucose residues present in an anti-KID31 antibody are modified to alter their composition to the extent required to enhance ADCC compared to the original, unmodified antibody.

[0128] In yet another embodiment, anti-KID31 antibody be conjugated to or associated with a radioactive molecule, toxin (*e.g.*, calicheamicin), chemotherapeutic molecule, liposomes or other vesicles containing chemotherapeutic compounds and administered to an individual in need of such treatment to target these compounds to the cancer cell containing the antigen recognized by the antibody and thus eliminate cancerous or diseased cells. Without being limited to any particular theory, the anti-KID31 antibody is internalized by the cell bearing KID31 at its surface, thus delivering the conjugated moiety to the cell to induce the therapeutic effect. In yet another embodiment, the antibody can be employed as adjuvant therapy at the time of the surgical removal of a cancer expressing the antigen in order to delay the development of metastasis. The antibody can also be administered before surgery (neoadjuvant therapy) in an individual with a tumor expressing the antigen in order to decrease the size of the tumor and thus enable or simplify surgery, spare tissue during surgery, and/or decrease the resulting disfigurement.

[0129] Cell cycle dosing is contemplated in the practice of this invention. In such embodiments, a chemotherapeutic agent is used to synchronize the cell cycle of the tumor or other target diseased cells at a pre-determined stage. Subsequently, administration of the anti-KID31 antibody of this invention (alone or with an additional therapeutic moiety) is made. In alternative embodiments, an anti-KID31 antibody is used to synchronize the cell cycle and reduce cell division prior to administration of a second round of treatment; the second round may be administration of an anti-KID31 antibody and/or an additional therapeutic moiety.

[0130] Chemotherapeutic agents include radioactive molecules, toxins, also referred to as cytotoxins or cytotoxic agents, which includes any agent that is

detrimental to the viability of cancerous cells, agents, and liposomes or other vesicles containing chemotherapeutic compounds. Examples of suitable chemotherapeutic agents include but are not limited to 1-dehydrotestosterone, 5-fluorouracil decarbazine, 6-mercaptopurine, 6-thioguanine, actinomycin D, adriamycin, aldesleukin, alkylating agents, allopurinol sodium, altretamine, amifostine, anastrozole, anthramycin (AMC)), anti-mitotic agents , cis-dichlorodiamine platinum (II) (DDP) cisplatin), diamino dichloro platinum, anthracyclines, antibiotics, antimetabolites, asparaginase, BCG live (intravesical), betamethasone sodium phosphate and betamethasone acetate, bicalutamide, bleomycin sulfate, busulfan, calcium leucouorin, calicheamicin, capecitabine, carboplatin, lomustine (CCNU), carmustine (BSNU), Chlorambucil, Cisplatin, Cladribine, Colchicin, conjugated estrogens, Cyclophosphamide, Cyclothosphamide, Cytarabine, Cytarabine, cytochalasin B, Cytoxan, Dacarbazine, Dactinomycin, dactinomycin (formerly actinomycin), daunirubicin HCL, daunorucbicin citrate, denileukin diftitox, Dexrazoxane, Dibromomannitol, dihydroxy anthracin dione, Docetaxel, dolasetron mesylate, doxorubicin HCL, dronabinol, E. coli L-asparaginase, emetine, epoetin alfa, Erwinia L-asparaginase, esterified estrogens, estradiol, estramustine phosphate sodium, ethidium bromide, ethinyl estradiol, etidronate, etoposide citrororum factor, etoposide phosphate, filgrastim, floxuridine, fluconazole, fludarabine phosphate, fluorouracil, flutamide, folinic acid, gemcitabine HCL, glucocorticoids, goserelin acetate, gramicidin D, granisetron HCL, hydroxyurea, idarubicin HCL, ifosfamide, interferon alfa-2b, irinotecan HCL, letrozole, leucovorin calcium, leuprolide acetate, levamisole HCL, lidocaine, lomustine, maytansinoid, mechlorethamine HCL, medroxyprogesterone acetate, megestrol acetate, melphalan HCL, mercaptipurine, mesna, methotrexate, methyltestosterone, mithramycin, mitomycin C, mitotane, mitoxantrone, nilutamide, octreotide acetate, ondansetron HCL, paclitaxel, pamidronate disodium, pentostatin, pilocarpine HCL, plimycin, polifeprosan 20 with carmustine implant, porfimer sodium, procaine, procarbazine HCL, propranolol, rituximab, sargramostim, streptozotocin, tamoxifen, taxol, teniposide, tenoposide, testolactone, tetracaine, thioepa chlorambucil, thioguanine, thiotepa, topotecan HCL, toremifene citrate,

trastuzumab, tretinoin, valrubicin, vinblastine sulfate, vincristine sulfate, and vinorelbine tartrate.

[0131] In a preferred embodiment, the cytotoxin is especially effective in dividing or rapidly dividing cells, such that non-dividing cells are relatively spared from the toxic effects.

[0132] The antibodies of the invention can be internalized within the diseased or carcinoma cells to which they bind and are therefore particularly useful for therapeutic applications, for example, delivering into the cells toxins that need to be internalized for their adverse activity. Examples of such toxins include, but not limited to, saporin, calicheamicin, auristatin, and maytansinoid.

[0133] The antibodies or polypeptides of the invention can be associated (including conjugated or linked) to a radioactive molecule, a toxin, or other therapeutic agents, or to liposomes or other vesicles containing therapeutic agents covalently or non-covalently, directly or indirectly. The antibody may be linked to the radioactive molecule, the toxin, or the chemotherapeutic molecule at any location along the antibody so long as the antibody is able to bind its target KID31.

[0134] A toxin or a chemotherapeutic agent may be coupled (e.g., covalently bonded) to a suitable monoclonal antibody either directly or indirectly (e.g., via a linker group, or, alternatively, via a linking molecule with appropriate attachment sites, such as a platform molecule as described in U.S. patent 5,552,391). The toxin and chemotherapeutic agent of the present invention can be coupled directly to the particular targeting proteins using methods known in the art. For example, a direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

[0135] The antibodies or polypeptides can also be linked to a chemotherapeutic agent via a microcarrier. Microcarrier refers to a biodegradable or a non-biodegradable particle which is insoluble in water and which has a size of less

than about 150, 120 or 100 mm in size, more commonly less than about 50-60 μm, preferably less than about 10, 5, 2.5, 2 or 1.5 μm. Microcarriers include "nanocarriers", which are microcarriers have a size of less than about 1 μm, preferably less than about 500 nm. Such particles are known in the art. Solid phase microcarriers may be particles formed from biocompatible naturally occurring polymers, synthetic polymers or synthetic copolymers, which may include or exclude microcarriers formed from agarose or cross-linked agarose, as well as other biodegradable materials known in the art. Biodegradable solid phase microcarriers may be formed from polymers which are degradable (*e.g.*, poly(lactic acid), poly(glycolic acid) and copolymers thereof) or erodible (*e.g.*, poly(ortho esters such as 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5.5]undecane (DETOSU) or poly(anhydrides), such as poly(anhydrides) of sebacic acid) under mammalian physiological conditions. Microcarriers may also be liquid phase (*e.g.*, oil or lipid based), such liposomes, iscoms (immune-stimulating complexes, which are stable complexes of cholesterol, and phospholipid, adjuvant-active saponin) without antigen, or droplets or micelles found in oil-in-water or water-in-oil emulsions, provided the liquid phase microcarriers are biodegradable. Biodegradable liquid phase microcarriers typically incorporate a biodegradable oil, a number of which are known in the art, including squalene and vegetable oils. Microcarriers are typically spherical in shape, but microcarriers that deviate from spherical shape are also acceptable (*e.g.*, ellipsoid, rod-shaped, etc.). Due to their insoluble nature (with respect to water), microcarriers are filterable from water and water-based (aqueous) solutions.

[0136] The antibody or polypeptide conjugates of the present invention may include a bifunctional linker that contains both a group capable of coupling to a toxic agent or chemotherapeutic agent and a group capable of coupling to the antibody. A linker can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker can be cleavable or non-cleavable. A linker can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be

possible. The bifunctional linker can be coupled to the antibody by means that are known in the art. For example, a linker containing an active ester moiety, such as an N-hydroxysuccinimide ester, can be used for coupling to lysine residues in the antibody via an amide linkage. In another example, a linker containing a nucleophilic amine or hydrazine residue can be coupled to aldehyde groups produced by glycolytic oxidation of antibody carbohydrate residues. In addition to these direct methods of coupling, the linker can be indirectly coupled to the antibody by means of an intermediate carrier such as an aminodextran. In these embodiments the modified linkage is via either lysine, carbohydrate, or an intermediate carrier. In one embodiment, the linker is coupled site-selectively to free thiol residues in the protein. Moieties that are suitable for selective coupling to thiol groups on proteins are well known in the art. Examples include disulfide compounds, α-halocarbonyl and α-halocarboxyl compounds, and maleimides. When a nucleophilic amine function is present in the same molecule as an α-halo carbonyl or carboxyl group the potential exists for cyclization to occur via intramolecular alkylation of the amine. Methods to prevent this problem are well known to one of ordinary skill in the art, for example by preparation of molecules in which the amine and α-halo functions are separated by inflexible groups, such as aryl groups or trans-alkenes, that make the undesired cyclization stereochemically disfavored. See, for example, U.S. Patent No. 6,441,163 for preparation of conjugates of maytansinoids and antibody via a disulfide moiety.

[0137] One of the cleavable linkers that can be used for the preparation of antibody-drug conjugates is an acid-labile linker based on cis-aconitic acid that takes advantage of the acidic environment of different intracellular compartments such as the endosomes encountered during receptor mediated endocytosis and the lysosomes. See, for example, Shen et al., *Biochem. Biophys. Res. Commun.* 102:1048-1054 (1981) for the preparation of conjugates of daunorubicin with macromolecular carriers; Yang et al., *J. Natl. Canc. Inst.* 80:1154-1159 (1988) for the preparation of conjugates of daunorubicin to an anti-melanoma antibody; Dillman et al., *Cancer Res.* 48:6097-6102 (1988) for using an acid-labile linker in a similar fashion to prepare conjugates of daunorubicin with

an anti-T cell antibody; Trouet et al., *Proc. Natl. Acad. Sci.* 79:626-629 (1982) for linking daunorubicin to an antibody via a peptide spacer arm.

[0138] An antibody (or polypeptide) of this invention may be conjugated (linked) to a radioactive molecule by any method known to the art. For a discussion of methods for radiolabeling antibody see "Cancer Therapy with Monoclonal AntibodiesT", D. M. Goldenberg ed. (CRC Press, Boca Raton, 1995).

[0139] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980. The formation of cross-linked antibodies can target the immune system to specific types of cells, for example, cancer or diseased cells expressing KID31.

[0140] This invention also provides methods of delaying development of metastasis in an individual with cancer (including, but not limited to, lung, kidney, pancreatic, ovarian, and colon cancer) using an anti-KID31 antibody or other embodiments that bind to KID31 linked to a chemotherapeutic agent. In some embodiments, the antibody is a humanized or chimeric form of a non-human anti-KID31 antibody.

[0141] In yet another embodiment, the antibody can be employed as adjuvant therapy at the time of the surgical removal of a cancer expressing the antigen in order to delay the development of metastasis. The antibody or antibody associated with a chemotherapeutic agent can also be administered before surgery (neoadjuvant therapy) in an individual with a tumor expressing the antigen in order to decrease the size of the tumor and thus enable or simplify surgery, spare tissue during surgery, and/or decrease the resulting disfigurement.

[0142] In yet another embodiment, any of the KID31 binding embodiments described herein can bind to KID31-expressing cancerous cells and induces an active immune response against the cancerous cells expressing KID31. In some cases, the active immune response can cause the death of the cancerous cells (*e.g.*, antibody binding to cancer cells inducing apoptotic cell death), or inhibit the growth (*e.g.*, block cells cycle progression) of the cancerous cells. In other cases, any of the novel antibodies described herein can bind to cancerous cells and

antibody dependent cellular cytotoxicity (ADCC) can eliminate cancerous cells to which anti-KID31 binds. Accordingly, the invention provides methods of stimulating an immune response comprising administering any of the compositions described herein.

[0143] In some cases, antibody binding can also activate both cellular and humoral immune responses and recruit more natural killer cells or increased production of cytokines (*e.g.*, IL-2, IFN-g, IL-12, TNF-a, TNF-b, etc.) that further activate an individual's immune system to destroy cancerous cells. In yet another embodiment, anti-KID31 antibodies can bind to cancerous cells, and macrophages or other phagocytic cell can opsonize the cancerous cells.

[0144] Various formulations of anti-KID31 antibodies or fragments thereof may be used for administration. In some embodiments, anti-KID31 antibodies or fragments thereof may be administered neat. In addition to the pharmacologically active agent, the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries that are well known in the art and are relatively inert substances that facilitate administration of a pharmacologically effective substance or which facilitate processing of the active compounds into preparations that can be used pharmaceutically for delivery to the site of action. For example, an excipient can give form or consistency, or act as a diluent. Suitable excipients include but are not limited to stabilizing agents, wetting and emulsifying agents, salts for varying osmolarity, encapsulating agents, buffers, and skin penetration enhancers.

[0145] Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate for oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension and include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally,

the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

[0146] The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulation may be used simultaneously to achieve systemic administration of the active ingredient. Excipients as well as formulations for parenteral and nonparenteral drug delivery are set forth in *Remington, The Science and Practice of Pharmacy* 20th Ed. Mack Publishing (2000).

[0147] Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

[0148] Generally, these agents are formulated for administration by injection (*e.g.*, intraperitoneally, intravenously, subcutaneously, intramuscularly, etc.), although other forms of administration (*e.g.*, oral, mucosal, etc) can be also used. Accordingly, anti-KID31 antibodies are preferably combined with pharmaceutically acceptable vehicles such as saline, Ringer's solution, dextrose solution, and the like.

[0149] The particular dosage regimen, *i.e.*, dose, timing and repetition, will depend on the particular individual and that individual's medical history. Generally, a dose of at least about 100 ug/kg body weight, more preferably at least about 250 ug/kg body weight, even more preferably at least about 750 ug/kg body weight, even more preferably at least about 3 mg /kg body weight, even more preferably at least about 5 mg /kg body weight, even more preferably at least about 10 mg/kg body weight is administered.

[0150] Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. Antibodies, which are compatible with the human immune system, such as humanized antibodies or fully human antibodies, may be used to prolong half-life of the antibody and to prevent the antibody being attacked by the host's immune system. Frequency of administration may be determined and adjusted over the course of therapy, and is based on reducing the number of cancerous cells, maintaining the reduction of

cancerous cells, reducing the proliferation of cancerous cells, or delaying the development of metastasis. Alternatively, sustained continuous release formulations of anti-KID31 antibodies may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

[0151] In one embodiment, dosages for anti-KID31 antibodies may be determined empirically in individuals who have been given one or more administration(s). Individuals are given incremental dosages of an anti-KID31 antibody. To assess efficacy of anti-KID31 antibodies, a marker of the specific cancer disease state can be followed. These include direct measurements of tumor size via palpation or visual observation, indirect measurement of tumor size by x-ray or other imaging techniques; an improvement as assessed by direct tumor biopsy and microscopic examination of the tumor sample; the measurement of an indirect tumor marker (*e.g.*, PSA for prostate cancer), a decrease in pain or paralysis; improved speech, vision, breathing or other disability associated with the tumor; increased appetite; or an increase in quality of life as measured by accepted tests or prolongation of survival. It will be apparent to one of skill in the art that the dosage will vary depending on the individual, the type of cancer, the stage of cancer, whether the cancer has begun to metastasize to other location in the individual, and the past and concurrent treatments being used.

[0152] Other formulations include suitable delivery forms known in the art including, but not limited to, carriers such as liposomes. See, for example, Mahato et al. (1997) *Pharm. Res.* 14:853-859. Liposomal preparations include, but are not limited to, cytofectins, multilamellar vesicles and unilamellar vesicles.

[0153] In some embodiments, more than one antibody may be present. The antibodies can be monoclonal or polyclonal. Such compositions may contain at least one, at least two, at least three, at least four, at least five different antibodies that are reactive against carcinomas, adenocarcinomas, sarcomas, or adenosarcomas. Anti-KID31 antibody can be admixed with one or more antibodies reactive against carcinomas, adenocarcinomas, sarcomas, or adenosarcomas in organs including but not limited to ovary, breast, lung, prostate, colon, kidney, skin, thyroid, bone, upper digestive tract, and pancreas.

In one embodiment, a mixture of different anti-KID31 antibodies are used. A mixture of antibodies, as they are often denoted in the art, may be particularly useful in treating a broader range of population of individuals.

[0154] The following examples are provided to illustrate, but not to limit, the invention.

EXAMPLES

Example 1. Preparation of human kidney cells as an immunogen

[0155] Human fetal kidneys of gestational age between 10 to 18 weeks were obtained from Advanced Biosciences Research at Alameda County, California. Kidneys were procured and shipped to the lab in tissue culture medium on wet ice. Immediately upon arrival, the kidneys were transferred to wash medium (cold PBS containing penicillin/streptomycin and gentamycin). The outer membranes were removed with forceps and the kidneys were briefly washed in 70% ethanol then rinsed twice in wash medium. The kidneys were minced into 1 mm cubes with surgical scissors in a 100 mm dry culture dish. The tissue pieces were plated in 10 ml of a defined serum-free medium referred to herein as I/3F. This media is described in U.S. Provisional Application No. 60/504,674, the disclosure of which is hereby incorporated by reference. While a variety of commonly used cell culture media may be used in the practice of this invention, presently preferred embodiments use serum-free, fructose-based cell culture media.

[0156] The tissue pieces were transferred into a 15 ml centrifuge tube and the tissue pieces were centrifuged at 1000xg for 5 minutes. The tissue pieces were resuspended in I/3F medium containing insulin (10 ug/ml), transferrin (10 ug/ml), epidermal growth factor (20 ng/ml), somatotropin (0.005 IU/ml), pig pituitary extract (0.2%), chicken serum (0.1%), gentamycin (100 ug/ml), penicillin/streptomycin (1x) and collagenase/dispase (0.1%) and incubated at 4°C overnight. The following day, centrifuge the digested tissue pieces were centrifuged at 1000xg for 5 minutes and washed twice with I/3F medium. The pellet was resuspended in 10 ml I/3F medium containing insulin (10 ug/ml), transferrin (10 ug/ml), epidermal growth factor (20 ng/ml), somatotropin (0.005

IU/ml), pig pituitary extract (0.2%) and chicken serum (0.1%) and cultured in fibronectin-precoated 10 cm plates.

[0157] Under these culture conditions, the human fetal kidney cells attached to the substrate-coated plates and grew as a monolayer. Culture medium was changed twice weekly.

[0158] To harvest the cells, the cell monolayers were rinsed once with calcium- and magnesium-free Hanks saline solution, incubated in 10mM EDTA in Hanks saline solution at 37C for 15 minutes. The cells were detached from the culture surface by gentle pipetting. The cell suspension was pelleted by centrifugation at 1000xg for 5 minutes. The supernatant was removed and cells were resuspended in serum-free medium (I/3F medium) with non-denaturing adjuvant as appropriate.

Example 2. Generation of monoclonal antibodies

[0159] A non-denaturing adjuvant (Ribi, R730, Corixa, Hamilton MT) was rehydrated to 2 ml in phosphate buffered saline. 100 μl of this rehydrated adjuvant was then gently mixed with some of the cell pellet from Example 1 to be used for immunization. Approximately 10^6 human fetal kidney cells per mouse were injected into Balb/c mice via footpad, approximately once or twice a week. The precise immunization schedule is as follows: Day zero, immunization plus Ribi. Day 3, immunization plus Ribi. Day 7, immunization plus Ribi. Day 24, immunization minus Ribi. Day 29, immunization minus Ribi. Day 32, immunization minus Ribi. Day 36, immunization minus Ribi. Day 44, immunization minus Ribi. Day 51, immunization minus Ribi. Day 69, bleed for titer test. Day 71. immunization plus Ribi. Day 74, immunization plus Ribi. Day 81, immunization plus Ribi. Day 91, prefusion boost (no Ribi). Day 104, harvest nodes for fusion.

[0160] At Day 69, a drop of blood was drawn from the tail of each immunized animal to test the titer of antibodies against human fetal kidney cells using FACS analysis. When the titer reached at least 1:2000, the mice were sacrificed using

CO_2 followed by cervical dislocation. Lymph nodes were harvested for hybridoma preparation.

[0161] Lymphocytes from mice were fused with the mouse myeloma line X63-Ag8.653 using 35% polyethylene glycol 4000. On day 10 following the fusion, the hybridoma supernatants were screened for the presence of human fetal kidney cells- specific monoclonal antibodies by fluorescence activated cell sorting (FACS). Conditioned medium from each hybridoma was incubated for 30 minutes with an aliquot of human fetal kidney cells. After incubation, the cell samples were washed, resuspended in 0.1 ml diluent and incubated with 1 μg/ml of FITC conjugated F(ab')2 fragment of goat anti-mouse IgG for 30 min at 4°C. The cells were washed, resuspended in 0.2 ml FACS diluent and analyzed using a FACScan cell analyzer (Becton Dickinson; San Jose, CA). Hybridoma clones were selected for further expansion, cloning, and characterization based on their binding to the surface of the human fetal kidney cells by FACS. A hybridoma making a monoclonal antibody designated mu-anti-KID31 which binds an antigen designated KID31 was selected.

Example 3. Purification of anti-KID31 antibodies, including mu-anti-KID31

[0162] Those hybridomas that produce antibodies that continue to be reactive against the immunizing cells or cell line are scaled for purification of the antibody. The following method was used, although other methods commonly known in the field can also be applicable. Positive hybridomas were scaled into three T75 flasks. Once confluent, the cells and supernatant were collected into 50 ml conical tubes and centrifuged. The supernatant was aspirated and the cell pellet was washed with F12/DME (50:50) media and re-centrifuged. The cell pellet was resuspended in 50 ml growth medium, F12/DME (50:50) containing 1% ultra-low IgG FBS (GIBCO, Invitrogen Corp.), 10 µg/ml rh insulin and 10 µg/ml transferring. Cells were then inoculated into the pouch of a CL1000 (IBS Integra BioScience), and the outer chamber was filled with 500ml of the same growth medium. The outer chamber is changed on day 7 and day 14 with fresh growth medium.

[0163] The antibody-containing medium was harvested from the cell pouch on day 21. 25ml of the harvested material is mixed 1:1 with load buffer (3M NaCl, 1.5M glycine pH9.0). The material was flowed over a prepared 5ml column of protein A resin (Amersham). The column was then washed with 20 column volumes of phosphate buffered saline (PBS). The antibodies were eluted with 0.1M glycine, pH 2.8 and neutralized in 20 µl of 3M Tris, pH 9.0. The antibodies were then dialyzed and protein concentration was determined by A_{280} readings on a Beckman DU530 spectrophotometer.

[0164] In other experiments, the binding of the mu-anti-KID31 antibody to KID31 was tested using live cell ELISA. The following method was used, although other methods commonly known in the field are applicable. Cells (HT-29, SKOV3, SKMES-1, SW480, SKBR-3, and HPAFII) were grown in 10% fetal bovine serum (FBS) containing media to confluency on tissue culture treated 96-well plates (Falcon). Cells were washed with PBS and then incubated with 50µl of desired antibodies at a desired concentration in Hank's Balanced Salt Solution (HBSS) containing 1% BSA and 0.1% sodium azide for 1 hour at room temperature. The cells were then washed three times with 100µl per well of HBSS before incubating with horseradish peroxidase (HRP) secondary antibody (50µl per well diluted in HBSS) for 30 minutes at room temperature. The cells were finally washed three times with HBSS and the color change substrate (TMB substrate, KPL) was added to each well at 100µl per well. The color change reaction was stopped with the addition of 100µl per well of 1M phosphoric acid. The plates were then read at O.D. 450nm.

Example 4. Biotinylated immunoprecipitation analysis of KID31 expression in cancer cell lines Colo205 and ES-2.

[0165] Colorectal adenocarcinoma cell line Colo205 and ovarian carcinoma cell line ES-2 were grown to confluency on 175 cm^2 culture dishes. The confluent monolayer was washed three times with Hank's Balanced Salt Solution (HBSS+ containing no sodium bicarbonate or phenol red; buffered with 10mM HEPES, pH 7.4; Sigma Chemicals) and biotinylated with 200 µg of sulfo-NHS-LC-biotin (Pierce Endogen) for 30 minutes at room temperature. The cells were then

washed with HBSS+ containing 0.1M Tris, pH 7.4 (Sigma Chemicals) and incubated in HBSS+ containing 0.1M Tris, pH 7.4 for 15 minutes at room temperature. The cells were finally washed three times with HBSS+ and lysed by incubation for 5 minutes, on ice, in lysis buffer (HBSS+ with 2% Triton X-100, 2mM PMSF, 0.1% sodium azide, and 1 tablet per 5ml lysis buffer of EDTA free complete mini-protease cocktail (Roche Molecular Biochemicals)).

[0166] Cells were scraped in lysis buffer and lysates collected. Lysates were centrifuged at 14,000 x g for one hour at 4°C. The clarified lysate was then pre-cleared for 2 hours at 4°C with 5 µl of human IgG conjugated (1mg/ml) CNBr 4MB Sepharose beads (Amersham Pharmacia). Human IgG beads were centrifuged and removed, and then the pre-cleared lysate was then incubated with monoclonal antibody mu-anti-KID31 conjugated to CNBr 4MB sepharose beads (conjugated at 1mg/ml) for 2 hours at 4°C. The mu-anti-KID31 beads were centrifuged and removed after the 2 hour incubation. Both the human IgG and the mu-anti-KID31 beads were individually washed three times with 1 ml of lysis buffer and then washed three times with 1ml HBSS+. The washed beads were eluted by the addition of 30 µl of SDS-PAGE sample buffer and boiling at 99°C for 5 minutes.

[0167] The samples were then resolved on a 4-20% Novex gradient gel (Invitrogen), and transferred onto 0.2µm nitrocellulose membrane (Invitrogen) and visualized by western blotting with 5 µg/blot of mu-anti-KID31.

[0168] For detection with HRP conjugated streptavidin, the nitrocellulose was first blocked for 1 hour with blocking buffer (5% non-fat dry milk in Tris buffered saline containing 0.05% Tween-20 (TBST, Sigma Chemicals). HRP conjugated streptavidin was diluted into PBST at 1 µg/ml and exposed to the nitrocellulose for 30 minutes at room temperature. The nitrocellulose was then washed three times with PBST before visualization with ECL+.

[0169] Results using this protocol and mu-anti-KID31 antibodies show a smear of molecular weight bands at approximately 60 kDa to 80 kDa under reducing conditions. The smear pattern and molecular weight of the mu-anti-KID31 specific bands are consistent with the molecular weight of carboxypeptidase M, a glycoprotein with a molecular weight of approximately 62 kD.

Example 5. Immunohistochemistry methods

[0170] Frozen tissue samples from cancer patients were embedded in OCT compound and quick-frozen in isopentane with dry ice. Cryosections were cut with a Leica 3050 CM microtome at thickness of 8-10 μm and thaw-mounted on SuperFrost Plus slides (VWR #48311-703). The sections were fixed with 75% acetone/25% ethanol at 10°C and allowed to air-dry 2-4 hours at room temperature. The fixed sections were stored at -80°C until use.

[0171] For immunohistochemistry, the tissue sections were retrieved washed in Tris buffered 0.05% Tween (TB-T) and blocked in blocking buffer (TB-T, 5% normal goat serum and 100μg/ml avidin) for 30 minutes at room temperature. The slides were then incubated with the mu-anti-KID31 and control monoclonal antibodies diluted in blocking buffer (1 μg/ml) for 60-90 minutes at room temperature. The sections were then washed three times with the blocking buffer. The bound monoclonal antibodies were detected with a goat anti-mouse IgG + IgM (H+L) $F(ab')^2$-peroxidase conjugates and the peroxidase substrate diaminobenzidine (1 mg/ml, Sigma cat. No. D 5637) in 0.1 M sodium acetate buffer pH 5.05 and 0.003% hydrogen peroxide (Sigma cat. No. H1009). The stained slides were counter-stained with hematoxylin and examined under Nikon microscope.

[0172] In some cases, paraffin embedded formaldehyde-fixed tissues were used for immunohistochemistry after appropriate antigen retrieval methods were employed. One such antigen retrieval method is described in Mangham and Isaacson, *Histopathology* 35:129-33 (1999). Other methods of antigen retrieval and/or detection may be used by one skilled in the art. Results from similar experiments performed using frozen tissues or, where appropriate, fixed tissue with antigen retrieval and polyMICA detection were performed. The binding of anti-KID31 antibody to a variety of normal and cancer tissues was assessed. In all cases, antibody binding in control fixed tissues was correlated with that of frozen tissues. The results from frozen tissues were only used if the two did not match in the controls.

[0173] For convenience, a summary of the combined results of several experiments using frozen surgical tissue from different sources is shown below in Table 1 and Table 2.

Table 1. Distribution of KID31 in normal human tissues

Tissue Type	Results
Skin	1+ sweat ducts; 1+ to 2+ focal staining on sebaceous glands
Liver	Negative
Kidney	1+ staining on scattered tubules
Lung	Negative
Duodenum	Negative
Pancreas	Negative (1+ staining on a few ducts)
Breast	Negative

Table 2. Distribution of KID31 in human tumor tissues

Tissue Type	Results
Colon	Negative (1+ focal staining on interstitium)
Breast	Negative
Pancreas	1+ to 2+ on tumor
Endometrium	+/- staining on tumor
Kidney	+/- staining on tumor; 1+ to 2+ staining on a subset of small vessels
Lung	Negative to 2+ focal staining on tumor
Ovary	Negative

Example 6. Immunocytochemistry results

[0174] Monoclonal antibody mu-anti-KID31 was used to test reactivity with various cell lines from different types of tissues. The results were scored as '+' for weak positive staining, '++' for moderate positive staining, '+++' for strong positive staining and '-' for negative staining.

[0175] Immunohistochemistry results were obtained using CellArray™ technology, as described in WO 01/43869. Cells from different established cell lines were removed from the growth surface without using proteases, packed

and embedded in OCT compound. The cells were frozen and sectioned, then stained using a standard IHC protocol.

[0176] Results of the binding of the mu-anti-KID31 antibody to various established human normal and tumor cell lines are compiled for convenience in Table 3. The experiments represented in Table 3 include Live-cell ELISA and CellArray™ binding experiments using the methods described herein.

Table 3. Immunocytochemistry results

Cell line	ATCC#	Organ	Cell Type	Reactivity Cell Array	Reactivity Live Cell ELISA
BT474	HTB-20	Breast	Ductal carcinoma	-	
MCF7	HTB-22	Breast	Adenocarcinoma	-	
ES-2	CRL-1978	Ovary	Carcinoma	+	
SKOV3	HTB-77	Ovary	Adenocarcinoma	-	+++
SKMES1	HTB-58	Lung	Squamous carcinoma	+	+++
CA130	Raven	Lung	Small cell carcinoma	-	
CaLu3	HTB-55	Lung	Adenocarcinoma	-	
9926	Raven	Pancreas	Adenocarcinoma	-	
AsPC-1	CRL-1682	Pancreas	Adenocarcinoma	-	
Hs700T	HTB-147	Pancreas	Adenocarcinoma	-	
Colo205	CCL-222	Colon	Ascites colorectal adenocarcinoma	-	
HT-29	HTB-38	Colon	Colorectal adenocarcinoma		+
Cos 7	CRL-1651	Kidney (African Green Monkey)	SV40 Transformed	-	
RL-65	CRL-10345	Lung (Rat)		-	

A204	HTB-82	Muscle	Rhabdomyosarcoma	+/-	
G292	CRL-1423	Bone	Osteosarcoma	+/-	
HT1080	CCL-121	Connective Tissue	Fibrosarcoma	-	
MG63	CRL-1427	Bone	Osteosarcoma	-	
RD	CCL-136	Muscle	Rhabdomyosarcoma	+/-	
RD-ES	HTB-166	Bone	Ewing's sarcoma	-	
SKES-1	HTB-86	Bone	Sarcoma	-	
SKLMS-1	HTB-88	Vulva	Leiomyosarcoma	-	
SKUT-1	HTB-114	Uterus	Mesodermal tumor (mixed)	-	
SW684	HTB-91	Connective Tissue	Fibrosarcoma	-	
SW872	HTB-92	Connective Tissue	Liposarcoma	-	
786-O	CRL-1932	Kidney	Renal cell carcinoma	-	
A498	HTB-44	Kidney	Carcinoma	-	
Caki-2	HTB-47	Kidney	Clear cell carcinoma	++	
22RV1	CRL-2505	Prostate	Carcinoma	-	
DU145	HTB-81	Prostate	Adenocarcinoma	-	
LNCaP	CRL-1740	Prostate	Carcinoma	-	

Example 9 Effect of mu-anti-KID31 on cancer cell line H322M

[0177] The ability of the antibodies to reduce cell number *in vitro* when grown as a monolayer can be assessed using cell monolayers grown in the presence or absence of varying amounts of test or control purified antibody and the change in cell number assessed using MTT. MTT is a dye that measures the activity of mitochondrial enzymes and correlates with relative viable cell number. Cells of

interest were plated and grown in F12/DMEM (1:1) growth medium supplemented with 10% fetal bovine serum in 96 well plates. H322M cells were plated at 500, 1000, 2000 or 4000 cells/well in triplicate wells of a 96 well dish. Immediately after plating, mu-anti-KID31 was added. The cells were incubated at 37°C in a humidified incubator at 5% CO_2 for 5 days. At the end of the assay, MTT was dissolved in PBS (5mg/ml) and added directly to wells at 1:10 dilution. Plates were placed back in incubator for 4 hours. After the incubation, medium was removed and 100 μl DMSO was added to solubilize the MTT precipitate. Plates were read at O.D. 540nm.

[0178] At 20μg/ml mu-anti-KID31 inhibited the growth of cancer cell line H322M approximately 10% at the plating densities of 1000 and 2000 cells/well.

Example 10. Anti-tumor efficacy of mu-anti-KID31 antibody in a sub-renal capsule tumor model

[0179] This study was designed to test the dose-responsive anti-tumor data for an anti-KID31 antibody in a sub-renal capsule tumor model using H322M, a non-small cell lung cancer-derived cell line and Caki-2, a renal tumor-derived cell line. H322M and Caki-2 cells were resuspended at 100 million cells/ml of media.

[0180] Type 1 rat-tail collagen was prepared by a standard method. Briefly, tails from mature breeding rats were removed and sterilized by soaking in 70% ethanol. The tail skin was removed and the tendons were isolated and weighed. One gram of tendon produces 100 ml collagen solution, and each tail yields approximately 1 to 1.5 grams of tendon. To extract the collagen, the tendons were placed in a dilute acetic acid solution (200 μl glacial acetic acid per gram of tendon in 100 ml water) containing penicillin, streptomycin and fungizone and stirred gently at 4°C for at least 1 week. The solution was then centrifuged and the collagen supernatant was stored at 4°C until use.

[0181] For this study, 50 μl collagen buttons were prepared by polymerizing the rat-tail collagen in a setting solution containing Earle's Balanced Salt Solution (EBSS), NaOH and $NaHCO_3$. Following polymerization, 5×10^5 cells were added per collagen button. The cells were incubated in collagen overnight at 37°C prior to implantation.

[0182] For implantation of the cells under the kidney capsule, mice were fully anesthetized with tribromoethanol. A pocket was made in the kidney capsule to allow for the placement of cells, which was made through a paralumbar surgical approach to the right and/or left kidney. In some studies, both kidneys received xenografts. Following surgery, the animals were allowed to recover on a heated surface and observed until fully recovered from the anesthesia. Wound clips were removed ten days post-surgery.

[0183] For each treatment dose group, mu-anti-KID31 was diluted in PBS to the appropriate concentration to administer 0.01-ml/gram body weight. Control groups received PBS (0.01 ml/gram body weight). Dosing was initiated on Day 2 following implantation, and doses of mu-anti-KID31 or PBS control were administered three times weekly as single rapid injections into the intraperitoneal cavity.

[0184] At the end of the dosing period, animals were euthanized and the tumors and adjacent tissue were removed and incubated in a digestion buffer containing proteinase K (1.45 mg/ml) and RNase A (0.07 mg/ml) overnight at 55°C for PCR analysis.

[0185] To generate a template for PCR analysis, genomic DNA was isolated from tumors using Wizard SV Genomic Purification System (Promega) according to manufacturer's instructions. Each DNA sample was resuspended in a final volume of 200 µl.

[0186] The amount of human DNA in the tumors was quantitated using real-time PCR on an Applied Biosystems SDS7000 system, with primers and probe specific for the human ribosomal gene RPL19.

[0187] Each sample was initially digested with BstX1 to ensure efficient amplification. Each reaction mix contained 5 µl of template DNA, 5 µl Taqman Gold 10x reaction buffer (Applied Biosystems), 5 units BstX1, 4 mM $MgCl_2$, 2.5 mM each deoxynucleotide mix, 250 nM each primer, 150 nM probe, 1.5 units Taqgold polymerase (Applied Biosystems) and water to a final volume of 50 µl. The thermal cycle conditions used were 30 minutes at 45°C for BstX1 digestion, 10 minutes at 95°C for BstX1 inactivation and Taq hot start, followed by 40 cycles of: 95°C for 20 seconds (denaturation) and 60°C for 1 minute (elongation).

[0188] A standard curve was generated using four-fold serial dilutions of human genomic DNA (BD Biosciences Clonetech) ranging from 400 to 0.09 ng DNA/reaction. Sample DNA concentrations were interpolated from the standard curve. Each tumor sample was analyzed in triplicate PCR reactions and average DNA concentrations were determined. The results are shown in Figure 1. From these experiments, H322M tumors treated with mu-anti-KID31 showed a 70.4% decrease in the amount of human DNA when compared to control PBS treated tumors. Caki-2 tumors treated with mu-anti-KID31 showed a 20.6% decrease in the amount of human DNA when compared to control PBS treated tumors.

[0189] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application. All publications, patents and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent or patent application were specifically and individually indicated to be so incorporated by reference.

We claim:

1. A substantially purified immunoglobulin polypeptide or an antigen-binding fragment thereof, that specifically binds to KID31, and has at least one or more of the following characteristics:

a. the ability to bind to KID31 on a cancer cell;

b. the ability to bind to a portion of KID31 that is exposed on the surface of a living cell *in vitro* or *in vivo*;

c. the ability to deliver a therapeutic agent or detectable marker to a cancer cell expressing KID31; and

d. the ability to deliver a therapeutic agent or detectable marker into a cancer cell expressing KID31.

2. The purified immunoglobulin polypeptide or antigen binding fragment of claim 1, wherein said cancer cell is selected from the group consisting of cancer cells from adrenal gland tumors, AIDS-associated cancers, alveolar soft part sarcoma, astrocytic tumors, bladder cancer (squamous cell carcinoma and transitional cell carcinoma), bone cancer (adamantinoma, aneurismal bone cysts, osteochondroma, osteosarcoma), brain and spinal cord cancers, metastatic brain tumors, breast cancer, carotid body tumors, cervical cancer, chondrosarcoma, dhordoma, chromophobe renal cell carcinoma, clear cell carcinoma, colon cancer, colorectal cancer, cutaneous benign fibrous histiocytomas, desmoplastic small round cell tumors, ependymomas, Ewing's tumors, extraskeletal myxoid chondrosarcoma, fibrogenesis imperfecta ossium, fibrous dysplasia of the bone, gallbladder and bile duct cancers, gestational trophoblastic disease, germ cell tumors, head and neck cancers, islet cell tumors, Kaposi's Sarcoma, kidney cancer (nephroblastoma, papillary renal cell carcinoma), leukemias, lipoma/benign lipomatous tumors, liposarcoma/malignant lipomatous tumors, liver cancer (hepatoblastoma, hepatocellular carcinoma), lymphomas, lung cancer, medulloblastoma, melanoma, meningiomas, multiple endocrine neoplasia, multiple myeloma, myelodysplastic syndrome, neuroblastoma, neuroendocrine tumors, ovarian

cancer, pancreatic cancers, papillary thyroid carcinomas, parathyroid tumors, pediatric cancers, peripheral nerve sheath tumors, phaeochromocytoma, pituitary tumors, prostate cancer, posterious unveal melanoma, rare hematologic disorders, renal metastatic cancer, rhabdoid tumor, rhabdomysarcoma, sarcomas, skin cancer, soft-tissue sarcomas, squamous cell cancer, stomach cancer, synovial sarcoma, testicular cancer, thymic carcinoma, thymoma, thyroid metastatic cancer, and uterine cancers (carcinoma of the cervix, endometrial carcinoma, and leiomyoma).

3. An isolated nucleic acid sequence coding for the immunoglobulin polypeptide or antigen-binding fragment thereof of claim 1.

4. The nucleic acid of claim 3, wherein the nucleic acid is operably linked to a promoter.

5. The nucleic acid of claim 4, wherein the promoter and the nucleic acid are contained in an expression vector.

6. The nucleic acid of claim 3, wherein the polypeptide is a monoclonal antibody.

7. A cell line transfected, transformed, or infected with a vector containing a nucleic acid of claim 3.

8. A method of producing a substantially purified immunoglobulin polypeptide, or an antigen binding fragment thereof, comprising the steps of:

a. growing a cell line transformed with the nucleic acid of claim 3 under conditions in which the immunoglobulin polypeptide or antigen binding fragment is expressed; and

b. harvesting the expressed immunoglobulin polypeptide or fragment.

9. The method of claim 8, wherein the cell line is a hybridoma.

10. The method of claim 9, wherein the hybridoma is ATCC No. PTA# 6516.

11. The method of claim 8, wherein the immunoglobulin polypeptide is a monoclonal antibody.

12. A pharmaceutical composition comprising a therapeutically effective dose of the purified immunoglobulin or antigen-binding fragment of claim 1, together with a pharmaceutically acceptable carrier.

13. A pharmaceutical composition comprising a therapeutically effective dose of a monoclonal antibody or an antigen binding fragment thereof that specifically binds to KID31, and has at least one or more of the following characteristics:

a. the ability to bind to KID31 on a cancer cell;

b. the ability to bind to a portion of KID31 that is exposed on the surface of a living cell *in vitro* or *in vivo*;

c. the ability to deliver a therapeutic agent or detectable marker to a cancer cell expressing KID31; and

d. the ability to deliver a therapeutic agent or detectable marker into a cancer cell expressing KID31;

together with a pharmaceutically acceptable carrier.

14. The pharmaceutical composition of claim 13, wherein the composition comprises an additional therapeutic moiety.

15. An isolated cell line consisting of ATCC No. PTA# 6516, or progeny thereof.

16. A method for delivering a chemotherapeutic agent to a cancer cell comprising administering a composition comprising an anti-KID31 antibody associated with the chemotherapeutic agent, wherein the cancer cell is selected from the group consisting of cancer cells from adrenal gland tumors, AIDS-associated cancers, alveolar soft part sarcoma, astrocytic tumors, bladder cancer (squamous cell carcinoma and transitional cell carcinoma), bone cancer (adamantinoma, aneurismal bone cysts, osteochondroma, osteosarcoma), brain and spinal cord cancers, metastatic brain tumors, breast cancer, carotid body tumors, cervical cancer, chondrosarcoma, dhordoma, chromophobe renal cell carcinoma, clear cell carcinoma, colon cancer, colorectal cancer, cutaneous benign fibrous histiocytomas, desmoplastic small round cell tumors, ependymomas, Ewing's tumors, extraskeletal myxoid chondrosarcoma, fibrogenesis imperfecta ossium, fibrous dysplasia of the bone, gallbladder and bile duct cancers, gestational trophoblastic disease, germ cell tumors, head and neck cancers, islet cell tumors, Kaposi's Sarcoma, kidney cancer (nephroblastoma, papillary renal cell carcinoma), leukemias, lipoma/benign lipomatous tumors, liposarcoma/malignant lipomatous tumors, liver cancer (hepatoblastoma, hepatocellular carcinoma), lymphomas, lung cancer, medulloblastoma, melanoma, meningiomas, multiple endocrine neoplasia, multiple myeloma, myelodysplastic syndrome, neuroblastoma, neuroendocrine tumors, ovarian cancer, pancreatic cancers, papillary thyroid carcinomas, parathyroid tumors, pediatric cancers, peripheral nerve sheath tumors, phaeochromocytoma, pituitary tumors, prostate cancer, posterious unveal melanoma, rare hematologic disorders, renal metastatic cancer, rhabdoid tumor, rhabdomysarcoma, sarcomas, skin cancer, soft-tissue sarcomas, squamous cell cancer, stomach cancer, synovial sarcoma, testicular cancer, thymic carcinoma, thymoma, thyroid metastatic cancer, and uterine cancers (carcinoma of the cervix, endometrial carcinoma, and leiomyoma).

17. The method of claim 16, wherein the chemotherapeutic agent is administered to an individual.

18. The method of claim 16, wherein the hybridoma is ATCC No. PTA# 6516 or progeny thereof.

19. A method of inhibiting growth of cancer cells in an individual comprising administering to the individual an effective amount of a composition comprising an anti-KID31 antibody associated with a chemotherapeutic agent to the individual, wherein the cancer cells are selected from the group consisting of cancer cells from adrenal gland tumors, AIDS-associated cancers, alveolar soft part sarcoma, astrocytic tumors, bladder cancer (squamous cell carcinoma and transitional cell carcinoma), bone cancer (adamantinoma, aneurismal bone cysts, osteochondroma, osteosarcoma), brain and spinal cord cancers, metastatic brain tumors, breast cancer, carotid body tumors, cervical cancer, chondrosarcoma, dhordoma, chromophobe renal cell carcinoma, clear cell carcinoma, colon cancer, colorectal cancer, cutaneous benign fibrous histiocytomas, desmoplastic small round cell tumors, ependymomas, Ewing's tumors, extraskeletal myxoid chondrosarcoma, fibrogenesis imperfecta ossium, fibrous dysplasia of the bone, gallbladder and bile duct cancers, gestational trophoblastic disease, germ cell tumors, head and neck cancers, islet cell tumors, Kaposi's Sarcoma, kidney cancer (nephroblastoma, papillary renal cell carcinoma), leukemias, lipoma/benign lipomatous tumors, liposarcoma/malignant lipomatous tumors, liver cancer (hepatoblastoma, hepatocellular carcinoma), lymphomas, lung cancer, medulloblastoma, melanoma, meningiomas, multiple endocrine neoplasia, multiple myeloma, myelodysplastic syndrome, neuroblastoma, neuroendocrine tumors, ovarian cancer, pancreatic cancers, papillary thyroid carcinomas, parathyroid tumors, pediatric cancers, peripheral nerve sheath tumors, phaeochromocytoma, pituitary tumors, prostate cancer, posterious unveal melanoma, rare hematologic disorders, renal metastatic cancer, rhabdoid tumor, rhabdomysarcoma, sarcomas, skin cancer, soft-tissue sarcomas, squamous cell cancer, stomach cancer, synovial sarcoma, testicular cancer, thymic carcinoma, thymoma, thyroid metastatic cancer, and uterine cancers (carcinoma of the cervix, endometrial carcinoma, and leiomyoma).

20. The method of claim 19, wherein the chemotherapeutic agent is delivered into the cancer cells.

21. The method of claim 19, wherein the anti-KID31 antibody is a monoclonal antibody expressed by hybridoma ATCC No. PTA# 6516 or progeny thereof.

22. A method for detecting the presence or absence of a cancer cell in an individual comprising contacting cells from the individual with an anti-KID31 antibody, and detecting a complex of KID31 from the cells and the antibody, if any, wherein the cancer cell is selected from the group consisting of cancer cells from adrenal gland tumors, AIDS-associated cancers, alveolar soft part sarcoma, astrocytic tumors, bladder cancer (squamous cell carcinoma and transitional cell carcinoma), bone cancer (adamantinoma, aneurismal bone cysts, osteochondroma, osteosarcoma), brain and spinal cord cancers, metastatic brain tumors, breast cancer, carotid body tumors, cervical cancer, chondrosarcoma, dhordoma, chromophobe renal cell carcinoma, clear cell carcinoma, colon cancer, colorectal cancer, cutaneous benign fibrous histiocytomas, desmoplastic small round cell tumors, ependymomas, Ewing's tumors, extraskeletal myxoid chondrosarcoma, fibrogenesis imperfecta ossium, fibrous dysplasia of the bone, gallbladder and bile duct cancers, gestational trophoblastic disease, germ cell tumors, head and neck cancers, islet cell tumors, Kaposi's Sarcoma, kidney cancer (nephroblastoma, papillary renal cell carcinoma), leukemias, lipoma/benign lipomatous tumors, liposarcoma/malignant lipomatous tumors, liver cancer (hepatoblastoma, hepatocellular carcinoma), lymphomas, lung cancer, medulloblastoma, melanoma, meningiomas, multiple endocrine neoplasia, multiple myeloma, myelodysplastic syndrome, neuroblastoma, neuroendocrine tumors, ovarian cancer, pancreatic cancers, papillary thyroid carcinomas, parathyroid tumors, pediatric cancers, peripheral nerve sheath tumors, phaeochromocytoma, pituitary tumors, prostate cancer, posterious unveal melanoma, rare hematologic disorders, renal metastatic cancer, rhabdoid tumor, rhabdomysarcoma, sarcomas, skin cancer, soft-tissue sarcomas,

squamous cell cancer, stomach cancer, synovial sarcoma, testicular cancer, thymic carcinoma, thymoma, thyroid metastatic cancer, and uterine cancers (carcinoma of the cervix, endometrial carcinoma, and leiomyoma).

23. An agent that blocks at least one of the following interactions between KID31 and a KID31 binding partner:

a. the ability to bind to KID31 on a cancer cell;

b. the ability to bind to a portion of KID31 that is exposed on the surface of a living cell *in vitro* or *in vivo*;

c. the ability to deliver a therapeutic agent or detectable marker to a cancer cell expressing KID31; and

d. the ability to deliver a therapeutic agent or detectable marker into a cancer cell expressing KID31;

24. A pharmaceutical composition comprising a therapeutically effective dose of an agent according to claim 23, together with a pharmaceutically acceptable carrier.

25. A KID31 modulator, having at least one of the following characteristics:

a. the capability of disrupting or blocking the interaction between human KID31 and a native KID31 ligand;

b. the capability of disrupting or blocking the interaction between human KID31 and an anti-KID31 antibody;

c. the capability of binding to human KID31;

d. the capability of binding to a native ligand for human KID31;

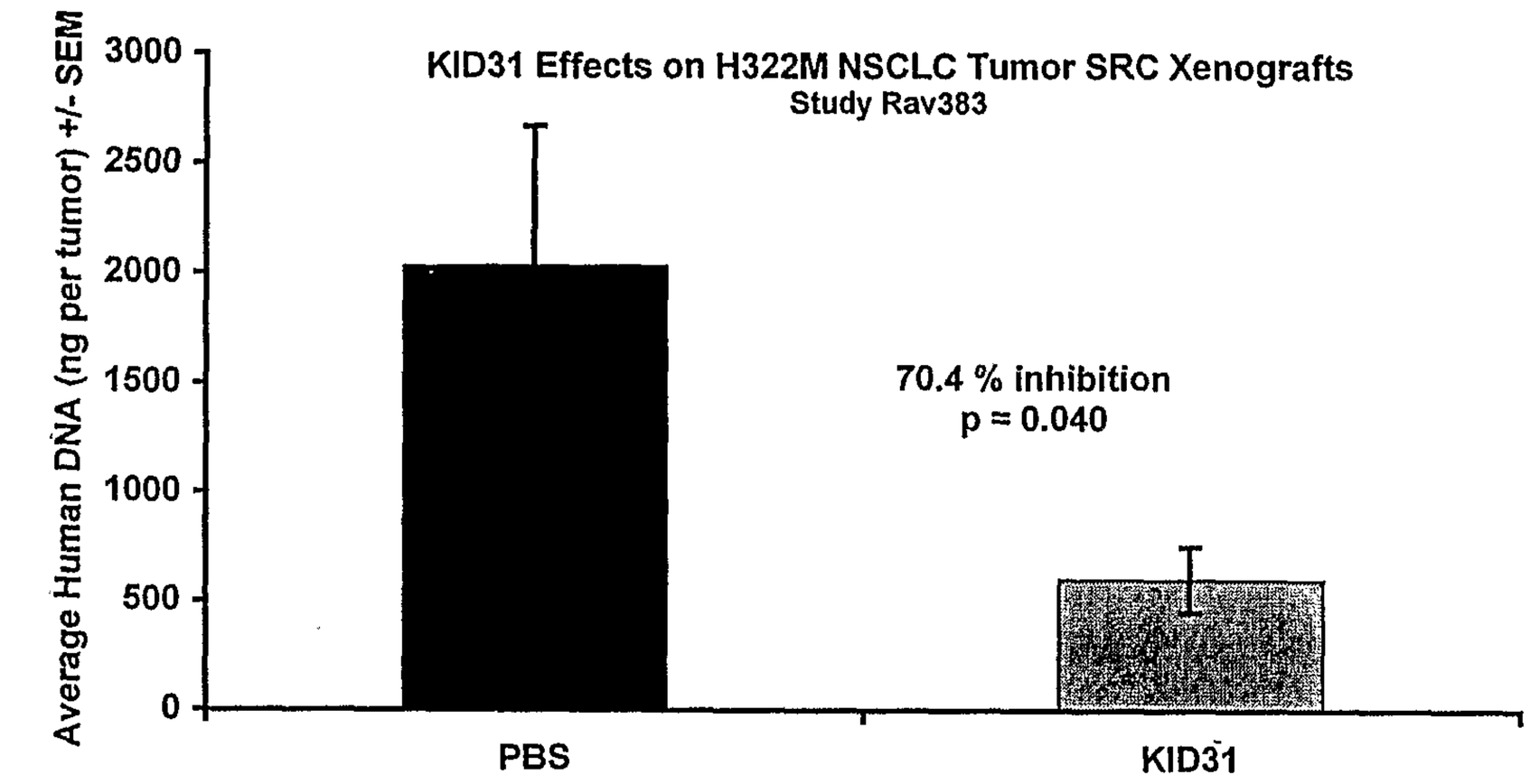
e. the capability of binding to an anti-KID31 antibody;

f. contains an antigenic site that can be used in the raising of antibodies capable of binding to human KID31, a native KID31 ligand or an anti-KID31 antibody;

g. contains an antigenic site that can be used in the screening of antibodies capable of binding to human KID31, native KID31 ligands or an anti-KID31 antibody;

h. contains an antigenic site that an be used in the raising of antibodies capable of disrupting or blocking the interaction between human KID31 and a native KID31 ligand or between KID31 and an anti-KID31 antibody;

i. contains an antigenic site that can be used in the screening of antibodies capable of disrupting or blocking the interaction between human KID31 and a native KID31 ligand or between KID31 and an anti-KID31 antibody.


KID31 Effects on H322M NSCLC Tumor SRC Xenografts
Study Rav383
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2000
1500
1000
500
0
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p = 0.040
PBS
KID31

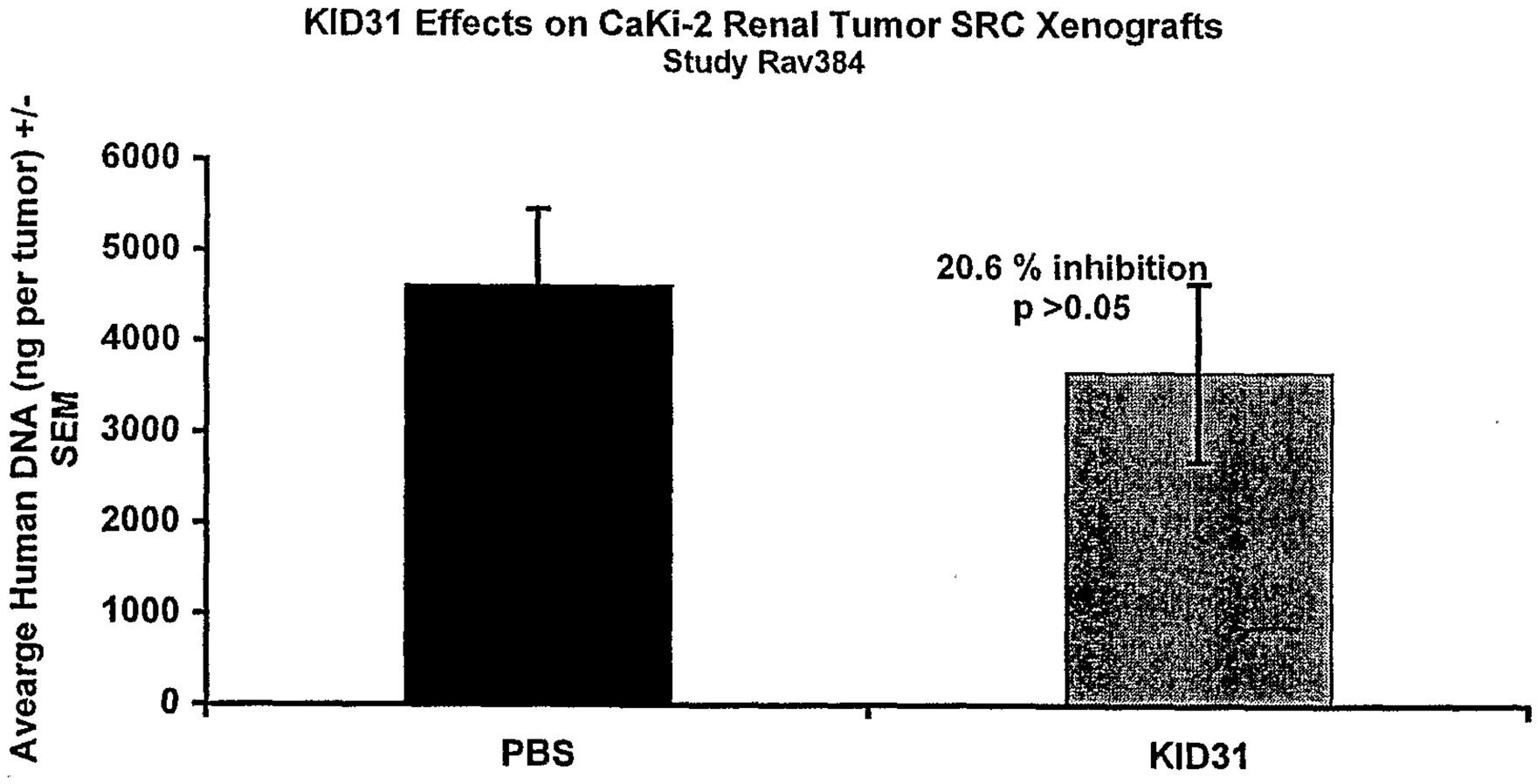
KID31 Effects on CaKi-2 Renal Tumor SRC Xenografts
Study Rav384
Avearge Human DNA (ng per tumor) +/- SEM
6000
5000
4000
3000
2000
1000
0
20.6 % inhibition
p >0.05
PBS
KID31

Figure 1