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(54) **MONOCLONAL ANTIBODY PAM4 AND ITS USE FOR DIAGNOSIS AND THERAPY OF PANCREATIC CANCER**

MONOKLONALER ANTIKÖRPER PAM4 UND DESSEN VERWENDUNG IN DER DIAGNOSE UND THERAPIE VON PANKREASKREBS

ANTICORPS MONOCLONAL PAM4 ET SON UTILISATION POUR DIAGNOSTIQUER ET TRAITER UN CANCER DU PANCREAS

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Description

FIELD OF THE INVENTION

[0001] The present invention is related to a chimeric PAM4 antibody or fragment thereof; a chimeric antibody or fragment thereof, comprising the complementarity-determining regions (CDRs) and framework regions (FR) of a murine PAM4 monoclonal antibody (MAb) and the light and heavy chain constant regions of a human antibody; a cancer cell targeting diagnostic or therapeutic conjugate comprising an antibody component that comprises the chimeric antibody or fragment thereof, comprising the complementarity-determining regions (CDRs) and framework regions (FR) of a murine PAM4 monoclonal antibody (MAb) and the light and heavy chain constant regions of a human antibody that binds to said cell; the cancer cell targeting diagnostic or therapeutic conjugate for use in intraoperative, endoscopic, or intravascular tumor diagnosis; a multivalent, multispecific antibody or fragment thereof comprising one or more antigen binding sites having affinity towards the PAM4 target epitope recognized by PAM4 antibody; an antibody fusion protein or fragment thereof comprising at least two chimeric PAM4 antibodies or fragments thereof or at least two chimeric antibodies or fragments thereof, comprising the complementarity-determining regions (CDRs) and framework regions (FR) of a murine PAM4 monoclonal antibody (MAb) and the light and heavy chain constant regions of a human antibody; the multivalent, multispecific antibody or fragment thereof for use in a method for diagnosing or treating cancer; the chimeric antibody or fragment thereof, comprising the complementarity-determining regions (CDRs) and framework regions (FR) of a murine PAM4 monoclonal antibody (MAb) and the light and heavy chain constant regions of a human antibody for use in the treatment of a malignancy in a subject; the antibody fusion protein or fragment thereof for use in the treatment of a malignancy in a subject; a diagnostic conjugate comprising the chimeric PAM4 Mab or fragment thereof or the PAM4 antibody fusion protein or fragment thereof for use in a method of diagnosing a malignancy in a subject; the chimeric antibody or fragment thereof being naked for use in a method for treating a cancer cell in a subject; the antibody fusion protein or fragment comprising the naked chimeric PAM4 antibody or fragment thereof for use in a method for treating a cancer cell in a subject; a method of diagnosing a pancreatic cancer in a subject comprising performing an *in vitro* diagnosis assay on a specimen from said subject with a composition comprising the naked PAM4 Mab or fragment thereof or the naked PAM4 Mab antibody fusion protein or fragment thereof; a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue expressing PAM4 antigen and at least one other arm that specifically binds a targetable conjugate, wherein said one arm that specifically binds a targeted tissue is the cPAM4 antibody or fragment thereof, for use in a method of identifying diseased tissue expressing a PAM4 antigen; a bispecific antibody F(ab)2 or F(ab')2 fragment, diabody, triabody, or tetrabody, wherein the bispecific antibody or fragment has a first antibody binding site which specifically binds to a PAM4 antigen, wherein the antibody is the PAM4 antibody, for use in a method of detection of lesions during an endoscopic, intravascular catheter, or surgical procedure; and a cPAM4 immunoconjugate or fragment thereof binding with the same antigen that is recognized by the full-length cPAM4 antibody for use in a method for close-range lesion detection, wherein the immunoconjugate comprises the cPAM4 antibody.

BACKGROUND OF THE INVENTION

[0002] The pancreas produces insulin to assist the body in converting glucose to energy and enzymes to assist the body in digesting food. Pancreatic cancer is a malignant growth of the pancreas that mainly occurs in the cells of the pancreatic ducts. This disease is the ninth most common form of cancer, yet it is the fourth and fifth leading cause of cancer deaths in men and women, respectively. Cancer of the pancreas is almost always fatal, with a five-year survival rate that is less than 3%.

[0003] The most common symptoms of pancreatic cancer include jaundice, abdominal pain, and weight loss, which, together with other presenting factors, are nonspecific in nature. Thus, diagnosing pancreatic cancer at an early stage of tumor growth is often difficult and requires considerable suspicion and extensive diagnostic work-up, often times including exploratory surgery. Endoscopic ultrasonography and computed tomography are the best noninvasive means available today for diagnosis of pancreatic cancer. However, reliable detection of small tumors, as well as differentiation of pancreatic cancer from focal pancreatitis, is troublesome. Unfortunately, the vast majority of patients are presently diagnosed at a late stage when the tumor has already extended outside of the capsule to invade surrounding organs and/or has metastasized extensively. Gold et al., Crit. Rev. Oncology/Hematology, 39:147-54 (2001). Late detection of the disease is common, and "early" pancreatic cancer diagnosis is rare in the clinical setting.

[0004] Current treatment procedures available for pancreatic cancer have not led to a cure, nor to a substantially improved survival time. Surgical resection has been the only modality that offers a chance at survival. However, due to a large tumor burden, only 10% to 25% of patients are candidates for "curative resection." For those patients undergoing a surgical treatment, the five-year survival rate is still poor, averaging only about 10%.

[0005] Early detection and diagnosis of pancreatic cancer, as well as appropriate staging of the disease, would provide

an increased survival advantage. A number of laboratories are proceeding on the development of a diagnostic procedure based upon the release of a tumor-associated marker into the bloodstream as well as detection of the marker substance within biopsy specimens. The best tumor associated marker for pancreatic cancer has been the immunoassay for CA19.9. Elevated levels of this sialylated Le^a epitope structure were found in 70% of pancreatic cancer patients but were not found in any of the focal pancreatitis specimens examined. However, CA19.9 levels were found to be elevated in a number of other malignant and benign conditions, so that currently the assay cannot be used for diagnosis. However, the assay is useful for monitoring, the continued increase in CA19.9 serum levels after surgery being indicative of a poor prognosis. Many other monoclonal antibodies (MAbs) have been reported with immunoassays for diagnosis in varying stages of development. These include but are not limited to DUPAN2, SPAN1, B72.3, Ia3, and various anti-CEA antibodies.

[0006] Man-made antibodies, in particular MAbs and engineered antibodies or antibody fragments, have been tested widely and shown to be of value in detection and treatment of pancreatic cancer, as well as other various human disorders, including cancers, autoimmune diseases, infectious diseases, inflammatory diseases, and cardiovascular diseases [Filpula and McGuire, Exp. Opin. Ther. Patents (1999) 9: 231-245]. The clinical utility of an antibody or an antibody-derived agent is primarily dependent on its ability to bind to a specific targeted antigen associated with a specific disorder. Selectivity is valuable for delivering a diagnostic or therapeutic agent, such as isotopes, drugs, toxins, cytokines, hormones, hormone antagonists, enzymes, enzyme inhibitors, oligonucleotides, growth factors, radionuclides, an angingenesis inhibitor, or metals, to a target location during the detection and treatment phases of a human disorder, particularly if the diagnostic or therapeutic agent is toxic to normal tissue in the body. Radiolabeled antibodies have been used with some success in numerous malignancies, including ovarian cancer, colon cancer and lymphoma. This technology may also prove useful for pancreatic cancer. However, other than the application of anti-CEA antibodies and B72.3, little clinical information exists.

[0007] The potential limitations of such antibody systems are discussed in Goldenberg, The American Journal ofMedicine, 94: 298-299 (1993). The important parameters in the detection and treatment techniques are the amount of the injected dose specifically localized at the site(s) where target cells are present and the uptake ratio, i.e. the ratio of the concentration of specifically bound antibody to that of the radioactivity present in surrounding normal tissues. When an antibody is injected into the blood stream, it passes through a number of compartments as it is metabolized and excreted. The antibody must be able to locate and bind to the target cell antigen while passing through the rest of the body. Factors that control antigen targeting include location, size, antigen density, antigen accessibility, cellular composition of pathologic tissue, and the pharmacokinetics of the targeting antibodies. Other factors that specifically affect tumor targeting by antibodies include expression of the target antigens, both in tumor and other tissues, and bone marrow toxicity resulting from the slow blood-clearance of the radiolabeled antibodies. The amount of targeting antibodies accreted by the targeted tumor cells is influenced by the vascularization of the tumor, barriers to antibody penetration of tumors, and intratumoral pressure. Non-specific uptake by non-target organs such as the liver, kidneys or bone-marrow is another potential limitation of the technique, especially for radioimmunotherapy, where irradiation of the bone marrow often causes the dose-limiting toxicity.

[0008] One suggested approach for delivering agents to a target site, referred to as direct targeting, is a technique designed to target specific antigens with antibodies carrying diagnostic or therapeutic radioisotopes. In the context of tumors, the direct targeting approach utilizes a radiolabeled anti-tumor monospecific antibody that recognizes the target tumor through its antigens. The technique involves injecting the labeled monospecific antibody into the patient and allowing the antibody to localize at the target tumor to obtain diagnostic or therapeutic benefits. The unbound antibody clears the body. This approach can be used to diagnose or treat additional mammalian disorders.

[0009] Another suggested solution, referred to as the "Affinity Enhancement System" (AES), is a technique especially designed to overcome deficiencies of tumor targeting by antibodies carrying diagnostic or therapeutic radioisotopes [US-5,256,395 (1993), Barbet et al., Cancer Biotherapy & Radiopharmaceuticals 14: 153-166 (1999)]. The AES utilizes a radiolabeled divalent hapten and an anti-tumor/anti-hapten bispecific antibody that recognizes both the target tumor and the radioactive hapten. Haptens with higher valency and antibodies with higher specificity may also be utilized for this procedure. The technique involves injecting the antibody into the patient and allowing it to localize at the target tumor. After a sufficient amount of time for the unbound antibody to clear from the blood stream, the radiolabeled hapten is administered. The hapten binds to the antibody-antigen complex located at the site of the target cell to obtain diagnostic or therapeutic benefits, while the unbound hapten rapidly clears from the body. Barbet mentions the possibility that a bivalent hapten may crosslink with a bispecific antibody, when the latter is bound to the tumor surface. As a result, the radiolabeled complex is more stable and stays at the tumor for a longer period of time. This system can be used to diagnose or treat mammalian disorders.

[0010] There remains a need in the art for production of multivalent, monospecific antibodies that are useful in a direct targeting system and for production of multivalent, multispecific antibodies that are useful in an affinity enhancement system. Specifically, there remains a need for a antibody that performs as a useful diagnostic tool for pancreatic cancer and that exhibits enhanced uptake at targeted antigens, decreased concentration in the blood, and optimal protection

of normal tissues and cells from toxic pharmaceuticals.

Gold D et al. (Int. J. Cancer: 71, no. 4, pp. 660-667 (1997)) describe radioimmunotherapy of experimental pancreatic cancer with 13I-labeled monoclonal antibody PAM4.

Cardillo TM et al. (Clinical Cancer Research, vol. 7, no. 10, pp. 3186-3192 (2001)) describe a therapeutic advantage of 90Y- versus 13I-labeled PAM4 antibody in experimental pancreatic cancer.

Mariani G et al. (Cancer Research (Suppl.) 55: 5911S-5915S (December 1, 1995)) describe initial tumor targeting, biodistribution and pharmacokinetic evaluation of the monoclonal antibody PAM4 in patients with pancreatic cancer.

Gold DV et al. (Int. J. Cancer: 57, 204-210 (1994)) describe the characterization of monoclonal antibody PAM4 reactive with a pancreatic cancer mucin.

Cardillo TM et al. (Int. J. Cancer: 97, 386-392 (2002)) describe combined gemcitabine and radioimmunotherapy for the treatment of pancreatic cancer.

Gold DV et al. (Cancer Research 55: 1105-1110 (March 1, 1995)) describe targeting of xenografted pancreatic cancer with a new monoclonal antibody, PAM4.

Price MR et al. ("Summary Report on the ISOBM TD-1 Workshop: Analysis of 56 Monoclonal Antibodies against the MUC1 Mucin", Tumor Biology, Karger, Basel CH, vol. 19, no. Suppl 1, 1998, pp. 1-20)) report on the analysis of a variety of monoclonal antibodies against mucin MUC1.

SUMMARY OF THE INVENTION

[0011] The problem underlying the present invention is solved by the subject matter of the attached independent claims; preferred embodiments may be taken from the attached dependent claims.

[0012] More specifically, the problem underlying the present invention is solved in a first aspect which is also the first embodiment of the first aspect, by a chimeric PAM4 antibody or fragment thereof, wherein said antibody or fragment thereof comprises the PAM4 V_κ polypeptide sequence of Fig. 2A or Fig. 2C, and the PAM4 V_h polypeptide sequence of Fig. 2B or Fig. 2D.

[0013] The problem underlying the present invention is solved in a second aspect which is also a first embodiment of the second aspect, by a chimeric antibody or fragment thereof, comprising the complementarity-determining regions (CDRs) and framework regions (FR) of a murine PAM4 monoclonal antibody (MAb) and the light and heavy chain constant regions of a human antibody, wherein the CDRs of the light chain variable region of the chimeric PAM4 MAb comprise CDR1 comprising an amino acid sequence of SASSSVSSSYLY; CDR2 comprising an amino acid sequence of STSNLAS; and CDR3 comprising an amino acid sequence of HQWNRYPYT; and the CDRs of the heavy chain variable region of the chimeric PAM4 MAb comprises CDR1 comprising an amino acid sequence of SYVLH; CDR2 comprising an amino acid sequence of YINPYNDGTQYNEKFKG and CDR3 comprising an amino acid sequence of GFGGSYGFAY.

[0014] The problem underlying the present invention is solved in a third aspect which is also a first embodiment of the third aspect, by a cancer cell targeting diagnostic or therapeutic conjugate comprising an antibody component that comprises an antibody or fragment thereof of the second aspect that binds to said cell, wherein said antibody component is bound to at least one diagnostic/detection and/or at least one therapeutic agent.

[0015] In an embodiment of the third aspect the said diagnostic/detection agent is selected from the group comprising a radionuclide, a contrast agent, and a photoactive diagnostic/detection agent.

[0016] In an embodiment of the third aspect said diagnostic agent is a radionuclide.

[0017] In an embodiment of the third aspect said radionuclide has an energy between 20 and 4,000 keV.

[0018] In an embodiment of the third aspect said radionuclide is a gamma-, beta- or a positron-emitting isotope.

[0019] In an embodiment of the third aspect said radionuclide is selected from the group consisting of ^{110}In, ^{111}In, ^{177}Lu, ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{89}Zr, ^{94m}Tc, ^{94}Tc, ^{99m}Tc, ^{120}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154\text{-}158}$Gd, ^{32}P, ^{11}C, ^{13}N, ^{15}O, ^{186}Re, ^{188}Re, ^{51}Mn, ^{52m}Mn, ^{55}Co, ^{72}As, ^{75}Br, ^{76}Br, ^{82m}Rb, ^{83}Sr, or other gamma-, beta-, or positron-emitters.

[0020] In an embodiment of the third aspect said diagnostic/detection agent is a contrast agent.

[0021] In an embodiment of the third aspect said contrast agent is a paramagnetic ion.

[0022] In an embodiment of the third aspect said paramagnetic ion is a metal comprising chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III),

vanadium (II), terbium (III), dysprosium (III), holmium (III) or erbium (III).

[0023] In an embodiment of the third aspect said contrast agent is a metal comprising lanthanum (III), gold (III), lead (II) or bismuth (III).

[0024] In an embodiment of the third aspect said contrast agent is an ultrasound enhancing agent.

[0025] In an embodiment of the third aspect said ultrasound enhancing agent is a liposome that comprises the chimeric antibody or fragment according to the second aspect.

[0026] In an embodiment of the third aspect said liposome is gas filled.

[0027] In an embodiment of the third aspect said contrast agent is a radiopaque material selected from the group comprising iodine compounds, barium compounds, gallium compounds, and thallium compounds.

[0028] In an embodiment of the third aspect said radiopaque material is selected from the group comprising barium, diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide, iodipamide, iodoxamic acid, iogulamide, iohexol, iopamidol, iopanoic acid, ioprocemic acid, iosefamic acid, ioseric acid, iosulamide meglumine, iosemetic acid, iotasul, iotetric acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizoic acid, ipodate, meglumine, metrizamide, metrizoate, propyliodone, and thallous chloride.

[0029] In an embodiment of the third aspect said diagnostic/detection agent is a photactive diagnostic/detection agent.

[0030] In an embodiment of the third aspect said photoactive diagnostic/detection agent is a fluorescent labeling compound selected from the group comprising fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

[0031] In an embodiment of the third aspect said photoactive diagnostic/detection agent is a chemiluminescent labeling compound selected from the group comprising luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester.

[0032] In an embodiment of the third aspect said photoactive diagnostic/detection agent is a bioluminescent compound selected from the group comprising luciferin, luciferase and aequorin.

[0033] In a fourth aspect which is also a first embodiment of the fourth aspect the conjugate of the third aspect is for use in intraoperative, endoscopic, or intravascular tumor diagnosis.

[0034] In an embodiment of the third aspect said therapeutic agent is selected from the group consisting of a radionuclide, an immunomodulator, a hormone, a hormone antagonist, an enzyme, an enzyme inhibitor, an oligonucleotide, a photoactive therapeutic agent, a cytotoxic agent, an antibody, an angiogenesis inhibitor, and a combination thereof.

[0035] In an embodiment of the third aspect said oligonucleotide is an antisense oligonucleotide.

[0036] In an embodiment of the third aspect said oligonucleotide is an antisense oligonucleotide against an oncogene.

[0037] In an embodiment of the third aspect said oncogene is bcl-2 or p53.

[0038] In an embodiment of the third aspect said therapeutic agent is a cytotoxic agent.

[0039] In an embodiment of the third aspect said cytotoxic agent is a drug or a toxin.

[0040] In an embodiment of the third aspect said drug possesses the pharmaceutical property selected from the group consisting of antimitotic, alkylating, antimetabolite, antiangiogenic, apoptotic, alkaloid, and antibiotic agents and combinations thereof.

[0041] In an embodiment of the third aspect said drug is selected from the group consisting of nitrogen mustards, gemcitabine, ethylenimine derivatives, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, anthracyclines, SN-38, taxanes, COX-2 inhibitors, pyrimidine analogs, purine analogs, antibiotics, enzymes, enzyme inhibitors, epipodophyllotoxins, platinum coordination complexes, vinca alkaloids, substituted ureas, methyl hydrazine derivatives, adrenocortical suppressants, hormone antagonists, endostatin, taxols, camptothecins, doxorubicins and their analogs, antimetabolites, alkylating agents, antimitotics, antiangiogenic, apoptotoic agents, methotrexate, CPT-11, and a combination thereof.

[0042] In an embodiment of the third aspect said toxin derived from a source selected from the group comprising an animal, a plant, and a microbial source.

[0043] In an embodiment of the third aspect said toxin is selected from the group consisting of ricin, abrin, alpha toxin, saporin, ribonuclease (RNase), DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtherin toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin.

[0044] In an embodiment of the third aspect therapeutic agent is an immunomodulator.

[0045] In an embodiment of the third aspect said immunomodulator is selected from the group consisting of a cytokine, a stem cell growth factor, a lymphotoxin, a hematopoietic factor, a colony stimulating factor (CSF), an interferon (IFN), a stem cell growth factor, erythropoietin, thrombopoietin and a combination thereof.

[0046] In an embodiment of the third aspect said lymphotoxin is tumor necrosis factor (TNF), said hematopoietic factor is an interleukin (IL), said colony stimulating factor is granulocyte-colony stimulating factor (G-CSF) or granulocyte macrophage-colony stimulating factor (GM-CSF)), said interferon is interferons-α, -β or -γ, and said stem cell growth factor is designated "S1 factor".

[0047] In an embodiment of the third aspect said immunomodulator comprises IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, IL-21, interferon-γ, TNF- a or a combination thereof.

[0048] In an embodiment of the third aspect said therapeutic agent is a radionuclide.

[0049] In an embodiment of the third aspect said radionuclide has an energy between 60 and 700 keV.

[0050] In an embodiment of the third aspect said radionuclide is selected from the group consisting of ^{32}P, ^{33}P, ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{86}Y, ^{90}Y, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{211}At, ^{223}Ra and ^{225}Ac, and combinations thereof.

[0051] In an embodiment of the third aspect said therapeutic agent is a photoactive therapeutic agent.

[0052] In an embodiment of the third aspect said photoactive therapeutic agent is selected from the group comprising chromogens and dyes.

[0053] In an embodiment of the third aspect said therapeutic agent is an enzyme.

[0054] In an embodiment of the third aspect said enzyme is selected from the group comprising malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, α -glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, ß-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

[0055] The problem underlying the present invention is solved in a fifth aspect which is also a first embodiment of the fifth aspect, by a multivalent, multispecific antibody or fragment thereof comprising one or more antigen binding sites having affinity towards the PAM4 target epitope recognized by PAM4 antibody, wherein the CDRs of the light chain variable region of the PAM4 antibody comprise CDR1 comprising an amino acid sequence of SASSSVSSSYLY; CDR2 comprising an amino acid sequence of STSNLAS; and CDR3 comprising an amino acid sequence of HQWNRYPYT; and the CDRs of the heavy chain variable region of the PAM4 MAb comprises CDR1 comprising an amino acid sequence of SYVLH; CDR2 comprising an amino acid sequence of YINPYNDGTQYNEKFKG and CDR3 comprising an amino acid sequence of GFGGSYGFAY, preferably the antibody according to the second aspect, or fragment thereof, and one or more hapten binding sites having affinity towards hapten molecules.

[0056] In an embodiment of the fifth aspect the antibody or fragment thereof comprises further a diagnostic or therapeutic agent.

[0057] The problem underlying the present invention is solved in a sixth aspect which is also a first embodiment of the sixth aspect, by an antibody fusion protein or fragment thereof comprising at least two chimeric PAM4 MAbs or fragments thereof of the first aspect of the second aspect.

[0058] The problem underlying the present invention is solved in a seventh aspect which is also a first embodiment of the seventh aspect, by the multivalent, multispecific antibody or fragment thereof according to the fifth aspect, for use in a method for diagnosing or treating cancer comprising: (a) administering to a subject in need thereof the antibody or fragment thereof of the multivalent, multispecific antibody or fragment thereof; (b) waiting a sufficient amount of time is waited for an amount of the non-antibody to clear the subject's blood stream; and (c) administering to said subject a carrier molecule comprising a diagnostic/detection agent, a therapeutic agent, or a combination thereof, that binds to a binding site of the antibody.

[0059] In an embodiment of the seventh aspect said method can be used for intraoperative identification of diseased tissues, endoscopic identification of diseased tissues, or intravascular identification of diseased tissues.

[0060] The problem underlying the present invention is solved in an eighth aspect which is also a first embodiment of the eighth aspect, by the chimeric antibody or fragment thereof according to the second aspect or an antibody fusion protein and fragment thereof comprising PAM4 antibody, wherein said PAM4 is the antibody according to the second aspect, and wherein said antibody or fragment thereof or antibody fusion protein of fragment thereof is bound to at least one therapeutic agent, for use in the treatment of a malignancy in a subject wherein said antibody or fragment is optionally formulated in a pharmaceutically suitable excipient.

[0061] In an embodiment of the eighth aspect the antibody fusion protein or fragment thereof further comprises a second Mab or fragment thereof that is not a PAM4 Mab or fragment thereof.

[0062] In an embodiment of the eighth aspect said second Mab or fragment thereof is a naked Mab or fragment thereof.

[0063] In an embodiment of the eighth aspect said second Mab or fragment thereof is selected from the group consisting of CAI9.9, SUPAN2, SPAN1, Nd2, B72.3, CC49, CEA, aLe^a, antibodies defined by the Lewis antigen Le(y), CSAp, MUC-2, MUC-3, MUC-4, TAG-72, EGFR, CD40, angiogenesis factors (e.g. VEGF), products of oncogenes, insulin like growth factor (1GF), tenascin, platelet derived growth factor, IL-6, and HER2/neu.

[0064] In an embodiment of the eighth aspect said second Mab is conjugated to a therapeutic or diagnostic/detection agent.

[0065] In an embodiment of the eighth aspect the antibody fusion protein or fragment thereof further comprises a second PAM4 Mab or fragment thereof.

[0066] In an embodiment of the eighth aspect said PAM4 antibody is administered parentally.

[0067] In an embodiment of the eighth aspect said PAM4 antibody is administered in a dosage of 20 to 2000 milligrams protein per dose.

[0068] In an embodiment of the eighth aspect said dosage is repeatedly administered.

[0069] In an embodiment of the eighth aspect said chimerized PAM4 antibody constant and hinge regions comprise constant and hinge regions of a human IgG.

[0070] In an embodiment of the eighth aspect said PAM4 antibody is administered before, in conjunction with, or after a second naked or conjugated antibody reactive with a second tumor marker expressed by said malignancy is administered to said subject.

[0071] In an embodiment of the eighth aspect said PAM4 antibody is administered before, concurrently, or after at least one therapeutic or diagnostic/detection agent is administered to said subject.

[0072] The problem underlying the present invention is solved in a ninth aspect which is also a first embodiment of the ninth aspect, by a diagnostic conjugate comprising a PAM4 Mab or fragment thereof or a PAM4 antibody fusion protein or fragment thereof, wherein said PAM4 Mab or fragment thereof or antibody fusion protein or fragment thereof is conjugated to at least one diagnostic/detection agent and wherein the PAM4 antibody is the antibody according to the second aspect, for use in a method of diagnosing a malignancy in a subject comprising:

administering to said subject a diagnostically effective amount of the diagnostic conjugate, and optionally formulating said PAM4 antibody or fragment thereof or said fusion protein or fragment thereof in a pharmaceutically suitable excipient.

[0073] The problem underlying the present invention is solved in a tenth aspect which is also a first embodiment of the tenth aspect, by the chimeric antibody or fragment thereof of the second aspect, wherein the chimeric antibody or fragment thereof is a naked chimeric antibody or fragment thereof, or a PAM4 Mab antibody fusion protein or fragment thereof comprising naked PAM4 Mab wherein in the PAM4 Mab is the antibody according to the second aspect, wherein the naked chimeric antibody or fragment thereof or the naked antibody fusion protein or fragment thereof is comprised in a composition, for use in a method for treating a cancer cell in a subject, wherein such method comprises administering to said subject a therapeutically effective amount of the composition, wherein said PAM4 antibody or fragment is optionally formulated in a pharmaceutically suitable excipient.

[0074] In an embodiment of the tenth aspect said composition further comprises a second naked antibody or fragment thereof.

[0075] In an embodiment of the tenth aspect said second antibody or fragment thereof is not a PAM4 Mab or fragment thereof.

[0076] In an embodiment of the tenth aspect said second antibody or fragment thereof is selected from the group consisting of CA19.9, DUPAN2, SPAN1, Nd2, B72.3, CC49, CEA, aLe^a , antibodies defined by the Lewis antigen Le(y), CSAp, MUC-2, MUC-3, MUC-4, TAG-72, EGFR, CD40, angiogenesis factors (e.g. VEGF), insulin-like growth factor (IGF), tenasin, platelet derived growth factor, IL-6, products of oncogenes and HER2/neu.

[0077] In an embodiment of the tenth aspect said naked PAM4 antibody is administered parentally.

[0078] In an embodiment of the tenth aspect said naked PAM4 antibody is administered in a dosage of 20 to 2000 milligrams protein per dose.

[0079] In an embodiment of the tenth aspect said dosage is repeatedly administered.

[0080] In an embodiment of the tenth aspect said naked PAM4 antibody constant and hinge regions comprise constant and hinge regions of a human IgG.

[0081] In an embodiment of the tenth aspect said second naked PAM4 antibody is administered before, in conjunction with, or after a naked antibody is administered to said subject.

[0082] In an embodiment of the tenth aspect said naked PAM4 antibody is administered before, concurrently or after a therapeutic and/or diagnostic/detection agent.

[0083] The problem underlying the present invention is solved in an eleventh aspect which is also a first embodiment of the eleventh aspect, by a method of diagnosing a pancreatic cancer in a subject comprising performing an *in vitro* diagnosis assay on a specimen from said subject with a composition comprising a naked PAM4 Mab or fragment thereof or a naked PAM4 Mab antibody fusion protein or fragment thereof wherein the PAM4 Mab antibody is the antibody according to the second aspect.

[0084] In an embodiment of the eleventh aspect said in vitro diagnosis is selected from the group consisting of immunoassays and immunohistochemistry.

[0085] In an embodiment of the eleventh aspect said in vitro diagnosis assay is or immunoassays.

[0086] In an embodiment of the eleventh aspect said specimen is body fluid or a tissue.

[0087] In an embodiment of the eleventh aspect said diagnosis assay is immunohistochemistry.

[0088] In an embodiment of the eleventh aspect said specimen is a population of cells or a tissue.

[0089] The problem underlying the present invention is solved in a twelfth aspect which is also a first embodiment of the twelfth aspect, by a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue expressing PAM4 antigen and at least one other arm that specifically binds a targetable conjugate, wherein said one arm that specifically binds a targeted tissue is a cPAM4 antibody or fragment thereof, wherein the

CDRs of the light chain variable region of the cPMA4 antibody comprises CDR1 comprising an amino acid sequence of SASSSVSSSYLY; CDR2 comprising an amino acid sequence of STSNLAS; and CDR3 comprising an amino acid sequence of HQWNRYPYT; and the CDRs of the heavy chain variable region of the cPAM4 MAb comprises CDR1 comprising an amino acid sequence of SYVLH; CDR2 comprising an amino acid sequence of YINPYNDGTQYNEKFKG and CDR3 comprising an amino acid sequence of GFGGSYGFAY, for use in a method of identifying diseased tissues expressing a PAM4 antigen, in a subject, comprising:

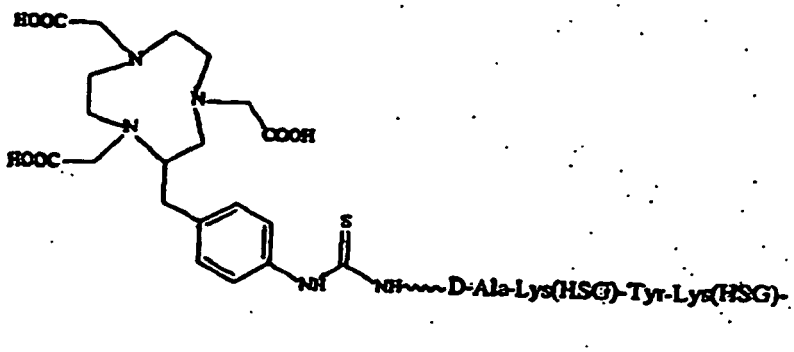
(a) administering an effective amount of the bispecific antibody or antibody fragment; and
(b) administering a targetable conjugate selected from the group consisting of

(i) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2

(ii) DOTA-Phe-Lye(HSG)-Tyr-Lys-(HSG)-NH_2

(iii) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(HSG)-Lys(Tscg-Cys)-NH_2

(iv)



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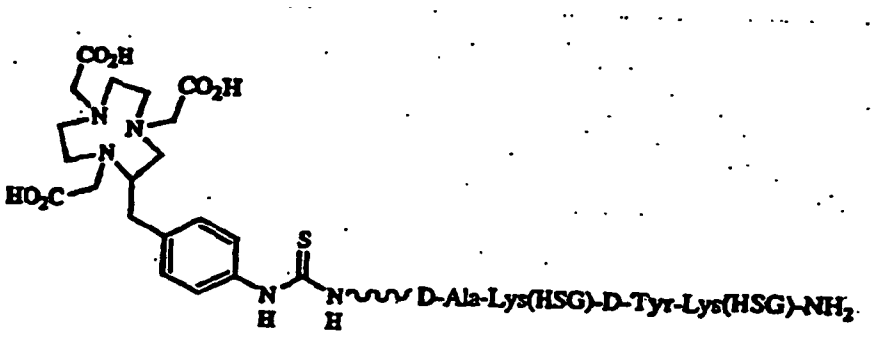


wherein the method of identifying diseased tissues expressing a PAM4 antigen is a method of intraoperatively identifying diseased tissues, a method for endoscopical identification of diseased tissues or a method for intravascular identification of diseased tissues.

[0090] The problem underlying the present invention is solved in an 13th aspect which is also a first embodiment of the 13th aspect, by a bispecific antibody F(ab)2 or F(ab')2 fragment, diabody, triabody, or tetrabody, wherein the bispecific antibody or fragment has a first antibody binding site which specifically binds to a PAM4 antigen, wherein the antibody is a PAM4 antibody and wherein the CDRs of the light chain variable region of the PMA4 antibody comprises CDR1 comprising an amino acid sequence of SASSSVSSSYLY; CDR2 comprising an amino acid sequence of STSNLAS; and CDR3 comprising an amino acid sequence of HQWNRYPYT; and the CDRs of the heavy chain variable region of the PAM4 antibody comprises CDR1 comprising an amino acid sequence of SYVLH; CDR2 comprising an amino acid sequence of YINPYNDGTQYNEKFKG and CDR3 comprising an amino acid sequence of GFGGSYGFAY, and has a

second antibody binding site which specifically binds to a hapten for use in a method of detection of lesions during an endoscopic, intravascular catheter, or surgical procedure, wherein the method comprises:

injecting a subject who is to undergo such a procedure with the bispecific antibody F(ab)2 or F(ab')2 fragment, diabody, triabody, or tetrabody, and permitting the antibody fragment to accrete at target sites; optionally clearing non-targeted antibody fragments using a galactosylated anti-idiotype clearing agent if the bispecific fragment is not largely cleared from circulation within about 24 hours of injection, and injecting a bivalent labeled hapten, which quickly localizes at the target site and clears through the kidneys; detecting the presence of the hapten by close-range detection of elevated levels of accreted label at the target sites with detection means, within 48 hours of the first injection, and conducting said procedure, wherein said detection is performed without the use of a contrast agent or subtraction agent.

[0091] The problem underlying the present invention is solved in a 14th aspect which is also a first embodiment of the 14th aspect by a cPAM4 immunoconjugate or fragment thereof binding with the same antigen that is recognized by the full-length cPAM4 antibody for use in a method for close-range lesion detection, during an operative, intravascular, or endoscopic procedure, wherein the method comprises:

injecting a subject to such a procedure parentally with an effective amount of the cPAM4 immunoconjugate or fragment thereof;

conducting the procedure within 48 hours of the injection;

scanning the accessed interior of the subject at close range with a detection means for detecting the presence of said labelled antibody or fragment thereof; and locating the sites of accretion of said labelled antibody or fragment thereof by detecting elevated levels of said labelled antibody or fragment thereof at such sites with the detection means,

wherein the immunoconjugate comprises a cPAM4 antibody, wherein the PAM4 antibody comprises CDR1 comprising an amino acid sequence of SASSSVSSSYLY; CDR2 comprising an amino acid sequence of STSNLAS; and CDR3 comprising an amino acid sequence of HQWNRYPYT; and the CDRs of the heavy chain variable region of the PAM4 MAb comprises CDR1 comprising an amino acid sequence of SYVLH; CDR2 comprising an amino acid sequence of YINPYNDGTQYNEKFKG and CDR3 comprising an amino acid sequence of GFGGSYGFAY.

[0092] Contemplated in the present invention is an antibody, fusion protein, and fragments thereof that bind a domain located between the amino terminus and start of the repeat domain of MUC1 which is a PAM4 antibody as defined in the claims. The PAM4 antibody, fusion protein, or fragment thereof of the present or invention is derived by immunization and/or selection with or against mucin of pancreatic cancer. Accordingly, the PAM4 antibody, fusion protein, and fragments thereof of the present invention preferably bind an antigen associated with pancreatic cancer cells.

[0093] The PAM4 antibody or fragment thereof is chimerized or the PAM4 fusion protein comprises a chimerized PAM4 antibody or fragment thereof. Also preferred, the PAM4 antibody, fusion protein, and fragments thereof can be conjugated to at least one therapeutic and/or diagnostic agent.

[0094] Contemplated herein is a murine PAM4 antibody or fragement thereof and disclosed herein and claimed is a chimerized PAM4 antibody or fragment thereof comprising the complementarity-determining regions (CDRs) and framework regions (FR) of a murine PAM4 MAb and the light and heavy chain constant regions of a human antibody, wherein the CDRs of the light chain variable region of the chimerized PAM4 MAb comprise CDR1 comprising an amino acid sequence of SASSSVSSSYLY; CDR2 comprising an amino acid sequence of STSNLAS; and CDR3 comprising an amino acid sequence of HQWNRYPYT; and the CDRs of the heavy chain variable region of the chimerized PAM4 MAb comprise CDR1 comprising an amino acid sequence of SYVLH; CDR2 comprising an amino acid sequence of YINPYNDGTQYNEKFKG and CDR3 comprising an amino acid sequence of GFGGSYGFAY. Most preferably, the chimerized PAM4 antibody or fragment thereof comprises a PAM4 Vκ nucleotide sequence of figure 1A and a PAM4 VH nucleotide sequence of figure 1B and/or comprises a cPAM4 V amino acid sequence of figure 2A and a cPAM4 V_H amino acid sequence of figure 2B. Also preferred, the murine PAM4 antibody or fragment thereof comprises a PAM4 Vκ nucleotide sequence of figure 1A and a PAM4 VH nucleotide sequence of figure 1B.

[0095] Another embodiment of the present invention is a cancer cell targeting diagnostic immunoconjugate comprising an antibody component that comprises an antibody or fragment thereof of any one of the antibodies, fusion proteins, or fragments thereof of the present invention, wherein the antibody, fusion protein, or fragment thereof is bound to at least one diagnostic/detection agent.

[0096] Preferably, the diagnostic/detection agent is selected from the group comprising a radionuclide, a contrast

agent, and a photoactive diagnostic/detection agent. Still preferred, the diagnostic/detection agent is a radionuclide with an energy between 20 and 4,000 keV or is a radionuclide selected from the group consisting of ^{110}In, ^{111}In, ^{177}Lu, ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{89}Zr, ^{94m}Tc, ^{94}Tc, ^{99m}Tc, ^{120}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154\text{-}158}$Gd, ^{32}P, ^{11}C, ^{13}N, ^{15}O, ^{186}Re, ^{18}Re, ^{51}Mn, ^{52m}Mn, ^{55}Co, ^{72}As, ^{75}Br, ^{76}Br, ^{82m}Rb, ^{83}Sr, or other gamma-, beta-, or positron-emitters. Also preferred, the diagnostic/detection agent is a paramagnetic ion, such as the a metal comprising chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (II), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and erbium (III), or a radioopaque material, such as barium, diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide, iodipamide, iodoxamic acid, iogulamide, iohexol, iopamidol, iopanoic acid, ioprocemic acid, iosefamic acid, ioseric acid, iosulamide meglumine, iosemetic acid, iotasul, iotetric acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizoic acid, ipodate, meglumine, metrizamide, metrizoate, propyliodone, and thallous chloride.

[0097] Also preferred, the diagnostic/detection agent is a fluorescent labeling compound selected from the group comprising fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, *o*-phthaldehyde and fluorescamine, a chemi-luminescent labeling compound selected from the group comprising luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester, or a bioluminescent compound selected from the group comprising luciferin, luciferase and aequorin. In another embodiment, the diagnostic immunoconjugates of the present invention are used in intraoperative, endoscopic, or intravascular tumor diagnosis.

[0098] Another embodiment of the present invention is a cancer cell targeting therapeutic immunoconjugate comprising an antibody component that comprises an antibody or fragment thereof of any one of the antibodies, fusion proteins, or fragments thereof of the present invention, wherein the antibody, fusion protein, or fragment thereof is bound to at least one therapeutic agent.

[0099] Preferably, the therapeutic agent is selected from the group consisting of a radionuclide, an immunomodulator, a hormone, a hormone antagonist, an enzyme, oligonucleotides, an enzyme inhibitor, a photoactive therapeutic agent, a cytotoxic agent, an angiogenesis inhibitor, and a combination thereof.

[0100] In one embodiment, the therapeutic agent is an oligonucleotide. For example, the oligonucleotide can be an antisense oligonucleotide such as an antisense oligonucleotide against an oncogene like bcl-2 and p53.

[0101] In a preferred embodiment, the therapeutic agent is a cytotoxic agent, such a drug or a toxin. Also preferred, the drug is selected from the group consisting of nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas, gemcitabine, triazenes, folic acid analogs, anthracyclines, taxanes, COX-2 inhibitors, pyrimidine analogs, purine analogs, antibiotics, enzymes, enzyme inhibitors, epipodophyllotoxins, platinum coordination complexes, vinca alkaloids, substituted ureas, methyl hydrazine derivatives, adrenocortical suppressants, hormone antagonists, endostatin, taxols, SN-38, camptothecins, doxorubicins and their analogs, antimetabolites, alkylating agents, antimitotics, antiangiogenic, apoptotoic agents, methotrexate, CPT-11, and a combination thereof.

[0102] In another preferred embodiment, the therapeutic agent is a toxin selected from the group consisting of ricin, abrin, alpha toxin, saporin, ribonuclease (RNase), DNase I, *Staphylococcal* enterotoxin-A, pokeweed antiviral protein, gelonin, diphtherin toxin, *Pseudomonas* exotoxin, and *Pseudomonas* endotoxin, an immunomodulator is selected from the group consisting of a cytokine, a stem cell growth factor, a lymphotoxin, a hematopoietic factor, a colony stimulating factor (CSF), an interferon (IFN), a stem cell growth factor, erythropoietin, thrombopoietin and a combination thereof, a radionuclide selected from the group consisting of ^{32}P, ^{33}P, ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{86}Y, ^{90}Y, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{211}At, ^{223}Ra and ^{225}Ac, and combinations thereof, or a photoactive therapeutic agent selected from the group comprising chromogens and dyes.

[0103] Still preferred, the therapeutic agent is an enzyme selected from the group comprising malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, α-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, β-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

[0104] Contemplated herein is a multivalent, multispecific antibody or fragment thereof comprising more than one antigen binding site having an affinity toward a PAM4 target antigen and one or more hapten binding sites having affinity towards hapten molecules. Preferably, the antibody or fragment thereof is a chimerized PAM4 antibody or fragment thereof. Also preferred, the multivalent, multispecific antibody or fragment thereof further comprises a diagnostic/detection and/or therapeutic agent.

[0105] Also described herein is a bispecific antibody or fragment thereof comprising at least one bnding site with an affinity toward a PAM4 target antigen and at least one binding site with an affinity toward a targetable construct/conjugate selected from the group consisting of:

DOTA-D-Asp-D-Lys(HSG)-D-Asp-D-Lys(HSG)- NH_2 (IMP 271);

DOTA-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH_2 (IMP 277);

DOTA-D-Tyr-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH_2 (IMP 288);

DOTA-D-Ala-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH_2 (IMP 0281); and

DOTA-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-NH_2 (IMP 284),

that is capable of carrying at least one diagnostic and/or therapeutic agent. Other targetable constructs suitable for use in the present invention are disclosed in US Provisional Application entitled "D-Amino Acid Peptides" (McBride), Attorney Docket Number 018733/1206, filed June 13,2003.

[0106] Another embodiment of the present invention is an antibody fusion protein or fragment thereof comprising at least two PAM4 MAbs or fragments thereof, wherein the MAbs or fragments comprise any of the antibodies and fragments thereof of the present invention. Also preferred, the antibody fusion protein or fragment thereof comprises at least one first PAM4 MAb or fragment thereof of any one of the antibodies and fragments thereof of the present invention and at least one, second MAb or fragment thereof, other than the MAb or fragment thereof of the antibodies and fragments thereof of the present invention. Preferably, the second MAb is a carcinoma-associated antibody, preferably selected from the group consisting of CAI9.9, DUFAN2, SPAN1, Nd2, B72.3, CC49, CEA, aLe^a, antibodies defined by the Lewis antigen Le(y), and antibodies against CSAp, MUC1, MUC2, MUC3, MUC4, TAG-72, EGFR. CD40, angiogenesis factors (e.g., VEGF), insulin-like growth factor (IGF), tenascin, platelet derived growth factor, IL-6, products of oncogenes and HER2/neu. The antibody fusion protein or fragments thereof of the present invention may further comprise at least one diagnostic and/or therapeutic agent.

[0107] Also disclosed herein is a DNA sequence, comprising a nucleic acid encoding a MAb or fragment thereof selected from the group consisting of:

(a) a PAM4 antibody or fragment thereof of any one of the antibodies described in the present invention;

(b) an antibody fusion protein or fragment thereof comprising at least two of the MAbs or fragments thereof described in (a);

(c) an antibody fusion protein or fragment thereof comprising at least one first PAM4 MAb or fragment thereof comprising said MAb or fragment thereof of the PAM4 antibodies or fragments thereof of the present invention and at least one second MAb or fragment thereof, other than the MAb or fragment thereof of any one of the antibodies or fragments thereof of the present invention; and

(d) an antibody fusion protein or fragment thereof comprising at least one first MAb or fragment thereof comprising said MAb or fragment thereof of any one of the antibodies or fragments thereof of the present invention and at least one second MAb or fragment thereof, other than the MAb or fragment thereof of any one of antibodies or fragments thereof of the present invention, wherein the second MAb is a carcinoma associated antibody. Preferably, the carcinoma associated antibody is selected from the group consisting of CA19.9, DUPAN2, SPAN1, Nd2, B72.3, CC49, CEA, aLe^a, antibodies defined by the Lewis antigen Le(y), CD40, and antibodies against angiogenesis factors (e.g., VEGF), insulin-like growth factor (IGF), tenascin, platelet derived growth factor, IL-6, products of oncogenes, MUC1, MUC-2, MUC-3, MUC-4, TAG72, EGFR, and HER2/neu.

[0108] Also disclosed is an expression vector, and host cell comprising the DNA sequence of any one of the antibodies, fusion proteins or fragments thereof of the present invention.

[0109] Also disclosed is a method of delivering a diagnostic or therapeutic agent, or a combination thereof, to a target comprising (i) providing a composition that comprises a PAM4 antibody or fragment thereof conjugated to at least one diagnostic/detection and/or therapeutic agent and (ii) administering to a subject in need thereof the diagnostic or therapeutic conjugate of any one of antibodies, fusion proteins, or fragments thereof of the present invention. Preferably, the diagnostic/detection agent is selected from the group consisting of a radionuclide, a contrast agent, and a photoactive diagnostic/detection agent, and the therapeutic agent is preferably selected from the group consisting of a cytotoxic agent, drugs, toxins, cytokine, immunomodulator, hormone, hormone antagonist, growth factor, radionuclide, metal.

[0110] Also disclosed is a method of delivering a diagnostic/detection agent, a therapeutic agent, or a combination thereof to a target, comprising: (a) administering to a subject the antibody or fragment thereof of any one of the multivalent, multispecific antibodies or fragments thereof of the present invention that have an affinity toward a PAM4 antigen and comprise one or more hapten binding site; (b) waiting a sufficient amount of time for an amount of the non-antibody to clear the subject's blood stream; and (c) administering to said subject a carrier molecule comprising a diagnostic/detection agent, a therapeutic agent, or a combination thereof, that binds to a binding site of the antibody. Preferably, the carrier molecule binds to more than one binding site of the antibody. Still preferred, the diagnostic/detection agent or the

therapeutic agent is selected from the group comprising isotopes, drugs, toxins, cytokines, hormones, hormone antagonists, enzymes, enzyme inhibitors, growth factors, radionuclides, oligonucleotides, and metals.

[0111] In one embodiment, an oligonucleotide, such as an antisense molecule inhibiting bc1-2 expression is described in U.S. 5,734,033 (Reed), may be conjugated to, or form the therapeutic agent portion of an immunoconjugate or antibody fusion protein of the present invention. Alternatively, the oligonucleotide may be administered concurrently or sequentially with the PAM4 antibodies of the present invention. In a preferred embodiment, the oligonucleotides is an antisense oligonucleotide that preferably is directed against an oncogene or oncogene product of a B-cell malignancy, such as bcl-2.

[0112] As subject to the claims, the method for diagnosing or treating cancer, comprises (a) administering to a subject in need thereof the antibody or fragment thereof of any one of the multivalent, multispecific antibodies or fragments thereof of the present invention that have an affinity toward a PAM4 antigen and comprise one or more hapten binding site; (b) waiting a sufficient amount of time for an amount of the non-antibody to clear the subject's blood stream; and (c) administering to said subject a carrier molecule comprising a diagnostic/detection agent, a therapeutic agent, or a combination thereof, that binds to a binding site of the antibody. In a preferred embodiment cancer is pancreatic cancer. Also preferred, the method can be used for intraoperative identification of diseased tissues, endoscopic identification of diseased tissues, or intravascular identification of diseased tissues.

[0113] As subject to the claims the method of treating a malignancy in a subject comprises (a) administering to said subject a therapeutically effective amount of an antibody or fragment thereof comprising a PAM4 MAb or fragment thereof or an antibody fusion protein or fragment thereof of any one of the antibodies, fusion proteins or fragments thereof of the present invention, wherein said PAM4 MAb or fragment thereof or antibody fusion protein or fragment thereof is conjugated to at least one therapeutic agent, and (b) formulating said PAM4 MAb or fragment thereof or antibody fusion protein or fragment thereof in a pharmaceutically suitable excipient. Preferably, the method further comprises a second MAb or fragment thereof not in any one of the antibodies, fusion proteins or fragments thereof of the present invention. Still preferred, the second MAb or fragment thereof is a naked MAb or fragment thereof Also preferred, thesecond MAb or fragment thereof is selected from the group consisting of CA19.9, DUPAN2, SPAN1, Nd2, B72.3, CC49, CEA, aLe^a, antibodies defined by the Lewis antigen Le(y), CSAp, MUC1, MUC-2, MUC-3, MUC-4, TAG-72, EGFR, CD40, angiogenesis factors (e.g., VEGF), insulin-like growth factor (IGF), tenascin, platelet derived growth factor, IL-6, products of oncogenes and HER2/neu.

[0114] As subject to the claims the method of diagnosing a malignancy in a subject comprises (a) administering to said subject a diagnostically effective amount of a diagnostic conjugate comprising a PAM4MAb or fragment thereof or PAM4 antibody fusion protein or fragment thereof of any one of the antibodies, fusion proteins or fragments thereof of the present invention, wherein said PAM4 MAb or fragment thereof or PAM4 antibody fusion protein or fragment thereof is conjugated to at least one diagnostic/detection agent, and (b) optionally formulating said PAM4 MAb or fragment thereof or antibody fusion protein or fragment thereof in a pharmaceutically suitable excipient.

[0115] As subject to the claims the method of treating a cancer cell in a subject comprises (i) administering to said subject a therapeutically effective amount of a composition comprising a naked PAM4 MAb or fragment thereof or a naked antibody fusion protein or fragment thereof of any one of the naked antibodies, fusion proteins, or fragments thereof of the present invention (ii) formulating said naked PAM4 MAb or fragment thereof or antibody fusion protein or fragment thereof in a pharmaceutically suitable excipient Preferably, the method further comprises a second naked antibody or fragment thereof not any one of the naked antibodies, fusion proteins or fragments thereof of the present invention. For example, the second antibody or fragment thereof may be selected from the group consisting of CA19.9, DUPAN2, SPAN1, Nd2, B72.3, CC49, CEA, aLe^a, antibodies defined by the Lewis antigen Le(y), CSAp, MUC1, MUC-2, MUC-3, MUC-4, TAG-72, EGFR, CD40, angiogenesis factors (e.g., VEGF), insulin-like growth factor (IGF), tenascin, platelet derived growth factor, IL-6, products of oncogenes and HER2/neu.

[0116] As subject to the claims the method of diagnosing a malignancy in a subject comprises (i) performing an *in vitro* diagnosis assay on a specimen from said subject with a composition comprising a naked PAM4 MAb or fragment thereof or a naked antibody fusion protein or fragment thereof of any one of the naked antibodies, fusion proteins, or fragments thereof of the present invention. Preferably, the malignancy is a cancer. Still preferred, the cancer is pancreatic cancer.

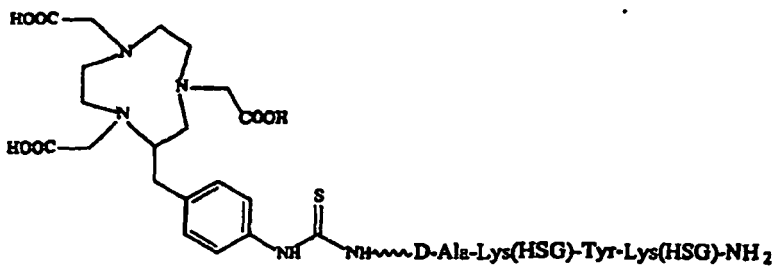
[0117] As subject to the claims the method of intraoperatively identifying diseased tissues expressing PAM4 antigen, in a subject, comprises: (A) administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue expressing PAM4-antigen and at least one other arm that specifically binds a targetable conjugate, wherein said one arm that specifically binds a targeted tissue is a cPAM4 antibody or fragment thereof; and (B) administering a targetable conjugate selected from the group consisting of:

(i) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(ii) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;

(iii) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(iv)



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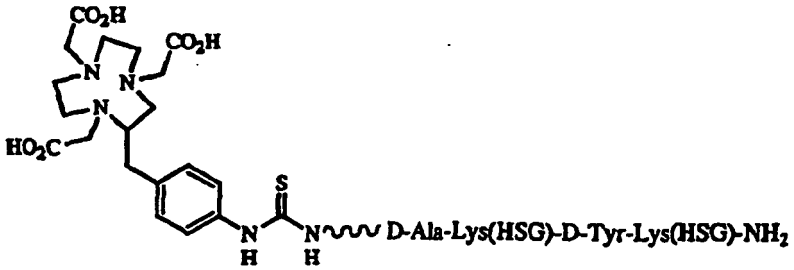


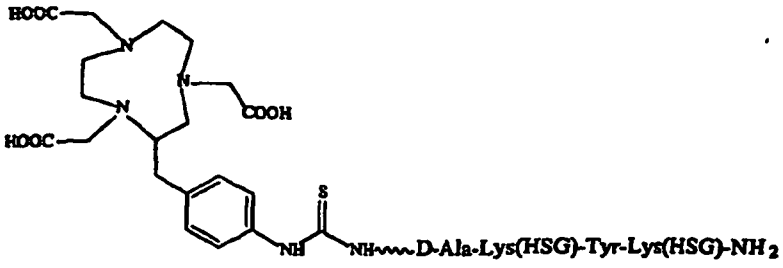
[0118] As subject to the claims the method for the endoscopic identification of diseased tissues expressing PAM4 antigen, in a subject, comprises: (A) administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue expressing PAM4-antigen and at least one other arm that specifically binds a targetable conjugate wherein said one arm that specifically binds a targeted tissue is a cPAM4 antibody or fragment thereof; and (B) administering a targetable conjugate selected from the group consisting of:

(i) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(ii) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;

(iii) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(iv)



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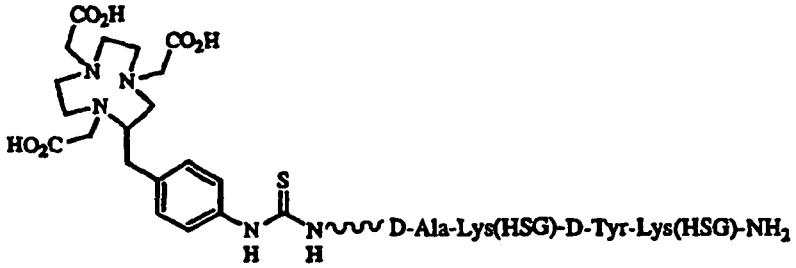


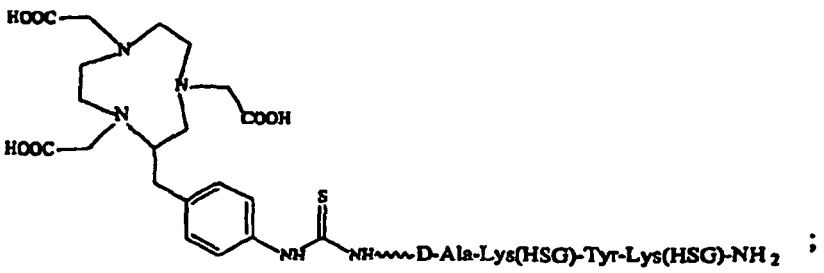
[0119] As subject to the claims the method for the intravascular identification of diseased tissues expressing PAM4 antigen, in a subject, comprises: (A) administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue expressing PAM4-antigen and at least one other arm that specifically binds a targetable conjugate wherein said one arm that specifically binds a targeted tissue is a cPAM4 antibody or fragment thereof; and (B) administering a targetable conjugate selected from the group consisting of

(i) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(ii) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;

(iii) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(iv)



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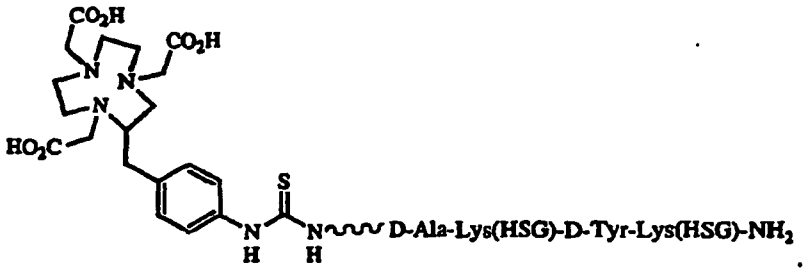
and

(v)



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[0120] As subject to the claims the method of detection of lesions during an endoscopic, intravascular catheter, or surgical procedure comprises: (a) injecting a subject who is to undergo such a procedure with a bispecific antibody $F(ab)_2$ or $F(ab)_2$ fragment thereof, diabody, triabody, or tetrabody, wherein said bispecific antibody or fragment thereof, diabody, triabody or tetrabody has a first antibody binding site which specifically binds to a PAM4 antigen, and has a second antibody binding site which specifically binds to a hapten, and permitting the antibody fragment to accrete at target sites; (b) optionally clearing non-targeted antibody fragments using a galactosylated anti-idiotype clearing agent if the bispecific fragment is not largely cleared from circulation within about 24 hours of injection, and injecting a bivalent labeled hapten, which quickly localizes at the target site and clears through the kidneys; (c) detecting the presence of the hapten by close-range detection of elevated levels of accreted label at the target sites with detection means, within 48 hours of the first injection, and conducting said procedure, wherein said detection is performed without the use of a

contrast agent or subtraction agent.

[0121] As subject to the claims, the method for close-range lesion detection, during an operative, intravascular, or endoscopic procedure comprises: (a) injecting a subject to such a procedure parenterally with an effective amount of a cPAM4 immunoconjugate or fragment thereof, (b) conducting the procedure within 48 hours of the injection; (c) scanning the accessed interior of the subject at close range with a detection means for detecting the presence of said labeled antibody or fragment thereof, and (d) locating the sites of accretion of said labeled antibody or fragment thereof by detecting elevated levels of said labeled antibody or fragment thereof at such sites with the detection means.

BRIEF DESCRIPTION OF THE FIGURES

[0122]

Figure 1 shows the cloned V genes and the deduced amino acid sequences of the murine PAM4. Figure 1A shows the DNA and amino acid sequences of the PAM4 Vk. Figure 1B shows the DNA and amino acid sequences of the PAM4VH. Amino acid sequences encoded by the corresponding DNA sequences are given as one-letter codes below the nucleotide sequence. Numbering of the nucleotide sequence is on the right side. The amino acid residues in the CDR regions are shown in bold and underlined. Kabat's Ig molecule numbering is used for amino acid residues as shown by the numbering above the amino acid residues. The amino acid residues numbered by a letter are the insertion residues defined by Kabat's numbering scheme. The insertion residues have the same preceding digits as that of the previous residue. For example, residues 82, 82A, 82B, and 82C in Figure 1B are indicated as 82, A, B, and C, respectively.

Figure 2 shows the amino acid sequences of the chimeric PAM4 (cPAM4) heavy and light chain variable regions expressed in Sp2/0 cells. Figure 2A shows the amino acid sequence of the cPAM4Vk. Figure 2B shows the amino acid sequence of the cPAM4VH. Another variation of a chimeric PAM4 VH and $V\kappa$ is represented in figures 2C and 2D. The amino acid differences are due to the sequences present in the vectors used to express the PAM4 variable regions. The sequences are given as one letter codes. The amino acid residues in the CDR regions are shown in bold and underlined. The numbering of amno acids is same as that in Figure 1.

Figure3 shows the binding activity of chimerized PAM4 antibody, cPAM4 (shown by closed squares), compared to the murine PAM4 (shown by diamonds). Results indicate comparable binding activity of the cPAM4 antibody and mPAM4 when competing with ^{131}I-mPAM4 binding to the antigen. A chimeric antibody is a recombinant protein that contains the variable domains including the complementarity determining regions (CDRs) of an antibody derived from one species while the constant domains of the antibody molecule is derived from those of a human antibody.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Overview

[0123] Unless otherwise specified, "a" or "an" means "one or more." As described herein, the term "PAM4 antibody" includes murine and chimerized PAM4 antibodies.

[0124] The present invention relates to a monoclonal antibody, PAM4, that is useful for the diagnosis, detection, staging, and therapy of pancreatic cancer. The PAM4 antibodies and fragments thereof of the present invention are chimerized. The murine PAM4 (mPAM44) antibody is a MUC1 antibody developed by employing a pancreatic cancer mucin derived from the xenografted RIP-1 human pancreatic carcinoma as immunogen. Gold et al., Int. J. Cancer, 57:204-210 (1994). The mPAM4 antibody recognizes a unique and novel epitopes on the target pancreatic cancer antigen. Immunohistochemical staining studies, such as those described in Example 1, have shown that the PAM4 MAb binds the domain located between the amino terminus and start of the repeat domain of a MUC1 antigen expressed by breast, pancreas and other cancer cells, with limited binding to normal human tissue. The PAM4 antibodies of the present invention are relatively specific to pancreatic cancer and therefore preferentially bind pancreatic cancer cells. In a preferred embodiment, the PAM4 antibodies and fragments thereof are chimerized. The PAM4 antibody is reactive with a target epitope expressed primarily by antigens associated with pancreatic cancer and not with pancreatitis. Localization and therapy studies using a radiolabeled PAM4 MAb in animal models have demonstrated tumor targeting and therapeutic efficacy.

[0125] The PAM4 antibodies of the present invention bind the PAM4 antigen, which is the domain located between the amino terminus and start of the repeat domain of MUC1, an antigen produced by many organs and tumor types. A preferred PAM4 antibody of the present invention preferentially binds pancreatic cancer cells. Studies with a PAM4 MAb, such as the PAM4 MAb in Example 2, indicate that the antibody exhibits several important properties, which make it a

candidate for clinical diagnostic and therapeutic applications. Since the PAM4 antigen provides a useful target for diagnosis and therapy, it is desirable to obtain a MAb that recognizes an epitope of a pancreatic cancer antigen that is distinct from the epitopes recognized by the non-PAM4 antibodies (CA19.9, DUPAN2, SPAN1, Nd2, B72.3, aLe^a,and the Lewis antigens) described in earlier studies.

[0126] Antibodies suitable for use in combination or conjunction with the PAM4 antibodies of the present invention include, for example, those against carcinoembryonic antigen (CEA), colon-specific antigen-p (CSAp), MUC1, MUC2, MUC3, MUC4, B72.3, Le(y), HER2/neu, EGFR, angiogenesis factors (e.g., VEGF), insulin-like growth factor (IGF), tenascin, platelet derived growth factor, IL-6, as well as products of oncogenes, and antibodies against tumor necrosis substances, such as described in patents by Epstein et al. (U.S. Pat Nos. 6,071,491, 6.017,514,5,019,368 and 5,882,626). Such antibodies would be useful for complementing current PAM4 antibody immunodetection and immunotherapy methods. In therapy applications, antibodies that are agonistic or antagonistic to immunomodulators involved in effector cell function against tumor cells could also be useful in combination with PAM4 antibodies alone or in combination with other tumor-associated antibodies, one example being antibodies against CD40. Todryk et al., J. Immunol Methods, 248:139-147 (2001); Turner et al., J Immunol, 166:89-94 (2001). Also of use are antibodies against markers or products of oncogenes, or antibodies against angiogenesis factors, such as VEGF. VEGF antibodies are described in Thorpe et al., U.S. Pat. Nos. 6,342,221,5,965,132 and 6,004,554.

[0127] Moreover, the availability of another PAM4-like antibody is essential for the development of a double-determinant enzyme-linked immunosorbent assay (ELISA), which is useful for detecting a PAM4 antigen in clinical samples. ELISA experiments are described in Example 5.

[0128] The present invention describes chimeric antibodies and fragments thereof that bind an epitope located between the amino terminus and the start of the repeat domain of a MUC1 antigen and can be used for diagnostic and therapeutic methods. In a preferred embodiment, the PAM4 antibody is chimerized. A chimeric antibody as disclosed herein is a recombinant protein that contains the variable domains including the complementarity determining regions (CDRs) of an antibody derived from one species, preferably a rodent antibody, while the constant domains of the antibody molecule are derived from those of a human antibody. For veterinary applications, the constant domains of the chimeric antibody may be derived from that of other species. Because non-human monoclonal antibodies can be recognized by the human host as a foreign protein, and repeated injections can lead to harmful hypersensitivity reactions, chimerization of a murine PAM4 antibody or fragment thereof can reduce the adverse immune response that patients may experience. For murine-based monoclonal antibodies, this is often referred to as a Human Anti-Mouse Antibody (HAMA) response.

[0129] The antibodies and fragments thereof of the present invention are preferably raised against a crude mucin preparation from a tumor of the human pancreas. In a related vein, the PALM4 antibody can be obtained using a substantially pure preparation of the PAM4 antigen. A substantially pure protein is a protein that is essentially free from contaminating cellular components, which are associated with the protein in nature.

Definitions

[0130] In the description that follows, a number of terms are used and the following definitions are provided to facilitate understanding of the present invention.

[0131] An antibody, as described herein, refers to a full-length (i.e., naturally occurring or formed by normal immunoglobulin gene fragment recombinatorial processes) immunoglobulin molecule (e.g., an IgG antibody) or an immunologically active (i.e., specifically binding) portion of an immunoglobulin molecule, like an antibody fragment An antibody fragment, is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, sFv and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the full-length antibody. For example, an anti-CD20 monoclonal antibody fragment binds with an epitope of CD20. The term "antibody fragment" also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex. For example, antibody fragments include isolated fragments consisting of the variable regions, such as the "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker ("scFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

[0132] A naked antibody is generally an antibody that is not conjugated to a therapeutic or diagnostic/detection agent. However, it may also be an antibody fragment that is not conjugated to a diagnostic/detection or therapeutic agent. This is so because the Fc portion of the antibody molecule provides effector functions, such as complement fixation and ADCC, (antibody dependent cell cytotoxicity), which set mechanisms into action that may result in cell lysis. However, it is possible that the Fc portion is not required for therapeutic function, with other mechanisms, such as apoptosis, coming into play. Naked antibodies include both polyclonal and monoclonal antibodies, as well as fusion proteins and certain recombinant antibodies, such as chimeric, humanized or human antibodies.

[0133] A chimeric antibody is a recombinant protein that contains the variable domains including the complementarity determining regions (CDRs) of an antibody derived from one species, preferably a rodent antibody, while the constant

domains of the antibody molecule are derived from those of a human antibody. For veterinary applications, the constant domains of the chimeric antibody may be derived from that of other species, such as a cat or dog.

[0134] A humanized antibody is a recombinant protein in which the CDRs from an antibody from one species; e.g., a rodent antibody, are transferred from the heavy and light variable chains of the rodent antibody into human heavy and light variable domains. The constant domains of the antibody molecule are derived from those of a human antibody.

[0135] A human antibody is an antibody obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green et al., Nature Genet. 7:13 (1994), Lonberg et al., Nature 368:856 (1994), and Taylor et al., Int. Immun. 6:579 (1994). A fully human antibody also can be constructed by genetic or chromosomal transfection methods, as well as phage display technology, all of which are known in the art. See for example, McCafferty et al, Nature 348:552-553 (1990) for the production of human antibodies and fragments thereof *in vitro,* from immunoglobulin variable domain gene repertoires from unimmunized donors. In this technique, antibody variable domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. In this way, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats, for their review, see e.g. Johnson and Chiswell, Current Opiniion in Structural Biology 3:5564-571 (1993).

[0136] Human antibodies may also be generated by *in vitro* activated B cells. See U.S. Patent Nos. 5,567,610 and 5,229,275, which are incoporated in their entirety by reference.

[0137] A therapeutic agent is a molecule or atom which is administered separately, concurrently or sequentially with an antibody moiety or conjugated to an antibody moiety, i.e., antibody or antibody fragment, or a subfragment, and is useful in the treatment of a disease. Examples of therapeutic agents include antibodies, antibody fragments, drugs, toxins, nucleases, hormones, immunomodulators, chelators, boron compounds, photoactive agents or dyes and radioisotopes.

[0138] A diagnostic/detection agent is a molecule or atom which is administered conjugated to an antibody moiety, i.e., antibody or antibody fragment, or subfragment, and is useful in diagnosing a disease by locating the cells containing the antigen. Useful diagnostic/detection agents include, but are not limited to, radioisotopes, dyes (such as with the biotin-streptavidin complex), contrast agents, fluorescent compounds or molecules and enhancing agents (e.g. paramagnetic ions) for magnetic resonance imaging (MRI). U.S. Patent No. 6,331,175 describes MRI technique and the preparation of antibodies conjugated to a MRI enhancing agent and is incoporated in its entirety by reference. Preferably, the diagnostic/detection agents are selected from the group consisting of radioisotopes, enhancing agents for use in magnetic resonance imaging, and fluorescent compounds. In order to load an antibody component with radioactive metals or paramagnetic ions, it may be necessary to react it with a reagent having a long tail to which are attached a multiplicity of chelating groups for binding the ions. Such a tail can be a polymer such as a polylysine, polysaccharide, or other derivatized or derivatizable chain having pendant groups to which can be bound chelating groups such as, *e.g.,* ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, polyoximes, and like groups known to be useful for this purpose. Chelates are coupled to the antibodies using standard chemistries. The chelate is normally linked to the antibody by a group, which enables formation of a bond to the molecule with minimal loss of immunoreactivity and minimal aggregation and/or internal cross-linking. Other, more unusual, methods and reagents for conjugating chelates to antibodies are disclosed in U.S. Patent 4,824,659 to Hawthorne, entitled "Antibody Conjugates," issued April 25,1989. Particularly useful metal-chelate combinations include 2-benzyl-DTPA and its monomethyl and cyclohexyl analogs, used with diagnostic isotopes in the general energy range of 60 to 4,000 keV, such as ^{125}I, ^{131}I, ^{123}I, ^{124}I, ^{62}Cu, ^{64}Cu, ^{18}F, ^{111}In, ^{67}Ga, ^{68}Ga, ^{99m}Tc, ^{94m}Tc, ^{11}C, ^{13}N, ^{15}O, ^{76}Br, for radio-imaging. The same chelates, when complexed with non-radioactive metals, such as manganese, iron and gadolinium are useful for MRI, when used along with the antibodies of the invention. Macrocyclic chelates such as NOTA, DOTA, and TETA are of use with a variety of metals and radiometals, most particularly with radionuclides of gallium, yttrium and copper, respectively. Such metal-chelate complexes can be made very stable by tailoring the ring size to the metal of interest. Other ring-type chelates such as macrocyclic polyethers, which are of interest for stably binding nuclides, such as ^{223}Ra for RAIT are encompassed by the invention.

[0139] An immunoconjugate is an antibody, fusion protein, or fragment thereof conjugated to at least one therapeutic and/or diagnostic/detection agent. The diagnostic/detection agent can comprise a radionuclide or non-radionuclide, a contrast agent (such as for magnetic resonance imaging, computed tomography or ultrasound), and the radionuclide can be a gamma-, beta-, alpha-, Auger electron-, or positron-emitting isotope.

[0140] An expression vector is a DNA molecule comprising a gene that is expressed in a host cell. Typically, gene

expression is placed under the control of certain regulatory elements, including constitutive or inducible promoters, tissue-specific regulatory elements and enhancers. Such a gene is said to be "operably linked to" the regulatory elements.

[0141] A recombinant host may be any prokaryotic or eukaryotic cell that contains either a cloning vector or expression vector. This term also includes those prokaryotic or eukaryotic cells, as well as transgenic animals, that have been genetically engineered to contain the cloned gene(s) in the chromosome or genome of the host cell or cells of the host cells. Suitable mammalian host cells include myeloma cells, such as SP2/0 cells, and NS0 cells, as well as Chinese Hamster Ovary (CHO) cells, hybridoma cell lines and other mammalian host cell useful for expressing antibodies. Also particularly useful to express mAbs and other fusion proteins, is a human cell line, PER.C6 disclosed in WO 0063403 A2, which produces 2 to 200-fold more recombinant protein as compared to conventional mammalian cell lines, such as CHO, COS, Vero, Hela, BHK and SP2- cell lines. Special transgenic animals with a modified immune system are particularly useful for making fully human antibodies.

[0142] As used herein, the term antibody fusion protein is a recombinantly produced antigen-binding molecule in which two or more of the same or different natural antibody, single-chain antibody or antibody fragment segments with the same or different specificities are linked. Valency of the fusion protein indicates the total number of binding arms or sites the fusion protein has to an antigen or epitope; i.e., monovalent, bivalent, trivalent or mutlivalent. The multivalency of the antibody fusion protein means that it can take advantage of multiple interactions in binding to an antigen, thus increasing the avidity of binding to the antigen. Specificity indicates how many antigens or epitopes an antibody fusion protein is able to bind; i.e., monospecific, bispecific, trispecific, multispecific. Using these definitions, a natural antibody, e.g., an IgG, is bivalent because it has two binding arms but is monospecific because it binds to one antigen. Monospecific, multivalent fusion proteins have more than one binding site for an epitope but only bind with the same or different epitopes on the same antigen, for example a diabody with two binding sites reactive with the same antigen. The fusion protein may comprise a multivalent or multispecific combination of different antibody components or multiple copies of the same antibody component. The fusion protein may additionally comprise a therapeutic agent. Examples of therapeutic agents suitable for such fusion proteins include immunomodulators ("antibody-immunomodulator fusion protein,") and toxins ("antibody-toxin fusion protein"). One preferred toxin comprises a ribonuclease (RNase), preferably a recombinant RNase.

[0143] A multispecific antibody is an antibody that can bind simultaneously to at least two targets that are of different structure, *e.g.*, two different antigens, two different epitopes on the same antigen, or a hapten and/or an antigen or epitope. One specificity would be for a B-cell, T-cell, myeloid-, plasma-, and mast-cell antigen or epitope. Another specificity could be to a different antigen on the same cell type, such as CD20, CD19, CD21, CD23, CD46, CD80, HLA-DR, CD74, and CD22 on B-cells. Multispecific, multivalent antibodies are constructs that have more than one binding site, and the binding sites are of different specificity. For example, a diabody, where one binding site reacts with one antigen and the other with the another antigen.

[0144] A bispecific antibody is an antibody that can bind simultaneously to two targets which are of different structure. Bispecific antibodies (bsAb) and bispecific antibody fragments (bsFab) have at least one arm that specifically binds to, for example, a B-cell, T-cell, myeloid-, plasma-, and mast-cell antigen or epitope and at least one other arm that specifically binds to a targetable conjugate that bears a therapeutic or diagnostic/detection agent A variety of bispecific fusion proteins can be produced using molecular engineering. In one form, the bispecific fusion protein is monovalent, consisting of, for example, a scFv with a single binding site for one antigen and a Fab fragment with a single binding site for a second antigen. In another form, the bispecific fusion protein is divalent, consisting of, for example, an IgG with a binding site for one antigen and two scFv with two binding sites for a second antigen.

Preparation of Chimerized PAM4 Antibodies

[0145] Monoclonal antibodies for specific antigens may be obtained by methods known to those skilled in the art *See,* for example, Kohler and Milstein, Nature 256: 495 (1975), and Coligan et al. (eds.), CURRENT PROTOCOLS IN IMMUNOLOGY, VOL. 1, pages 2.5.1-2.6.7 (John Wiley & Sons 1991) (hereinafter "Coligan"). Briefly, PAM4 MAbs can be obtained by injecting mice with a composition comprising the PAM4 antigen, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B-lymphocytes, fusing the B-lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones which produce antibodies to PAM4 antigen, culturing the clones that produce antibodies to PAM4 antigen, and isolating PAM4 antibodies from the hybridoma cultures. The PAM4 antibodies of the present invention bind the PAM4 antigen, a domain located between the amino terminus and the start of the repeat domain of MUC 1. The PAM4 antibodies of the present invention preferentially bind pancreatic cancer cells.

[0146] After the initial raising of antibodies to the immunogen, the antibodies can be sequenced and subsequently prepared by recombinant techniques. Chimerization of murine antibodies and antibody fragments are well known to those skilled in the art. The use of antibody components derived from chimerized monoclonal antibodies reduces potential problems associated with the immunogenicity of murine constant regions.

[0147] General techniques for cloning murine immunoglobulin variable domains are described, for example, by the publication of Orlandi et al., Proc. Nat'l Acad Sci. USA 86: 3833 (1989). In general, the V_κ (variable light chain) and V_H (variable heavy chain) sequences for PAM4 antibodies can be obtained by a variety of molecular cloning procedures, such as RT-PCR, 5'-RACE, and cDNA library screening. Specifically, the V_H and V_κ genes of the MAb PAM4 were cloned by PCR amplification from the hybridoma cells by RT-PCR, and their sequences determined by DNA sequenceing. To confirm their authenticity, the cloned V_L and V_H genes can be expressed in cell culture as a chimeric Ab as described by Orlandi et al., (Proc. Natl. Acad. Sci. USA, 86: 3833 (1989)).

[0148] Antibodies can generally be isolated from cell culture media as follows. Transfectoma cultures are adapted to serum-free medium. For production of chimerized antibody, cells are grown as a 500 ml culture in roller bottles using HSFM. Cultures are centrifuged and the supernatant filtered through a 0.2 μ membrane. The filtered medium is passed through a protein A column (1 x 3 cm) at a flow rate of 1 ml/min. The resin is then washed with about 10 column volumes of PBS and protein A-bound antibody is eluted from the column with 0.1 M glycine buffer (pH 3.5) containing 10 mM EDTA. Fractions of 1.0 ml are collected in tubes containing 10 μl of 3 M Tris (pH 8.6), and protein concentrations determined from the absorbance at 280/260 nm. Peak fractions are pooled, dialyzed against PBS, and the antibody concentrated, for example, with the Centricon 30 (Amicon, Beverly, MA). The antibody concentration is determined by ELISA, as before, and its concentration adjusted to about 1 mg/ml using PBS. Sodium azide, 0.01% (w/v), is conveniently added to the sample as preservative.

[0149] In one aspect of the invention the chimerized PAM4 antibody or antibody fragment comprises the complementarity-determining regions (CDRs) and framework regions (FR) of a murine PAM4 MAb and the light and heavy chain constant regions of a human antibody, wherein the CDRs of the light chain variable region of the chimerized PAM4 comprises CDR1 comprising an amino acid sequence ofSASSSVSSSYLY; CDR2 comprising an amino acid sequence of STSNLAS; and CDR3 comprising an amino acid sequence of HQWNRYPYT; and the CDRs of the heavy chain variable region of the chimerized PAM4 MAb comprises CDR1 comprising an amino acid sequence of SYVLH; CDR2 comprising an amino acid sequence of YINPYNDGTQYNEKFKG and CDR3 comprising an amino acid sequence of GFGGSYGFAY. PAM4 MAbs can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3. Also, see Baines et al., "Purification of Immunoglobulin G (IgG)," in METHODS IN MOLECULAR BIOLOGY, VOL. 10, pages 79-104 (The Humana Press, Inc. 1992).

[0150] PAM4 MAbs can be characterized by a variety of techniques that are well-known to those of skill in the art For example, the ability of a PAM4 MAb to bind to the PAM4 antigen can be verified using an indirect enzyme immunoassay, flow cytometry analysis, or Western analysis.

Production of PAM4 Antibody Fragments

[0151] The present invention contemplates the use PAM4 antibody fragments. Antibody fragments which recognize specific epitopes can be generated by known techniques. The antibody fragments are antigen binding portions of an antibody, such as $F(ab')_2$. Fab', Fab, Fv, sFv and the like. $F(ab')_2$ fragments, for example, can be produced by pepsin digestion of the antibody molecule and Fab' fragments can be generated by reducing disulfide bridges of the $F(ab)'_2$ fragments. These methods are described, for example, by Goldenberg, U.S. patent Nos. 4,036,945 and 4,331,647 and references contained therein. Also, see Nisonoff et al., Arch Biochem. Biophys. 89: 230 (1960); Porter, Biochem. J. 73. 119 (1959), Edelman et a/., in METHODS IN ENZYMOLOGY VOL. 1, page 422 (Academic Press 1967), and Coligan at pages 2.8.1-2.8.10 and 2.10.-2.10.4. Alternatively, Fab' expression libraries can be constructed (Huse et al., 1989, Science, 246:1274-1281) to allow rapid and easy identification of monoclonal Fab' fragments with the desired specificity. The present invention encompasses antibodies and antibody fragments.

[0152] A single chain Fv molecule (scFv) comprises a V_L domain and a V_H domain. The V_L and V_H domains associate to form a target-binding site. These two domains are further covalently linked by a peptide linker (L). A scFv molecule is denoted as either V_L-L-V_H if the V_L domain is the N-terminal part of the scFv molecule, or as V_H -L- V_L if the V_H domain is the N-terminal part of the scFv molecule. Methods for making scFv molecules and designing suitable peptide linkers are described in US Patent No. 4,704,692, US Patent No. 4,946,778, R. Raag and M. Whitlow, "single Chain Fvs." FASEB Vol 9:73-80 (1995) and R.E. Bird and B.W. Walker, "Single Chain Antibody Variable Regions," TIBTECH, Vol 9: 132-137 (1991).

[0153] An antibody fragment can be prepared by proteolytic hydrolysis of the full-length antibody or by expression in *E.coli* or another host of the DNA coding for the fragment An antibody fragment can be obtained by pepsin or papain digestion of full-length antibodies by conventional methods. For example, an antibody fragment can be produced by enzymatic cleavage of antibodies with pepsin to provide an approximate 100Kd fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionnally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce an approximate 50Kd Fab' monovalent fragment. Alternatively, an en-

zymatic cleavage using papain produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Patent Nos. 4,036,945 and 4,331,647 and references contained therein, Also, see Nisonoff et al., Arch Biochem. Biophys. 89: 230 (1960); Porter, Biochem. J. 73: 119 (1959), Edelman et al., in METHODS IN ENZYMOLOGY VOL. 1, page 422 (Academic Press 1967), and Coligan at pages 2.8.1-2.8.10 and 2.10.-2.10.4.

[0154] Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). A CDR is a segment of the variable region of an antibody that is complementary in structure to the epitope to which the antibody binds and is more variable than the rest of the variable region. Accordingly, a CDR is sometimes referred to as hypervariable region. A variable region comprises three CDRs. CDR peptides can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction (PCR) to synthesize the variable region from RNA of antibody-producing cells. See, for examples, Larrick et al. Methods: A Companion to Methods in Enzymology 2: 106 (1991); Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in MONOCLONAL ANTIBODIES: PRODUCTION, ENGINEERING AND CLINICAL APPLICATION, Ritter et al. (eds.), pages 166-179 (Cambridge University Press 1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in MONOCLONAL ANTIBODIES: PRINCIPLES AND APPLICATIONS, Birch et al., (eds.), pages 137-185 (Wiley-Liss, Inc. 1995).

[0155] Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

Production of Chimerized PAM4 Antibody Fusion Proteins

[0156] The antibody fusion proteins of the present invention can be prepared by a variety of conventional procedures, ranging from glutaraldehyde linkage to more specific linkages between functional groups. The antibodies and/or antibody fragments that comprise the fusion proteins described herein are preferably covalently bound to one another, directly or through a linker moiety, through one or more functional groups on the antibody or fragment, e.g., amine, carboxyl, phenyl, thiol, or hydroxyl groups. Various conventional linkers in addition to glutaraldehyde can be used, e.g., diisocyanates, diisothiocyanates, bis(hydroxysuccinimide) esters, carbodiimides, maleimidehydroxysuccinimide esters, and the like.

[0157] A simple method for producing chimerized PAM4 fusion proteins is to mix the antibodies or fragments in the presence of glutaraldehyde. The initial Schiff base linkages can be stabilized, e.g., by borohydride reduction to secondary amines. A diisothiocyanate or carbodiimide can be used in place of glutaraldehyde as a non-site-specific linker. In one embodiment of the present invention, an antibody fusion protein comprises one chimerized PAM4 MAb, or fragment thereof, wherein the MAb binds to the domain located between the amino terminus and the start of the repeat domain of the MUC1 antigen. This fusion protein and fragments thereof preferentially bind pancreatic cancer cells. This monovalent, monospecific MAb is useful for direct targeting of an antigen, where the MAb is attached to a therapeutic agent, a diagnostic/detection agent, or a combination thereof, and the protein is administered directly to a patient in need thereof. The PAM4 antibody fusion proteins and fragments thereof of the present invention may instead comprise at least two chimerized PAM4 MAbs, or fragments thereof, wherein at least two of the MAbs or fragments thereof bind to distinct epitopes of the PAM4 antigen. For example, the MAbs can produce antigen specific diabodies, triabodies and tetrabodies, which are multivalent but monospecific to the PAM4 antigen. The non-covalent association of two or more scFv molecules can form functional diabodies, triabodies and tetrabodies. Monospecific diabodies are homodimers of the same scFv, where each scFv comprises the V_H domain from the selected antibody connected by a short linker to the V_L domain of the same antibody. A diabody is a bivalent dimer formed by the non-covalent association of two scFvs, yielding two Fv binding sites. A triabody results from the formation of a trivalent trimer of three scFvs, yielding three binding sites, and a tetrabody is a tetravalent tetramer of four scFvs, resulting in four binding sites. Several monospecific diabodies have been made using an expression vector that contains a recombinant gene construct comprising V_{H1}-linker-V_{L1}. See Holliger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993); Atwell et al., Molecular Immunology 33: 1301-1302 (1996); Holliger et al., Nature Biotechnology 15: 632-631(1997); Helfrich et al., Int. J. Cancer 76: 232-239 (1998); Kipriyanov et al., Int. J. Cancer 77: 763-772 (1998); Holiger et al., Cancer Research 59: 2909-2916(1999)). Methods of constructing scFvs are disclosed in US-4,946,778 (1990) and US-5,132,405 (1992). Methods of producing multivalent, monospecific antibodies based on scFv are disclosed in US-5,837,242 (1998), US-5,844,094 (1998) and WO-98/44001 (1998). The multivalent, monospecific antibody fusion protein binds to two or more of the same type of epitopes that can be situated on the same antigen or on separate antigens. The increased valency allows for additional interaction, increased affinity, and longer residence times. These antibody fusion proteins can be utilized in direct targeting systems, where the antibody fusion protein is conjugated to a therapeutic agent, a diagnostic/detection agent, or a combination thereof, and administered directly to a patient in need thereof.

[0158] A preferred embodiment of the instant invention is a multivalent, multispecific antibody or fragment thereof

comprising more than one antigen binding site having an affinity toward a PAM4 target epitope and one or more additional epitopes associated with pancreatic cancer antigens. This fusion protein is multispecific because it binds at least two different epitopes, which can reside on the same or different antigens. For example, the fusion protein may comprise more than one antigen binding site, the first with an affinity toward one PAM4 antigen epitope and the second with an affinity toward another target antigen such as TAG-72 or CEA. Another example is a bispecific PAM4 antibody fusion protein which may comprise a CA19.9 MAb (or fragment thereof) and a PAM4 MAb (or fragment thereof). Such a fusion protein will have an affinity toward CA19.9 as well as the domain located between the amino terminus and start of the repeat domain of MUC1. Also contemplated in the present invention is a fusion protein comprising more than one antigen binding site having an affinity for at least two different PAM4 antigen epitopes.

[0159] The antibody fusion proteins and fragments thereof of the present invention can be utilized in direct targeting systems, where the antibody fusion protein is conjugated to a therapeutic agent, a diagnostic/detection agent, or a combination thereof, and administered directly to a patient in need thereof.

[0160] Another preferred embodiment of the instant invention is a multivalent, multispecific antibodies and fragments thereof comprising more than one antigen binding site having affinity toward a PAM4 target epitope and at least one hapten binding site having affinity towards hapten molecules. For example, a bispecific PAM4 antibody fusion protein may comprise the 679 MAb (or fragment thereof) and the PAM4 MAb (or fragment thereof). The monoclonal antibody, 679, binds with high affinity to molecules containing the tri-peptide moiety histamine succinyl glycyl (HSG). Such a bispecific PAM4 antibody fusion protein can be prepared, for example, by obtaining an $F(ab')_2$ fragment from 679, as described above. The interchain disulfide bridges of the 679 $F(ab')_2$ fragment are gently reduced with cystine, taking care to avoid light-heavy chain linkage, to form Fab'-SH fragments. The SH group(s) is (are) activated with an excess of bis-maleimide linker (1,1'-(methylenedi-4,1-phenylene)bis-malemide). The PAM4 MAb is converted to Fab'-SH and then reacted with the activated MR23 Fab'-SH fragment to obtain a bispecific PAM4 antibody fusion protein. Bispecific antibody fusion proteins such as this one can be utilized in affinity enhancing systems, where the target antigen is pretargeted with the fusion protein and is subsequently targeted with a diagnostic or therapeutic agent that binds with the antibody-antigen complex formed by pretargeting.

[0161] Bispecific antibodies can be made by a variety of conventional methods, e.g., disulfide cleavage and reformation of mixtures of whole IgG or, preferably $F(ab')_2$ fragments, fusions of more than one hybridoma to form polyomas that produce antibodies having more than one specificity, and by genetic engineering. Bispecific antibody fusion proteins have been prepared by oxidative cleavage of Fab' fragments resulting from reductive cleavage of different antibodies. This is advantageously carried out by mixing two different $F(ab')_2$ fragments produced by pepsin digestion of two different antibodies, reductive cleavage to form a mixture of Fab' fragments, followed by oxidative reformation of the disulfide linkages to produce a mixture of $F(ab')_2$ fragments including bispecific antibody fusion proteins containing a Fab' potion specific to each of the original epitopes. General techniques for the preparation of antibody fusion proteins may be found, for example, in Nisonoff et al., Arch Biochem. Biophys. 93: 470 (1961), Hämmerling et al., J. Exp. Med. 128: 1461 (1968), and U.S. patent No. 4,331,647. Contemplated in the present invention is an antibody fusion protein or fragment thereof comprising at least one first PAM4 MAb or fragment thereof and at least one second MAb or fragment thereof, other than the PAM4 MAbs or fragments thereof of the present invention

[0162] More selective linkage can be achieved by using a heterobifunctional linker such as maleimidehydroxysuccinimide ester. Reaction of the ester with an antibody or fragment will derivative amine groups on the antibody or fragment, and the derivative can then be reacted with, e.g., and antibody Fab fragment having free sulfhydryl groups (or, a larger fragment or intact antibody with sulfhydryl groups appended thereto by, e.g., Traut's Reagent). Such a linker is less likely to crosslink groups in the same antibody and improves the selectivity of the linkage.

[0163] It is advantageous to link the antibodies or fragments at sites remote from the antigen binding sites. This can be accomplished by, e.g., linkage to cleaved interchain sulfydryl groups, as noted above. Another method involves reacting an antibody having an oxidized carbohydrate portion with another antibody that has at lease one free amine function. This results in an initial Schiff base (mime) linkage, which is preferably stabilized by reduction to a secondary amine, e.g., by borohydride reduction, to form the final composite. Such site-specific linkages are disclosed, for small molecules, in U.S. patent No. 4,671,958, and for larger addends in U.S. patent No. 4,699,784.

[0164] A polyspecific PAM4 antibody fusion protein can be obtained by adding PAM4 antigen binding moieties to a bispecific chimerized PAM4 antibody fusion protein. For example, a bispecific antibody fusion protein can be reacted with 2-iminothiolane to introduce one or more sulfhydryl groups for use in coupling the bispecific fusion protein to a third PAM4 MAb or fragment, using the bis-maleimide activation procedure described above. These techniques for producing antibody fusion proteins are well known to those of skill in the art. See, for example, U.S. patent No. 4,925,648.

[0165] ScFvs with linkers greater than 12 amino acid residues in length (for example, 15-or 18-residue linkers) allow interacting between the VH and V_L domains on the same chain and generally form a mixture of monomers, dimers (termed diabodies) and small amounts of higher mass multimers, (Kortt et al., Eur. J. Biochem. (1994) 221: 151-157). ScFvs with linkers of 5 or less amino acid residues, however, prohibit intramolecular pairing of the V_H , and V_L domains on the same chain, forcing pairing with V_H and V_L domains on a different chain. Linkers between 3- and 12-residues

form predominantly dimers (Atwell et al., Protein Engineering (1999) 12:597-604). With linkers between 0 and 2 residues, trimeric (termed triabodies), tetrameric (termed tetrabodies) or higher oligomeric structures of scFvs are formed; however, the exact patterns of oligomerization appear to depend on the composition as well as the orientation of the V-domains, in addition to the linker length. For example, scFvs of the anti-neuraminidase antibody NC10 formed predominantly trimers (V_H to V_L orientation) or tetramers (V_L to V_H orientation) with 0-residue linkers (Dolezal et al., Protein Engineering (2000) 13: 565-574). For scFvs constructed from NC10 with 1- and 2-residue linkers, the V_H to V_L orientation formed predominantly diabodies (Atwell et al., Protein Engineering (1999) 12:597-604); in contrast, the V_L to V_H orientation formed a mixture of tetramers, trimers, dimers, and higher mass multimers (Dolezal et al., Protein Engineering (2000) 13: 565-574). For scFvs constructed from the anti-CD19 antibody HD37 in the V_H to V_L orientation, the 0-residue linker formed exclusively trimers and the 1-residue linker formed exclusively tetramers (Le Gall et al., FEBS Letters (1999) 453: 164-168).

Expression Vectors and Host Cells

[0166] An expression vector is a DNA molecule comprising a gene that is expressed in a host cell. Typically, gene expression is placed under the control of certain regulatory elements, including constitutive or inducible promoters, tissue-specific regulatory elements, and enhancers. Such a gene is said to be "operably linked to" the regulatory elements. A promoter is a DNA sequence that directs the transcription of a structural gene. A structural gene is a DNA sequence that is transcribed into messenger RNA (mRNA) which is then translated into a sequence of amino acids characteristic of a specific polypeptide. Typically, a promoter is located in the 5' region of a gene, proximal to the transcriptional start site of a structural gene. If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. An enhancer is a DNA regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

[0167] An isolated DNA molecule is a fragment of DNA that is not integrated in the genomic DNA of an organism. For example, a cloned PAM4 antigen gene is a DNA fragment that has been separated from the genomic DNA of a mammalian cell. Another example of an isolated DNA molecule is a chemically-synthesized DNA molecule that is not integrated in the genomic DNA of an organism. Complementary DNA (cDNA) is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a short synthetic oligo nucleotide complementary to a portion of the mRNA is employed as a primer for the initiation of reverse transcription to generate the first stand DNA. Those skilled in the art also use the term "cDNA" to refer to a double-stranded DNA molecule consisting of such a single-stranded DNA molecule and its complementary DNA strand.

[0168] A cloning vector is a DNA molecule, such as a plasmid, cosmid, or bacteriophage, that has the capability of replicating autonomously in a host cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites at which foreign DNA sequences can be inserted in a determinable fashion without loss of an essential biological function of the vector, as well as a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance or ampicillin resistance. A recombinant host may be any prokaryotic or eukaryotic cell that contains either a cloning vector or expression vector. This term also includes those prokaryotic or eukaryotic cells that have been genetically engineered to contain the cloned gene(s) in the chromosome or genome of the host cell. The term expression refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

[0169] Suitable host cells include microbial or mammalian host cells. A preferred host is the human cell line, PER.C6, which was developed for production of MAbs, and other fusion proteins. Accordingly, a preferred embodiment of the present disclosure is a host cell comprising a DNA sequence encoding the PAM4 MAb, conjugate, fusion protein or fragments thereof. PER.C6 cells (WO 97/00326) were generated by transfection of primary human embryonic retina cells, using a plasmid that contained the Adserotype 5 (Ad5) E1A-and E1B-coding sequences (Ad5 nucleotides 459-3510) under the control of the human phosphoglycerate kinase (PGK) promoter. E1A and E1B are adenovirus early gene activation protein 1A and 1B, respectively. The methods and compositions are particularly useful for generating stable expression of human recombinant proteins of interest that are modified post-translationally, e.g. by glycosylation. Several features make PER.C6 particularly useful as a host for recombinant protein production, such as PER.C6 is a fully characterized human cell line and it was developed in compliance with good laboratory practices. Moreover, PER.C6 can be grown as a suspension culture in defined serum-free medium devoid of any human- or animal-derived proteins and its growth is compatible with roller bottles, shaker flasks, spinner flasks and bioreactors with doubling times of about 35 hours. Finally, the presence of E1A causes an up regulation of expression of genes that are under the control of the CMV enhancer/promoter and the presence of E13 prevents p53-dependent apoptosis possibly enhanced through over expression of the recombinant transgene. In one embodiment, the cell is capable of producing 2 to 200-fold more recombinant protein and/or proteinaceous substance than conventional mammalian cell lines.

Chimerized PAM4 Antibodies Use for Treatment and Diagnosis

[0170] As subject to the claims, the method of diagnosing or treating a malignancy in a subject comprises administering to the subject a therapeutically effective amount of a therapeutic conjugate comprising a PAM4 MAb or fragment thereof or an antibody fusion protein or fragment thereof, wherein the PAM4 MAb or fragment thereof or antibody fusion protein or fragment thereof is bound to at least one diagnostic and/or therapeutic agent and then formulated in a pharmaceutically suitable excipient Also preferred is a method for diagnosing or treating cancer, comprising: administering a multivalent, multispecific antibody or fragment thereof comprising one or more antigen binding sites toward a PAM4 antigen and one or more hapten binding sites to a subject in need thereof, waiting a sufficient amount of time for an amount of the non-antibody to clear the subject's blood stream; and then administering to the subject a carrier molecule comprising a diagnostic/detection agent, a therapeutic agent, or a combination thereof that binds to the binding site of the multivalent, multispecific antibody or fragment thereof. In a preferred embodiment, the cancer is a pancreatic cancer. In another preferred embodiment, the antibody is a multivalent, monospecific antibody or fragment thereof.

[0171] The use of MAbs for *in vitro* diagnosis is well-known. See, for example, Carlsson et al., Bio/Technology 7 (6): 567 (1989). For example, MAbs can be used to detect the presence of a tumor-associated antigen in tissue from biopsy samples. MAbs also can be used to measure the amount of tumor-associated antigen in clinical fluid samples using techniques such as radioimmunoassay, enzyme-linked immunosorbent assay, and fluorescence immunoassay.

[0172] Contemplated herein also is the use of PAM4 antibodies and fragments thereof and PAM4 fusion proteins and fragments thereof for in vitro diagnosis of a malignancy. The use of MAbs for *in vitro* diagnosis is well-known. See, for example, Carlsson et al., Bio/Technology 7 (6): 567 (1989). For example, MAbs can be used to detect the presence of a tumor-associated antigen in tissue from biopsy samples. MAbs also can be used to measure the amount of tumor-associated antigen in clinical fluid samples using techniques such as radioimmunoassay, enzyme-linked immunosorbent assay, and fluorescence immunoassay.

[0173] Conjugates of tumor-targeted MAbs and toxins can be used to selectively kill cancer cells *in vivo* (Spalding, Bio/Technology 9(8): 701 (1991); Goldenberg, Scientific American Science & Medicine 1(1): 64 (1994)). For example, therapeutic studies in experimental animal models have demonstrated the anti-tumor activity of antibodies carrying cytotoxic radionuclides. See Example 3 and 5 for a discussion of animal models and therapeutic studies. (Goldenberg et al., Cancer Res. 41: 4354 (1981), Cheung et al., J. Nat'l Cancer Inst. 77: 739 (1986), and Senekowitsch et al., J. Nucl. Med. 30: 531 (1989)).

[0174] Chimerized antibodies and fragments thereof are suitable for use in therapeutic methods and diagnostic methods. Accordingly, contemplated in the present invention is a method of delivering a diagnostic or therapeutic agent, or a combination thereof to a target comprising (i) providing a composition that comprises a PAM4 antibody or fragment thereof conjugated to at least one diagnostic and/or therapeutic agent and (ii) administering to a subject in need thereof the diagnostic or therapeutic antibody conjugate. In a preferred embodiment, the PAM4 antibodies and fragments thereof are chimerized. In another embodiment, the chimerized PAM4 antibodies and fragments thereof of the present invention are used in methods for treating malignancies.

[0175] Also described herein is a cancer cell targeting diagnostic or therapeutic conjugate comprising an antibody component that comprises a PAM4 MAb or fragment thereof of any of the antibodies of the present invention, or an antibody fusion protein or fragment thereof wherein the antibody component is bound to at least one diagnostic or at least one therapeutic agent Preferably, the diagnostic conjugate is a photoactive diagnostic/detection agent, an ultrasound detectable agent or an MRI contrast agent Still preferred, the diagnostic/detection agent is a radionuclide with an energy between 20 and 4,000 keV.

[0176] As subject to the claims the method for diagnosing or treating a malignancy comprising administering a therapeutically or diagnostically effective amount of at least one naked PAM4 antibody or fragment thereof and/or PAM4 fusion protein or fragment thereof, and optionally formulating the PAM4 antibody, fusion protein, or fragments thereof in a pharmaceutical excipient

[0177] The compositions for treatment contain at least one chimerized PAM4 antibody or fragment thereof either alone and unconjugated, or conjugated or unconjugated and in combination with other antibodies or fragments thereof, such as other humanized or chimeric antibodies, human antibodies, therapeutic agents or immunomodulators. Naked or conjugated antibodies to the same or different epitope or antigen may also be combined with one or more of the PAM4 antibodies or fragments thereof of the present invention.

[0178] Accordingly and as subject to the claims, the present invention contemplates the administration of PAM4 antibodies and fragments thereof including PAM4 fusion proteins and fragments thereof, alone, as a naked antibody or antibody fragment, or administered as a multimodal therapy. Preferably, the antibody is a chimerized PAM4 antibody or fragment thereof. Multimodal therapies of the present invention further include immunotherapy with a naked PAM4 antibody supplemented with administration of other antibodies in the form of naked antibodies, fusion proteins, or as immunoconjugates. For example, a chimerized PAM4 antibody may be combined with another naked chimerized PAM4 or other antibody, or a humanized PAM4, or other antibody conjugated to an isotope, one or more chemotherapeutic

agents, cytokines, toxins or a combination thereof For example, the present invention contemplates treatment of a naked or conjugated PAM4 antibody or fragments thereof before, in combination with, or after other pancreatic tumor associated antibodies such as CA19.9, DUPAN2, SPAN1, Nd2, B72.3, CC49,1a3, aLe^a antibodies, and other Lewis antigens (e.g., Le(y)), as well as antibodies against carcinoembryonic antigen (CEA), colon-specific antigen-p (CSAp), MUC1, MUC2, MUC3, MUC4, HER2/neu, EGFR, angiogenesis factors (e.g., VEGF), insulin-like growth factor (IGF), tenascin, platelet derived growth factor, IL-6, as well as products of oncogenes and antibodies against tumor necrosis substances. These solid tumor antibodies may be naked or conjugated to, *inter alia,* drugs, toxins, isotopes, external radiation or immunomodulators. A fusion protein of a chimerized PAM4 antibody and a toxin or may also be used in this invention. Many different antibody combinations may be constructed, either as naked antibodies or as partly naked and partly conjugated with a therapeutic agent or immunomodulator. Alternatively, different naked antibody combinations may be employed for administration in combination with other therapeutic agents, such as a cytotoxic drug or with radiation, given consecutively, simultaneously, or sequentially.

[0179] The monospecific antibodies described herein that are linked to diagnostic or therapeutic agents directly target PAM4 positive tumors. The monospecific molecules bind selectively to targeted antigens and as the number of binding sites on the molecule increases, the affinity for the target cell increases and a longer residence time is observed at the desired location. Moreover, non-antigen bound molecules are cleared from the body quickly and exposure of normal tissues is minimized. A use of multispecific antibodies is in AES systems, where PAM4 pre-targets positive tumors for subsequent specific delivery of diagnostic or therapeutic agents. The agents are carried by histamine succinyl glycyl (HSG) containing peptides. The murine monoclonal antibody designated 679 (an IgG1, *K*) binds with high affinity to molecules containing the tri-peptide moiety, HSG (Morel et al, Molecular Immunology, 27,995-1000,1990). 679 MAb can form a bispecific antibody with cPAM4 that binds with HSG and the target antigen. Alternative haptens may also be utilized. These antibodies bind selectively to targeted antigens allowing for increased affinity and a longer residence time at the desired location. Moreover, non-antigen bound diabodies are cleared from the body quickly and exposure of normal tissues is minimized. PAM4 antibodies and fragments thereof and conjugates can be used to diagnose and treat mammalian disorders such as cancer.

[0180] Delivering a diagnostic or a therapeutic agent to a target for diagnosis or treatment in accordance with the invention includes providing the PAM4 antibody or fragments thereof with a diagnostic or therapeutic agent and administering to a subject in need thereof with the antibody. Diagnosis further requires the step of detecting the bound proteins with known techniques.

[0181] In the context of this application, the terms "diagnosis" or "detection" can be used interchangeably. Whereas diagnosis usually refers to defining a tissue's specific histological status, detection recognizes and locates a tissue, lesion or organism containing a particular antigen.

[0182] Administration of the antibodies and their fragments of the present invention with diagnostic or therapeutic agents can be effected in a mammal by intravenous, inttaarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, perfusion through a regional catheter, or direct intralesional injection. When administering the antibody by injection, the administration may be by continuous infusion or by single or multiple boluses.

[0183] The antibody with the diagnostic or therapeutic agent may be provided as a kit for human or mammalian therapeutic and diagnostic use in a pharmaceutically acceptable injection vehicle, preferably phosphate-buffered saline (PBS) at physiological pH and concentration. The preparation preferably will be sterile, especially if it is intended for use in humans. Optional components of such kits include stabilizers, buffers, labeling reagents, radioisotopes, paramagnetic compounds, second antibody for enhanced clearance, and conventional syringes, columns, vials and the like.

Naked Antibody Therapy

[0184] A therapeutically effective amount of a naked chimerized PAM4 antibody, or fragments thereof, or PAM4 fusion proteins or fragments thereof, can be formulated in a pharmaceutically acceptable excipient The efficacy of the naked chimerized PAM4 antibodies and their fragments can also be enhanced by supplementing these naked antibodies with one or more other naked antibodies, with one or more immunoconjugates of chimerized PAM4 antibodies, conjugated with one or more therapeutic agents, including drugs, toxins, immunomodulators, hormones, hormone antagonists, enzymes, enzyme inhibitors, oligonucleotides, therapeutic radionuclides, an angiogenesis inhibitor, etc., administered concurrently or sequentially or according to a prescribed dosing regimen, with the PAM4 antibodies or fragments thereof The naked antibodies that may supplement the naked PAM4 antibodies and fragments thereof may be directed against either the same tumor type or against immunomodulator cells (e.g., CD40$^+$ cells) that can be recruited to enhance the antitumor effects of the naked antibodies of choice.

[0185] In one embodiment, an oligonucleotide, such as an antisense molecule inhibiting bcl-2 expression is described in U.S. 5,734,033 (Reed), may be conjugated to, or form the therapeutic agent portion of an immunoconjugate or antibody fusion protein of the present invention. Alternatively, the oligonucleotide may be administered concurrently or sequentially with a naked or conjugated PAM4 antibody or antibody Fragment of the present invention. In a preferred embodiment,

the oligonucleotides is an antisense oligonucleotide that preferably is directed against an oncogene or oncogene product of a B-cell malignancy, such as bcl-2.

PAM4 Immunoconjugates

[0186] The present invention also contemplates the use of chimerized PAM4 antibodies and fragments thereof conjugated to at least one therapeutic and/or diagnostic/detection agent for therapy or diagnosis. For immunotherapy, the objective is to deliver cytotoxic doses of radioactivity, toxin, or drug to target cells, while minimizing exposure to non-target tissues. The PAM4 antibodies of the present invention can be used to diagnose and treat pancreatic tumors.

[0187] Any of the antibodies, antibody fusion proteins, and fragments thereof of the present invention can be conjugated with one or more therapeutic or diagnostic/detection agents. Generally, one therapeutic or diagnostic/detection agent is attached to each antibody, fusion protein or fragment thereof but more than one therapeutic agent and/or diagnostic/detection agent can be attached to the same antibody or antibody fragment If the Fc region is absent (for example when the antibody used as the antibody component of the immunoconjugate is an antibody fragment), it is possible to introduce a carbohydrate moiety into the light chain variable region of a full length antibody or antibody fragment. See, for example, Leung et al., J. Immunol. 154: 5919 (1995); Hansen et al., U.S. Patent No. 5,443,953 (1995), Leung et al., U.S. patent No. 6,254,868, The engineered carbohydrate moiety is used to attach the therapeutic or diagnostic/detection agent.

[0188] Methods for conjugating peptides to antibody components via an antibody carbohydrate moiety are well-known to those of skill in the art. See, for example, Shih et al., Int. J. Cancer 41: 832 (1988); Shih et al., Int. J. Cancer 46: 1101 (1990); and Shih et al., U.S. Patent No. 5,057,313. The general method involves reacting an antibody component having an oxidized carbohydrate portion with a carrier polymer that has at least one free amine function and that is loaded with a plurality of peptide. This reaction results in an initial Schiff base (imine) linkage, which can be stabilized by reduction to a secondary amine to form the final conjugate.

[0189] The antibody fusion proteins and fragments thereof of the present invention comprise two or more antibodies or fragments thereof and each of the antibodies that compose this fusion protein can contain at least one therapeutic agent and/or diagnostic/detection agent. For example, an antibody fusion protein may comprise one antibody (two antigen binding sites) and an antibody fragment, two antibody fragments, or two antibodies. The antibody fusion protien may then be conjugated to at least one diagnostic/detection and/or therapeutic agent.

[0190] Accordingly, one or more of the antibodies or fragments thereof of the antibody fusion protein can have more than one therapeutic and/or diagnostic/detection agent attached. Further, the therapeutic agents do not need to be the same but can be different therapeutic agents, for example, one can attach a drug and a radioisotope to the same fusion protein. Particularly, an IgG can be radiolabeled with ^{131}I and attached to a drug. The ^{131}I can be incorporated into the tyrosine of the IgG and the drug attached to the epsilon amino group of the IgG lysines. Both therapeutic and diagnostic/detection agents also can be attached to reduced SH groups and to the carbohydrate side chains.

[0191] A wide variety of diagnostic and therapeutic reagents can be administered concurrently or sequentially, or advantageously conjugated to the antibodies of the invention, for example, drugs, toxins, immunomodulators, hormones, hormone antagonists, enzymes, oligonucleotides, enzyme inhibitors, therapeutic radionuclides, an angiogenesis inhibitor, etc. The therapeutic agents recited here are those agents that also are useful for administration separately with the naked antibody as described above. Therapeutic agents include, for example, chemotherapeutic drugs such as vinca alkaloids, anthracyclines, epidophyllotoxins, taxanes, antimetabolites, alkylating agents, antibiotics, COX-2 inhibitors, SN-38, antimitotics, antiangiogenic and apoptotoic agents, particularly doxorubicin, methotrexate, taxol, CPT-11, camptothecans, and others from these and other classes of anticancer agents , and the like. Other useful cancer chemotherapeutic drugs for administering concurrently or sequentially, or for the preparation of immunoconjugates and antibody fusion proteins include nitrogen mustards, gemcitabine, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, COX-2 inhibitors, pyrimidine analogs, purine analogs, platinum coordination complexes, hormones, and the like. Suitable chemotherapeutic agents are described in REMINGTON'S PHARMACEUTICAL SCIENCES, 19th Ed. (Mack Publishing Co. 1995), and in GOODMAN AND GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 7th Ed. (MacMillan Publishing Co. 1985), as well as revised editions of these publications. Other suitable chemotherapeutic agents, such as experimental drugs, are known to those of skill in the art.

[0192] In one embodiment, an oligonucleotide, such as an antisense molecule inhibiting bcl-2 expression may be conjugated to, or form the therapeutic agent portion of an immunoconjugate or antibody fusion protein of the present invention. Alternatively, the oligonucleotide may be administered concurrently or sequentially with a naked or conjugated PAM4 antibody or antibody fragment of the present invention. In a preferred embodiment, the oligonucleotides is an antisense oligonucleotide that preferably is directed against an oncogene or oncogene product of a B-cell malignancy, such as bcl-2.

[0193] In one embodiment, the chimeric PAM4 antibodies and fragments thereof of the present invention is conjugated to gemcitabine. In another embodiment, gemcitabine is given before, after, or concurrently with a naked or conjugated

chimeric PAM4 antibody or fragment thereof of the present invention. Preferably, the conjugated chimeric PAM4 antibody or antibody fragment is conjugated to a radionuclide.

[0194] A toxin can be of animal, plant or microbial origin. A toxin, such as *Pseudomonas* exotoxin, may also be complexed to or form the therapeutic agent portion of an immunoconjugate of the PAM4 and cPAM4 antibodies of the present invention. Other toxins suitably employed in the preparation of such conjugates or other fusion proteins, include ricin, abrin, ribonuclease (RNase), DNase I, *Staphylococcal* enterotoxin-A, pokeweed antiviral protein, gelonin, diphtherin toxin, *Pseudomonas* exotoxin, and *Pseudomonas* endotoxin. See, for example, Pastan et al., Cell 47:641 (1986), and Goldenberg, CA - A Cancer Journal for Clinicians 44:43 (1994). Additional toxins suitable for use in the present invention are known to those of skill in the art and are disclosed in U.S. Patent 6,077,499.

[0195] An immunomodulator, such as a cytokine, may also be conjugated to, or form the therapeutic agent portion of the PAM4 and cPAM4 immunoconjugate, or may be administered with, but unconjugated to, the chimerized PAM4 antibody or fragment thereof, or PAM4 fusion protein or fragment thereof of the present invention. The PAM4 fusion protein or fragment thereof may comprise one or more antibodies or fragments thereof binding to different antigens. For example, the fusion protein may bind the PAM4 antigen as well as immunomodulating cells or factors. Alternatively, subjects can receive a naked PAM4 antibody, fusion protein, or fragment thereof and a separately administered cytokine, which can be administered before, concurrently, or after administration of the naked PAM4 antibodies. As used herein, the term "immunomodulator" includes cytokines, stem cell growth factors, lymphotoxins, such as tumor necrosis factor (TNF), and hematopoietic factors, such as interleukins (*e.g.,* interleukin-1 (IL-1), IL-2, IL-3, IL-6, IL-10, IL-12, IL-18 and IL-21), colony stimulating factors (*e.g.,* granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF)), interferon (*e.g.,* interferons-α, -β and -γ), the stem cell growth factor designated "S1 factor," erythropoietin and thrombopoietin. Examples of suitable immunomodulator moieties include IL-2, IL-6, IL-10, IL-12, IL-18, IL-21, interferon-γ, TNF-α, and the like.

[0196] Alternatively, the antibodies and fragments of the present invention can be detectably labeled by linking the antibody to an enzyme. When the antibody-enzyme conjugate is incubated in the presence of the appropriate substrate, the enzyme moiety reacts with the substrate to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or visual means. Examples of enzymes that can be used to detectably label antibody include malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, α-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, β-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

[0197] A therapeutic or diagnostic/detection agent can be attached at the hinge region of a reduced antibody component via disulfide bond formation. As an alternative, such agents can be attached to the antibody component using a heterobifunctional cross-linker, such as *N*-succinyl 3-(2-pyridyldithio)proprionate (SPDP). Yu et al., Int. J. Cancer 56: 244 (1994). General techniques for such conjugation are well-known in the art. See, for example, Wong, CHEMISTRY OF PROTEIN CONJUGATION AND CROSS-LINKING (CRC Press 1991); Upeslacis et al., "Modification of Antibodies by Chemical Methods," in MONOCLONAL ANTIBODIES: PRINCIPLES AND APPLICATIONS, Birch et al. (eds.), pages 187-230 (Wiley-Liss, Inc. 1995); Price, "Production and Characterization of Synthetic Peptide-Derived Antibodies," in MONOCLONAL ANTIBODIES: PRODUCTION, ENGINEERING AND CLINICAL APPLICATION, Ritter et al. (eds.), pages 60-84 (Cambridge University Press 1995). Alternatively, the therapeutic or diagnostic/detection agent can be conjugated via a carbohydrate moiety in the Fc region of the antibody. The carbohydrate group can be used to increase the loading of the same agent that is bound to a thiol group, or the carbohydrate moiety can be used to bind a different peptide.

[0198] In the methods as subject to the claims, the targetable construct may comprise one or more radioactive isotopes useful for detecting diseased tissue. Particularly useful diagnostic radionuclides include, but are not limited to, ^{110}In, ^{111}In, ^{177}Lu, ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{89}Zr, ^{94m}Tc, ^{94}Tc, ^{99m}Tc, ^{120}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154\text{-}158}$Gd, ^{32}P, ^{11}C, ^{13}N, ^{15}O, ^{186}Re, ^{188}Re, ^{51}Mn, ^{52m}Mn ^{55}Co, ^{72}As, ^{75}Br, ^{76}Br, ^{82m}Rb, ^{83}Sr, or other gamma-, beta-, or positron-emitters, preferably with a decay energy in the range of 20 to 4,000 keV, more preferably in the range of 25 to 4,000 keV, and even more preferably in the range of 25 to 1,000 keV, and still more preferably in the range of 70 to 700 keV. Total decay energies of useful positron-emitting radionuclides are preferably < 2,000 keV, more preferably under 1,000 keV, and most preferably < 700 keV. Radionuclides useful as diagnostic/detection agents utilizing gamma-ray detection include, but are not limited to: ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{67}Cu, ^{67}Ga, ^{75}Se, ^{97}Ru, ^{99m}Tc, ^{111}In, ^{114}In ^{123}I, ^{125}I, ^{131}I, ^{169}Yb, ^{197}Hg, and ^{201}Tl. Decay energies of useful gamma-ray emitting radionuclides are preferably 20-2000 keV, more preferably 60-600 keV, and most preferably 100-300 keV.

[0199] In the methods as subject to the claims? the targetable construct may comprise one or more radioactive isotopes useful for treating diseased tissue. Particularly useful therapeutic radionuclides include, but are not limited to ^{111}In, ^{177}Lu, ^{212}Bi, ^{213}Bi, ^{211}At, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{90}Y, ^{125}I, ^{131}I, ^{32}P, ^{33}P, ^{47}Sc, ^{111}Ag, ^{67}Ga, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{186}Re, ^{188}Re, ^{189}Re, ^{212}Pb, ^{223}Ra, ^{225}Ac, ^{59}Fe, ^{75}Se, ^{77}As, ^{89}Sr, ^{99}Mo, ^{105}Rh, ^{109}Pd, ^{143}Pr, ^{149}Pm, ^{169}Er, ^{194}Ir, ^{198}Au, ^{199}Au, and ^{211}Pb. The therapeutic radionuclide preferably has a decay energy in the range of 20 to 6,000 keV, preferably

in the ranges 60 to 200 keV for an Auger emitter, 100-2,500 keV for a beta emitter, and 4,000-6,000 keV for an alpha emitter. Maximum decay energies of useful beta-particle-emitting nuclides are preferably 20-5,000 keV, more preferably 100-4,000 keV, and most preferably 500-2,500 keV. Also preferred are radionuclides that substantially decay with Auger-emitting particles. For example, Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, I-125, Ho-161, Os-189m and Ir-192. Decay energies of useful beta-particle-emitting nuclides are preferably < 1,000 keV, more preferably < 100 keV, and most preferably < 70 keV. Also preferred are radionuclides that substantially decay with generation of alpha-particles. Such radionuclides include, but are not limited to: Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213 and Fm-255. Decay energies of useful alpha-particle-emitting radionuclides are preferably 2,000-10,000 keV, more preferably 3,000-8,000 keV, and most preferably 4,000-7,000 keV.

[0200] For example, ^{67}Cu, considered one of the more promising radioisotopes for radioimmunotherapy due to its 61.5 hour half-life and abundant supply of beta particles and gamma rays, can be conjugated to a PAM4 antibody using the chelating agent, p-bromoacetamido-benzyl-tetraethylaminetetraacetic acid (TETA). Chase, *supra.* Alternatively, ^{90}Y, which emits an energetic beta particle, can be coupled to a PAM4 antibody, fusion protein, or fragment thereof using diethylenetriaminepentaacetic acid (DTPA).

[0201] Additional potential radioisotopes include ^{11}C, ^{13}N, ^{15}O, ^{75}Br, ^{198}Au, ^{224}Ac, ^{126}I, ^{133}I, ^{77}Br, ^{113m}In, ^{95}Ru, ^{97}Ru, ^{103}Ru, ^{105}Ru, ^{107}Hg, ^{203}Hg, ^{121m}Te, ^{122m}Te, ^{125m}Te, ^{165}Tm, ^{167}Tm, ^{168}Tm, ^{197}Pt, ^{109}pd, ^{105}Rh, ^{142}Pr, ^{143}Pr, ^{161}Tb, ^{166}Ho, ^{199}Au, ^{57}Co, ^{58}Co, ^{51}Cr, ^{59}Fe, ^{75}Se, ^{201}Tl, ^{225}Ac, ^{76}Br, ^{169}Yb, and the like.

[0202] In another embodiment, a radiosensitizer can be used in combination with a naked or conjugated PAM4 antibody or antibody fragment of the present invention. For example, the radiosensitizercan be used in combination with a radiolabeled PAM4 antibody or antibody fragment. The addition of the radiosensitizer can result in enhanced efficacy when compared to treatment with the radiolabeled antibody or antibody fragment alone. Radiosensitizers are described in D.M. Goldenberg (ed.), CANCER THERAPY WITH RADIOLABELED ANTIBODIES, CRC Press (1995),

[0203] The PAM4 antibody or fragment thereof, or PAM4 fusion protein or fragment thereof of the present invention that have a boron addend-loaded carrier for thermal neutron activation therapy will normally be effected in similar ways. However, it will be advantageous to wait until non-targeted PAM4 immunoconjugate clears before neutron irradiation is performed. Clearance can be accelerated using an antibody that binds to the PAM4 antibody. See U.S. patent No. 4,624,846 for a description of this general principle. For example, boron addends such as carboranes, can be attached to PAM4 antibodies. Carboranes can be prepared with carboxyl functions on pendant side chains, as is well-known in the art. Attachment of carboranes to a carrier, such as aminodextran, can be achieved by activation of the carboxyl groups of the carboranes and condensation with amines on the carrier. The intermediate conjugate is then conjugated to the PAM4 antibody. After administration of the PAM4 antibody conjugate, a boron addend is activated by thermal neutron irradiation and converted to radioactive atoms which decay by α-emission to produce highly toxic, short-range effects.

[0204] In the methods of diagnosing cancer in a subject as subject to the claims, diagnosis may be accomplished by administering a diagnostically effective amount of a diagnostic conjugate, formulated in a pharmaceutically suitable excipient, and detecting said label. The PAM4 antibodies, fusion proteins, and fragments thereof may be conjugated to the diagnostic/detection agent or be administered unconjugated to the diagnostic/detection agent, but before, concurrently, or after administration of the diagnostic/detection agent Radioactive agents that can be used as diagnostic/detection agents were discussed above. A suitable non-radioactive diagnostic/detection agent is a contrast agent suitable for magnetic resonance imaging, X-rays, computed tomography or ultrasound. Magnetic imaging agents include, for example, non-radioactive metals, such as manganese, iron and gadolinium, complexed with metal-chelate combinations that include 2-benzyl-DTPA and its monomethyl and cyclohexyl analogs, when used along with the antibodies of the invention. See U.S. Serial No. 09/921,290 filed on October 10, 2001.

[0205] Contrast agents, such as MRI contrast agents, contemplated in the present invention include, for example, gadolinium ions, lanthanum ions, dysprosium ions, iron ions, manganese ions or other comparable label, CT contrast agents, and ultrasound contrast agents are suitable for use in the present invention.

[0206] Paramagnetic ions suitable for the present invention include include chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and erbium (III), with gadolinium being particularly preferred.

[0207] Ions useful in other contexts, such as X-ray imaging, include but are not limited to lanthanum (III), gold (III), lead (II), and especially bismuth (III). Fluorescent labels include rhodamine, fluorescein and renographin. Rhodamine and fluorescein are often linked via an isothiocyanate intermediate.

[0208] Metals are also useful in diagnostic/detection agents, including those for magnetic resonance imaging techniques. These metals include, but are not limited to: Gadolinium, manganese, iron, chromium, copper, cobalt, nickel, dysprosium, rhenium, europium, terbium, holmium and neodymium. In order to load an antibody component with radioactive metals or paramagnetic ions, it may be necessary to react it with a reagent having a long tail to which are attached a multiplicity of chelating groups for binding the ions. Such a tail can be a polymer such as a polylysine, polysaccharide, or other derivatized or derivatizable chain having pendant groups to which can be bound chelating groups such as, e.g.,

ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, polyoximes, and like groups known to be useful for this purposes. Chelates are coupled to the PAM 4 antibody, fusion protein, or fragments thereof using standard chemistries. The chelate is normally linked to the antibody by a group which enables formation of a bond to the molecule with minimal loss of immunoreactivity and minimal aggregation and/or internal cross-linking. Other, more unusual, methods and reagents for conjugating chelates to antibodies are disclosed in U.S. Patent 4,824,659 to Hawthorne, entitled "antibody Conjugates," issued April 25,1989. Particularly useful metal-chelate combinations include 2-benzyl-DTPA and its monomethyl and cyclohexyl analogs, used with diagnostic isotopes in the general energy range of 20 to 2,000 keV. The same chelates, when complexed with non-radioactive metals, such as manganese, iron and gadolinium are useful for MRI, when used along with the antibodies of the invention. Macrocyclic chelates such as NOTA, DOTA, and TETA are of use with a variety of metals and radiometals, most particularly with radionuclides of gallium, yttrium and copper, respectively. Such metal-chelate complexes can be made very stable by tailoring the ring size to the metal of interest Other ring-type chelates such as macrocyclic polyethers, which are of interest for stably binding nuclides, such as ^{223}Ra for RAIT are encompassed by the invention.

[0209] Radiopaque and contrast materials are used for enhancing X-rays and computed tomography, and include iodine compounds, barium compounds, gallium compounds, thallium compounds, etc. Specific compounds include barium, diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide, iodipamide, iodoxamic acid, iogulamide, iohexol, iopamidol, iopanoic acid, ioprocemic acid, iosefamic acid, ioseric acid, iosulamide meglumine, iosemetic acid, iotasul, iotetric acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizoic acid, ipodate, meglumine, metrizamide, metrizoate, propyliodone, and thallous chloride.

[0210] The antibodies, fusion proteins, and fragments thereof of the present invention also can be labeled with a fluorescent compound. The presence of a fluorescent-labeled MAb is determined by exposing the antibody to light of the proper wavelength and detecting the resultant fluorescence. Fluorescent labeling compounds include fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine. Fluorescently-labeled antibodies are Particularly useful for flow cytometry analysis.

[0211] Alternatively, the antibodies, fusion proteins, and fragments thereof of this invention can be detectably labeled by coupling the antibody to a chemiluminescent compound. The presence of the chemihuninescent-tagged MAb is determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of chemiluminescent labeling compounds include luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester.

[0212] Similarly, a bioluminescent compound can be used to label the antibodies and fragments thereof the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Bioluminescent compounds that are useful for labeling include luciferin, luciferase and aequorin.

[0213] Accordingly and as subject to the claims, a method of diagnosing a malignancy in a subject comprises performing an in vitro diagnosis assay on a specimen (fluid, tissue or cells) from the subject with a composition comprising a naked PAM4 MAb or fragment thereof or a naked antibody fusion protein or fragment thereof. Immunohistochemistry can be used to detect the presence of PAM4 in a cell or tissue. Preferably, the malignancy that is being diagnosed is a cancer. Most preferably, the cancer is pancreatic cancer.

[0214] Additionally, a chelator such as DTPA, DOTA, TETA, or NOTA or a suitable peptide, to which a detectable label, such as a fluorescent molecule, or cytotoxic agent, such as a heavy metal or radionuclide, can be conjugated. For example, a therapeutically useful immunoconjugate can be obtained by conjugating a photoactive agent or dye to an antibody fusion protein. Fluorescent compositions, such as fluorochrome, and other chromogens, or dyes, such as porphyrins sensitive to visible light, have been used to detect and to treat lesions by directing the suitable light to the lesion. In therapy, this has been termed photoradiation, phototherapy, or photodynamic therapy (Jori et al. (eds.), PHOTODYNAMIC THERAPY OF TUMORS AND OTHER DISEASES (Libreria Progetto 1985); van den Bergh, Chem. Britain 22:430 (1986)). Moreover, monoclonal antibodies have been coupled with photoactivated dyes for achieving phototherapy. Mew et al., J. Immunol. 130:1473 (1983); *idem.,* CancerRes. 45:4380 (1985); Oseroffet al., Proc. Natl. Acad Sci. USA 83:8744 (1986); *idem.,* Phorochem. Photobiol. 46:83 (1987); Hasan et al., Prog. Clin. Biol. Res. 288:471 (1989); Tatsuta et al., Lasers Surg. Med. 9:422 (1989); Pelegrin et al., Cancer 67:2529 (1991). However, these earlier studies did not include use of endoscopic therapy applications, especially with the use of antibody fragments or subfragments. Thus, the present invention contemplates the therapeutic use of immunoconjugates comprising photoactive agents or dyes.

[0215] For purposes of therapy, the PAM4 antibodies and fragments thereof of the present invention are administered to a patient in a therapeutically effective amount An antibody is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

[0216] A diagnostic/detection agent is a molecule or atom, which may be administered conjugated to an antibody

moiety, i.e., antibody or antibody fragment, or subfragment, fusion protein, and fragments thereof and is useful in diagnosing/detecting a disease by locating the cells containing the disease-associated antigen. Useful diagnostic/detection agents include, but are not limited to, radioisotopes, dyes (such as with the biotin-streptavidin complex), radiopaque materials (e.g., iodine, barium, gallium, and thallium compounds and the like), contrast agents, fluorescent compounds or molecules and enhancing agents (e.g., paramagnetic ions) for magnetic resonance imaging (MRI). U.S. Patent No. 6,331,175 describes MRI technique and the preparation of antibodies conjugated to a MRI enhancing agent. Preferably, the diagnostic/detection agents are selected from the group consisting of radioisotopes for nuclear imaging, endoscopic and intravascular detection, enhancing agents for use in magnetic resonance imaging or in ultrasonography, radiopaque and contrast agents for X-rays and computed tomography, and fluorescent compounds for fluoroscopy, including endoscopic fluoroscopy. Fluorescent and radioactive agents conjugated to antibodies or used in bispecific, pretargeting methods, are particularly useful for endoscopic, intraoperative or intravascular detection of the targeted antigens associated with diseased tissues or clusters of cells, such as malignant tumors, as disclosed in Goldenberg U.S. Pat. Nos. 5,716,595,6,096,289 and U.S. Application Serial No. 09/348,818, particularly with gamma-, beta-, and positron-emitters. Endoscopic applications may be used when there is spread to a structure that allows an endoscope, such as the colon. Radionuclides useful for positron emission tomography include, but are not limited to: F-18, Mn-51, Mn-52m, Fe-52, Co-55, Cu-62, Cu-64, Ga-68, As-72, Br-75, Br-76, Rb-82m, Sr-83, Y-86, Zr-89, Tc-94m, In-110, I-120, and I-124. Total decay energies of useful positron-emitting radionuclides are preferably < 2,000 keV, more preferably under 1,000 keV, and most preferably < 700 keV. Radionuclides useful as diagnostic/detection agents utilizing gamma-ray detection include, but are not limited to: Cr-51, Co-57, Co-58, Fe-59, Cu-67, Ga-67, Se-75, Ru-97, Tc-99m, In-111, In-114m, I-123, I-125, I-131, Yb-169, Hg-197, and Tl-201. Decay energies of useful gamma-ray emitting radionuclides are preferably 20-2000 keV, more preferably 60-600 keV, and most preferably 100-300 keV.

In-Vitro Diagnosis

[0217] The present invention contemplates the use of PAM4 antibodies, including PAM4 fusion proteins and fragments thereof, to screen biological samples *in vitro* for the presence of the PAM4 antigen. In such immunoassays, the PAM4 antibody, fusion protein, or fragment thereof may be utilized in liquid phase or bound to a solid-phase carrier, as described below. In a preferred embodiment, the PAM4 antibody or fragment thereof is chimerized. Still preferred, the PAM4 fusion protein comprises a chimerized PAM4 antibody.

[0218] One example of a screening method for determining whether a biological sample contains the PAM4 antigen is the radioimmunoassay (RIA). For example, in one form of RIA, the substance under test is mixed with PAM4 antigen MAb in the presence of radiolabeled PAM4 antigen. In this method, the concentration of the test substance will be inversely proportional to the amount of labeled PAM4 antigen bound to the MAb and directly related to the amount of free, labeled PAM4 antigen. Other suitable screening methods will be readily apparent to those of skill in the art.

[0219] Alternatively, *in vitro* assays can be performed in which a PAM4 antibody, fusion protein, or fragment thereof is bound to a solid-phase carrier. For example, MAbs can be attached to a polymer, such as aminodextran, in order to link the MAb to an insoluble support such as a polymer-coated bead, a plate or a tube.

[0220] Other suitable *in vitro* assays will be readily apparent to those of skill in the art. The specific concentrations of detectably labeled PAM4 antibody and PAM4 antigen, the temperature and time of incubation, as well as other assay conditions may be varied, depending on various factors including the concentration of the PAM4 antigen in the sample, the nature of the sample, and the like. The binding activity of a sample of PAM4 antibody may be determined according to well-known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

[0221] Other such steps as washing, stirring, shaking, filtering and the like may be added to the assays as is customary or necessary for the particular situation.

[0222] The presence of the PAM4 antigen in a biological sample can be determined using an enzyme-linked immunosorbent assay (ELISA). In the direct competitive ELISA, a pure or semipure antigen preparation is bound to a solid support that is insoluble in the fluid or cellular extract being tested and a quantity of detectably labeled soluble antibody is added to permit detection and/or quantitation of the binary complex formed between solid-phase antigen and labeled antibody.

[0223] In contrast, a "double-determinant" ELISA, also known as a "two-site ELISA" or "sandwich assay," requires small amounts of antigen and the assay does not require extensive purification of the antigen. Thus, the double-determinant ELISA is preferred to the direct competitive ELISA for the detection of an antigen in a clinical sample. See, for example, the use of the double-determinant ELISA for quantitation of the *c-myc* oncoprotein in biopsy specimens. Field et al., Oncogene 4: 1463 (1989); Spandidos et al., AntiCancer Res. 9: 821 (1989).

[0224] In a double-determinant ELISA, a quantity of unlabeled MAb or antibody fragment (the "capture antibody") is bound to a solid support, the test sample is brought into contact with the capture antibody, and a quantity of detectably labeled soluble antibody (or antibody fragment) is added to permit detection and/or quantitation of the ternary complex

formed between the capture antibody, antigen, and labeled antibody. An antibody fragment is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, and the like. In the present context, an antibody fragment is a portion of a PAM4 MAb that binds to an epitope of the PAM4 antigen. The term "antibody fragment" also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex. For example, antibody fragments include isolated fragments consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, and recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker. An antibody fusion protein is a recombinantly produced antigen-binding molecule in which two or more of the same or different single-chain antibody or antibody fragment segments with the same or different specificities are linked. The fusion protein may comprise a single antibody component, a multivalent or multispecific combination of different antibody components or multiple copies of the same antibody component. The fusion protein may additionally comprise an antibody or an antibody fragment conjugated to a diagnostic/detection and/or a therapeutic agent The term PAM4 antibody includes chimerized and murine antibodies, antibody fragments thereof, immunoconjugates and fragments thereof and antibody fusion proteins and fragments thereof.

[0225] Methods of performing a double-determinant ELISA are well-known. See, for example, Field *et al.*, *supra,* Spandidos *et al., supra,* and Moore et al., "Twin-Site ELISAs for fos and myc Oncoproteins Using the AMPAK System," in METHODS IN MOLECULAR BIOLOGY, VOL. 10, pages 273-281 (The Humana Press, Inc. 1992).

[0226] In the double-determinant ELISA, the soluble antibody or antibody fragment must bind to a PAM4 epitope that is distinct from the epitope recognized by the capture antibody. The double-determinant ELISA can be performed to ascertain whether the PAM4 antigen is present in a biopsy sample. Alternatively, the assay can be performed to quantitate the amount of PAM4 antigen that is present in a clinical sample of body fluid. The quantitative assay can be performed by including dilutions of purified PAM4 antigen.

[0227] The PAM4 Mabs, fusion proteins, and fragments thereof of the present invention also are suited for the preparation of an assay kit. Such a kit may comprise a carrier means that is compartmentalized to receive in close confinement one or more container means such as vials, tubes and the like, each of said container means comprising the separate elements of the immunoassay.

[0228] For example, there may be a container means containing the capture antibody immobilized on a solid phase support, and a further container means containing detectably labeled antibodies in solution. Further container means may contain standard solutions comprising serial dilutions of PAM4 antigen. The standard solutions of PAM4 antigen may be used to prepare a standard curve with the concentration of PAM4 antigen plotted on the abscissa and the detection signal on the ordinate. The results obtained from a sample containing PAM4 antigen may be interpolated from such a plot to give the concentration of PAM4 antigen in the biological sample.

[0229] PAM4 antibodies, fusion proteins, and fragments thereof of the present invention also can be used to detect the presence of the PAM4 antigen in tissue sections prepared from a histological specimen. Such *in situ* detection can be used to determine the presence of the PAM4 antigen and to determine the distribution of the PAM4 antigen in the examined tissue. *In situ* detection can be accomplished by applying a detectably-labeled PAM4 antibody to frozen tissue sections. Studies indicate that the PAM4 antigen is preserved in paraffin-embedded sections. General techniques of *in situ* detection are well-known to those of ordinary skill. See, for example, Ponder, "Cell Marking Techniques and Their Application," in MAMMALIAN DEVELOPMENT: A PRACTICAL APPROACH 113-38 Monk (ed.) (IRL Press 1987), and Coligan at pages 5.8.1-5.8.8.

[0230] PAM4 antibodies, fusion proteins, and fragments thereof can be detectably labeled with any appropriate marker moiety, for example, a radioisotope, an enzyme, oligonucleotides, a fluorescent label, a dye, a chromagen, a chemiluminescent label, a bioluminescent labels or a paramagnetic label. Methods of making and detecting such detectably-labeled PAM4 antibodies are well-known to those of ordinary skill in the art, and are described in more detail below.

[0231] The marker moiety can be a radioisotope that is detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography. In a preferred embodiment, the diagnostic conjugate is a gamma-, beta- or a positron-emitting isotope. A marker moiety in the present description refers to a molecule that will generate a signal under predetermined conditions. Examples of marker moieties include radioisotopes, enzymes, fluorescent labels, chemiluminescent labels, bioluminescent labels and paramagnetic labels. As used herein, a diagnostic or therapeutic agent is a molecule or atom which is conjugated to an antibody moiety to produce a conjugate which is useful for diagnosis and for therapy. Examples of diagnostic or therapeutic agents include drugs, toxins, immunomodulators, cytokines, hormones, hormone antagonists, enzymes, oligonucleotides, enzyme inhibitors, isotopes, other antibodies, chelators, dyes, chromagens, boron compounds, and marker moieties.

[0232] Those of skill in the art will know of other suitable labels, which can be employed in accordance with the present invention. The binding of marker moieties to PAM4 antibodies can be accomplished using standard techniques known to the art. Typical methodology in this regard is described by Kennedy et al., Clin. Chim. Acta 70: 1 (1976), Schurs et al., Clin. Chim. Acta 81:1 (1977), Shih et al., Int'l J. Cancer 46: 1101 (1990).

[0233] The above-described *in vitro* and *in situ* detection methods may be used to assist in the diagnosis or staging of a pathological condition. For example, such methods can be used to detect tumors that express the PAM4 antigen

such as pancreatic cancer.

In Vitro Diagnosis

[0234] The present invention also contemplates the use of PAM4 antibodies for *in vivo* diagnosis. The method of diagnostic imaging with radiolabeled MAbs is well-known. In the technique of immunoscintigraphy, for example, antibodies are labeled with a gamma-emitting radioisotope and introduced into a patient A gamma camera is used to detect the location and distribution of gamma-emitting radioisotopes. See, for example, Srivastava (ed.), RADIOLABELED MONOCLONAL ANTIBODIES FOR IMAGING AND THERAPY (Plenum Press 1988), Chase, "Medical Applications of Radioisotopes," in REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Edition, Gennaro et al. (eds.), pp. 624-652 (Mack Publishing Co., 1990), and Brown, "Clinical Use of Monoclonal Antibodies," in BIOTECHNOLOGY AND PHARMACY 227-49, Pezzuto et al. (eds.) (Chapman & Hall 1993).

[0235] For diagnostic imaging, radioisotopes may be bound to the PAM4 antibody either directly, or indirectly by using an intermediary functional group. Useful intermediary functional groups include chelators such as ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid. For example, see Shih *et al., supra,* and U.S. patent No. 5,057,313.

[0236] The radiation dose delivered to the patient is maintained at as low a level as possible through the choice of isotope for the best combination of minimum half-life, minimum retention in the body, and minimum quantity of isotope which will permit detection and accurate measurement. Examples of radioisotopes that can be bound to PAM4 antibody and are appropriate for diagnostic imaging include ^{99m}Tc and ^{111}In.

[0237] The PAM4 antibodies, fusion proteins, and fragments thereof also can be labeled with paramagnetic ions and a variety of radiological contrast agents for purposes of *in vivo* diagnosis. Contrast agents that are particularly useful for magnetic resonance imaging comprise gadolinium, manganese, dysprosium, lanthanum, or iron ions. Additional agents include chromium, copper, cobalt, nickel, rhenium, europium, terbium, holmium, or neodymium. PAM4 antibodies and fragments thereof can also be conjugated to ultrasound contrast/enhancing agents. For example, the ultrasound contrast agent is a liposome that comprises a chimerized PAM4 IgG or fragment thereof Also preferred, the ultrasound contrast agent is a liposome that is gas filled.

[0238] In a related vein, a bispecific antibody can be conjugated to a contrast agent. For example, the bispecific antibody may comprise more than one image-enhancing agent for use in ultrasound imaging. In a preferred embodiment, the contrast agent is a liposome. Preferably, the liposome comprises a bivalent DTPA-peptide covalently attached to the outside surface of the liposome. Still preferred, the liposome is gas filled.

Pharmaceutically suitable excipient

[0239] Additional pharmaceutical methods may be employed to control the duration of action of a PAM4 antibody in a therapeutic application. Control release preparations can be prepared through the use of polymers to complex or adsorb the PAM4 antibody, fusion protein, and fragment thereof. For example, biocompatible polymers include matrices of poly(ethylene-co-vinyl acetate) and matrices of a polyanhydride copolymer of a stearic acid dimer and sebacic acid. Sherwood et al., Bio/Technology 10: 1446 (1992). The rate of release of a PAM4 antibody, fusion protein, and fragment thereof from such a matrix depends upon the molecular weight of the PAM4 antibody, fusion protein, and fragment thereof the amount of PAM4 antibody within the matrix, and the size of dispersed particles. Saltzman et al., Biophys. J. 55: 163 (1989); Sherwood *et al., supra.* Other solid dosage forms are described in Ansel et al., PHARMACEUTICAL DOSAGE FORMS AND DRUG DELIVERY SYSTEMS, 5th Edition (Lea & Febiger 1990), and Gennaro (ed.), REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Edition (Mack Publishing Company 1990), and revised editions thereof

[0240] The chimerized PAM4 antibodies and fragments thereof to be delivered to a subject can consist of the antibody, immunoconjugate, fusion protein, or fragments thereof alone, or can comprise one or more pharmaceutically suitable excipients, one or more additional ingredients, or some combination of these.

[0241] The immunoconjugate, naked antibody, and fragments thereof of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the immunoconjugate or naked antibody is combined in a mixture with a pharmaceutically suitable excipient. Sterile phosphate-buffered saline is one example of a pharmaceutically suitable excipient. Other suitable excipients are well-known to those in the art. See, for example, Ansel et al., PHARMACEUTICAL DOSAGE FORMS AND DRUG DELIVERY SYSTEMS, 5th Edition (Lea & Febiger 1990), and Gennaro (ed.), REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Edition (Mack Publishing Company 1990), and revised editions thereof.

[0242] The immunoconjugate or naked antibody of the present invention can be formulated for intravenous administration via, for example, bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, e.g., in ampules or in multidose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as sus-

pending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0243] The immunoconjugate, naked antibody, and fragments thereof may also be administered to a mammal subcutaneously or even by other parenteral routes. In a preferred embodiment, the PAM4 antibody or fragment thereof is administered in a dosage of 20 to 2000 milligrams protein per dose. Moreover, the administration may be by continuous infusion or by single or multiple boluses. In general, the dosage of an administered immunoconjugate, fusion protein or naked antibody for humans will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition and previous medical history. Typically, it is desirable to provide the recipient with a dosage of immunoconjugate, antibody fusion protein or naked antibody that is in the range of from about 1mg/kg to 20 mg/kg as a single intravenous infusion, although a lower or higher dosage also may be administered as circumstances dictate. This dosage may be repeated as needed, for example, once per week for four to ten weeks, preferably once per week for eight weeks, and more preferably, once per week for four weeks. It may also be given less frequently, such as every other week for several months. The dosage may be given through various parenteral routes, with appropriate adjustment of the dose and schedule.

[0244] The PAM4 antibodies, fusion proteins, and fragments thereof of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby PAM4 antibodies, fusion proteins and fragments thereof are combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. See, for example, REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Ed. (1990).

[0245] For purposes of therapy, the immunoconjugate, or naked antibody is administered to a mammal in a therapeutically effective amount. A suitable subject for the present invention are usually a human, although a non-human animal subject is also contemplated. An antibody preparation is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient mammal.

EXAMPLES

[0246] The examples below are illustrative of embodiments of the current invention and should not be used, in any way, to limit the scope of the claims.

[0247] The following examples discuss experimental studies employing PAM4 MAb and the CaPan1 human pancreatic cancer. The CaPan1 human pancreatic cancer is carried as a xenograft in both subcutaneous and orthotopic sites. The MAb and agent have resulted in significantly improved survival time. High concentrations of PAM4 monoclonal antibody are shown to target xenografted human tumor models to target the majority of pancreatic tumors within an initial group of patients. Employing an *in vitro* immunoassay to quantitate PAM4-reactive antigen in the blood of patients appears promising in its ability to discriminate pancreatic cancer from pancreatitis, as well as other disease and normal groups.

[0248] Clinical studies with PAM4 MAb have shown that a majority of the lesions were targeted in patients and that there is no indication of uptake in normal tissues. Dosimetry indicated that it was possible to deliver 10 to 20 cGy/mCi to tumors, with a tumor to red marrow dose ratio of 3:1 to 10:1. These data suggest that PAM4 may be useful for development of a phase-I trial for the treatment of pancreatic cancer.

Example 1 - Immunohistochemistry Staining Studies

[0249] Immunohistochemistry on normal adult tissues showed that the PAM4 reactive epitope was restricted to the gastrointestinal tract where staining was weak, yet definitely positive (Table 1). Normal pancreatic tissue, including ducts, ductules, acini, and islet cells, were negative for staining. A PAM4 based enzyme immunoassay with tissue homogenates as antigens generally supported the immunohistology data (Table 2). The PAM4 epitope was absent from normal pancreas and other nongastrointestinal tissues. In neoplastic tissues, PAM4 was reactive with twenty one out of twenty five (85%) pancreatic cancers (Table 3). PAM4 reactivity appeared to correlate with the stage of tumor differentiation. For example, twenty out of twenty one well and moderately differentiated pancreatic tumors were positive whereas only one out of four poorly differentiated tumors were positive. Generally, poorly differentiated tumors represent less than 10% of all pancreatic cancers.

[0250] These studies have shown the PAM4 reactivity and tissue distribution (both normal and cancer) to be unlike that reported for CA19.9, DUPAN2, SPAN1, Nd2, B72.3, and the Lewis antigens. Together with crossblocking studies performed with certain of these MAbs, the data suggests that the PAM4 MAb recognizes a unique and novel epitope. When compared to CA19.9, DUPAN2, and aLe^a, PAM4 appears to be more restricted in its tissue distribution and it is reactive with a higher percentage of pancreatic tumors. Moreover, it gives a greater overall intensity of reaction at equivalent concentrations and is reactive with a higher percentage of cells within the tumors. Finally, PAM4 was found

to be only weakly reactive with three out of twelve chronic pancreatitis specimens, whereas CA19.9 and DUPAN2 were strongly reactive with all twelve specimens. Although it is recognized that specificity is dependent upon the type of assay employed and the range and number of tissues examined, the ability of PAM4 to discriminate between normal and neoplastic pancreatic tissue, its ability to react with a large percentage of the cancer specimens, as well as the high intensity of the reactions, were important rationales for pursuing developmental studies of clinical application.

Table 1 - Immunoperoxidase Staining of Normal Adult Tis

Tissue	Staining
	Reaction
Pancreas (22)[a]	
Ducts	-
Acini	-
Islets	-
Submaxillary gland (2)	-
Esophagus (2)	-
Stomach (3)	+ mucus secreting cells
Duodenum (3)	+ goblet cells
Jejunum (3)	+ goblet cells
Ileum (3)	+ goblet cells
Colon (5)	+ goblet cells
Liver (3)	-
Gallbladder (2)	-
Bronchus (3)	-
Lung (3)	-
Heart (3)	-
Spleen (3)	-
Kidney (3)	-
Bladder (3)	-
Prostate (2)	-
Testes (2)	-
Uterus (2)	-
Ovary (2)	-
a - ( ) number of individual specimens examined.	

Table 2 - Monoclonal Antibody PAM4 Reactivity with Normal Adult Tissue Homogenates by EIA

Tissue	ug/g tissue[a]
Pancreas	6.4
Esophagus	8.1
Stomach	61.3
Duodenum	44.7
Jejunum	60.6

(continued)

Tissue	ug/g tissue[a]
Colon	74.5
Liver	0.0
Gallbladder	5.6
Heart	3.7
Spleen	3.4
Kidney	6.6
Bladder	4.9
Thyroid	3.5
Adrenal	13
Ureter	2.6
Testes	39
CaPan1 Pancreatic Tumor	569
a - values are mean from two autopsy specimens	

Table 3 - Immunohistochemical Reactivity of Several Monoclonal Antibodies with Pancreatic Tumors

	Differentiation	PAM4	CA19.9	aLe[a]	DUPAN2
1	W	+++	-	-	+++
2	M	++	+++	+++	+
3	M	+	-	+	+
4	M	+++	+++	+++	+
5	M	++	+	-	-
6	M	+	ND	ND	ND
7	M	+++	+++	+++	+++
8	M	+	-	-	+++
9	M	++	+	++	-
10	M	++	++	++	+++
11	M	++	+++	+++	+
12	M	++	+	+	+++
13	M	+	+++	+++	+
14	M	++	+	+	++
15	M	+++	+	+	++
16	M	+	+	++	-
17	M	-	+	+	-
18	M	++	++	++	++
19	M	+++	+	+++	++
20	M	+	-	-	-
21	M	+++	+++	+	++

(continued)

	Differentiation	PAM4	CA19.9	aLe[a]	DUPAN2
22	P	+	+	+	+++
23	P	-	-	-	-
24	P	-	-	-	-
25	P	-	-	+	-
TOTAL		21/25	17/24	18/24	16/24
- : Negative; + : 5-20% of tissue is stained; ++ : 21-50% of tissue is stained; +++ : >50% of tissue is stained; W,M,P : Well, moderate, or poor differentiation; * :Metastatic tissue; ND : Not Done					

Table 4 - Immunoperoxidase Staining of Neoplastic Tissues with MAb PAM4

Tissue	Positive/Total
Pancreas	21/25
Colon	10/26
Stomach	1/5
Lung	1/15
Breast	0/30
Ovarian	0/10
Prostate	0/4
Liver	0/10
Kidney	0/4

Example 2 - *In Vivo* Biodistribution and Tumor Targeting of Radiolabeled PAM4

[0251] Initial biodistribution studies of PAM4 were carried out in a series of four different xenografted human pancreatic tumors covering the range of expected differentiation. Each of the four tumor lines employed, AsPcl, BxPc3, Hs766T and CaPanl, exhibited concentrations of ^{131}I-PAM4 within the tumors (range: 21% - 48% ID/g on day three) that was significantly ($p<0.01$-0.001) higher than concomitantly administered nonspecific, isotype-matched Ag8 antibody (range: 3.6% - 9.3% ID/g on day three). The biodistribution data were used to estimate potential radiation doses to the tumor of 12,230; 10,684; 6,835; and 15,843 cGy/mCi of injected dose to AsPcl, BxPc3, Hs766T and CaPan1, respectively. With an actual maximum tolerated dose (MTD) of 0.7mCi, PAM4 could provide substantial rad dose to each of the xenografted tumor models. In each tumor line the blood levels of radiolabeled PAM4 were significantly ($p<0.01$-0.001) lower than the nonspecific Ag8. Potential radiation doses to the blood from PAM4 were 1.4 - 4.4 fold lower than from Ag8. When radiation doses to the tumor from PAM4 were normalized to the blood doses from PAM4, the tumors received doses that were 2.2; 3.3; 3.4; and 13.1-fold higher than blood, respectively. Importantly, potential radiation doses to non-tumor tissues were minimal.

[0252] The biodistribution of PAM4 was compared with an anti-CEA antibody, MN14, using the CaPan1 tumor model. The concentration of PAM4 within the tumor was much greater than the MN14 at early timepoints, yielding tumor:blood ratios at day three of 12.7 $\pm$ 2.3 for PAM4 compared to 2.7 $\pm$ 1.9 for MN14. Although PAM4 uptake within the tumor was significantly higher than for MN14 at early timepoints (day one - $p<0.001$; day three - $p<0.01$), dosimetry analyses indicated only a 32-fold higher dose to the tumor from PAM4 as compared to MN14 over the fourteen day study period. This was due to a rapid clearance of PAM4 from the tumor, such that at later timepoints similar concentrations of the two antibodies were present within the tumors. A rapid clearance of PAM4 from the tumor was also noted in the BxPc3 and Hs766T but not AsPc1 tumor models. These observations were unlike those reported for other anti-mucin antibodies, as for example G9 and B72.3 in colorectal cancer, where each exhibited longer retention times as compared to the MN14 antibody. Results from studies on the metabolism of PAM4, indicate that after initial binding to the tumor cell, antibody is rapidly released, possibly being catabolized or being shed as an antigen:antibody complex. This might have had unfavorable implications for the use of the antibody in patients except that the blood clearance is also very rapid.

These data suggest that ^{131}I may not be the appropriate choice of isotope for therapeutic applications. A short-lived isotope, such as ^{90}Y or ^{188}Re, that can be administered frequently may prove to be a more effective reagent.

[0253] PAM4 showed no evidence of targeting to normal tissues, except in the CaPan1 tumor model, where a small but statistically significant splenic uptake was observed (range 3.1-7.5 %ID/g on day three). This type of splenic targeting has been observed in the clinical application of the anti-mucin antibodies B72.3 and CC49. Importantly, these studies also reported that splenic targeting did not affect tumor uptake of antibody nor did it interfere with interpretation of the nuclear scans. These studies suggested that splenic targeting was not due to crossreactive antigens in the spleen, nor to binding by F_c receptors, but rather to one or more of the following possibilities: direct targeting of antigen trapped in the spleen, or indirect uptake of antigen:antibody complexes formed either in the blood or released from the tumor site. The latter would require the presence of immune complexes in the blood; however, these were not observed when specimens as early as five minutes and as late as seven days were examined by gel filtration (HPLC, GF-250 column); radiolabeled antibody eluted as native material. The former explanation seems more likely in view of the fact that the CaPan1 tumor produced large quantities of PAM4-reactive antigen, 100 to 1000 - fold higher than for the other tumor cell lines examined. The lack of splenic targeting by PAM4 in these other tumor lines suggests that this phenomenon was related to excessive antigen production. In any event, splenic targeting can be overcome by increasing the protein dose to 10 ug from the original 2 ug dose. A greater amount of the splenic entrapped antigen presumably was complexed with unlabeled PAM4 rather than radiolabeled antibody. Increasing the protein dose had no adverse effect upon targeting of PAM4 to the tumor or nontumor tissues. In fact, an increase of the protein dose to 100 ug more than doubled the concentration of radiolabeled PAM4 within the CaPan1 tumor.

Example 3 - Development of Orthotopic Pancreatic Tumor Model in Athymic Nude Mice

[0254] In order to resemble the clinical presentation of pancreatic cancer in an animal model more closely, applicants developed an orthotopic model by injecting of tumor cells directly into the head of the pancreas. Orthotopic CaPan1 tumors grew progressively without overt symptoms until the development of ascites and death at ten to fourteen weeks. By three to four weeks post-implantation, animals developed a palpable tumor of approximately 0.2g. Within eight weeks of growth, primary tumors of approximately 1.2g along with metastases to the liver and spleen were observed (1-3 metastatic tumors/animal; each tumor <0.1g). At ten to fourteen weeks seeding of the diaphragm with development of ascites were evident. Ascites formation, and occasional jaundice, were usually the first overt indications of tumor growth. Ascites is an accumulation of fluid in the abdominal cavity and jaundice is a yellowing of the skin and eyes due to excessive bile pigments in the blood. At this time tumors were quite large, 1 to 2 g, and animals had at most only three to four weeks until death occurred.

[0255] Radiolabeled ^{131}I-PAM4, administered to animals bearing four week old orthotopic tumors (approximately 0.2g) showed specific targeting to the primary tumor with localization indices of 7.9 ± 3.0 at day one increasing to 22.8 ± 15.3 at day fourteen. No evidence of specific targeting to other tissues was noted. In one case where tumor metastases to the liver and spleen were observed, both metastases were targeted, and had high concentrations of radiolabeled antibody. In addition, approximately half of the animals developed a subcutaneous tumor at the incision site. No significant differences were noted in the targeting of orthotopic and subcutaneous tumors within the same animal, and no significant differences were observed in the targeting of orthotopic tumor whether or not the animal had an additional subcutaneous tumor. The estimated radiation doses from PAM4 were 6,704 and 1,655 cGy/mCi to the primary tumor and blood, respectively.

Example 4 - Development of an Enzyme Immunoassay for Quantification of Circulating Tumor Antigen

[0256] An enzyme immunoassay was developed employing PAM4 as the capture reagent with an unlabeled, purified IgG derived from rabbit polyclonal, anti-pancreatic mucin, followed by peroxidase labeled donkey anti-rabbit IgG as the detection reagent. The following results were obtained through use of this assay.

[0257] Within the range of antigen detected by the assay, coefficient of variation values were obtained of less than 10%. Sera from twenty five healthy individuals were examined and exhibited a mean $\pm$ S.D. of 4.0 ± 3.1 units. A cutoff value for positive response was then set to the mean + 2 S.D. = 10.2 units. Out of a total of thirty seven pancreatic cancer patients, thirty two or 86% were positive by this assay, whereas only three out of thirteen pancreatitis patients were positive. PAM4 antigen was elevated in 55% (18/33) of colorectal cancer patients, a number roughly similar to the 40% of colorectal cancer specimens reactive with PAM4 by immunohistochemistry. Amongst other cancers, PAM4 antigen was positive in four out of sixteen ovarian cancer, and five out of twenty breast cancer patients, all of whom had extensive disease. Also, as can be seen in Table 5 below the median value for pancreatic cancer (84.5 units) is on the order of ten fold greater than for all of the other cancer groups (except biliary cancer) even though the overwhelming majority of these cases were late stage, large tumor burden.

Table 5 - PAM4 Reactivity with Sera

		Units/ml				
	n	Mean	SD	Median	Range	% Positive[a]
Normal	25	4.0	3.1	4.7	0.0 - 9.4.	0%
Pancreatitis	13	14.6	20.3	6.8	0.4 - 66.7	23%
Pancreatic CA	37	317.5	427.1	84.5	0.9 - 1000	86%
Biliary CA	8	155.4	343.8	37.8	6.6 - 1000	63%
Hepatoma CA	30	7.9	8.0	6.4	0.0 - 32.8	30%
Colorectal CA	33	50.0	171.6	11.8	3.4 - 1000	55%
Lung 2A	38	25.8	44.6	9.3	0.0 - 196.0	39%
Breast CA	20	11.1	18.5	5.8	0.0 - 83.3	25%
Ovarian CA	16	68.9	248.4	5.5	0.0 - 1000	25%
Non-Hodgkin's Lymphoma	14	6.6	3.1	7.5	2.2 - 12.8	14%
[a] Cutoff 10.2 units/ml (mean + 2 S.D.)						

[0258] In addition to these findings, a preliminary study was performed in the orthotopic model to examine the potential use of this PAM4 assay in management. At two weeks post-implantation of orthotopic CaPan1 tumor (estimated tumor mass of 0.15g), none of the animals had detectable antigen in the blood. At four weeks (estimated tumor mass of 0.2g) one out of five animals had a detectable level of antigen, (72 units), and at six weeks (estimated tumor volume of 0.4g) four out of five had quantifiable antigen (range: 98 - 6080 units). A severe limiting factor in terms of determining the earliest time point at which serum borne antigen could be detected was the limited amount of blood obtainable, such that repeated bleedings could be performed. Thus sera were diluted 1:10 prior to assay.

Example 5 - Experimental Radioimmunotherapy of Pancreatic Cancer

[0259] The initial studies on the use of ^{131}I-PAM4 for therapy were carried out with the CaPan1 tumor, which was grown as a subcutaneous xenograft in athymic mice. Animals bearing a 0.25g tumor were administered 350μCi, ^{131}I-PAM4 in an experiment that also compared the therapeutic effects of a similar dose of nonspecific Ag8. The MTD for administration of ^{131}I-PAM4 to animals bearing 1cm^3 tumors is 700μCi. By weeks five and six, the PAM4 treated animals showed a dramatic regression of tumor, and even at week twenty seven, five out of eight remained tumor free. The untreated, as well as Ag8-treated animals, showed rapid progression of tumor growth although a significant difference was noted between these two control groups. At seven weeks, tumors from the untreated group had grown 20.0 $\pm$ 14.6-fold from the initial timepoint whereas the ^{131}I-Ag8-treated tumors had grown only 4.9 $\pm$ 1.8-fold. At this time point, the PAM4 tumors had regressed to 0.1 $\pm$ 0.1-fold of their original size, a significant difference from both untreated ($p<0.001$) and nonspecific Ag8-treated ($p<0.01$) animals.

[0260] Although the CaPan1 tumors were sensitive to treatment with ^{131}I-PAM4, the outcome, that is, regression or progression of the tumor, is dependent upon many factors including initial tumor size. Thus, groups of animals bearing CaPan1 tumor burdens of 0.25g, 0.5g, 1.0g, or 2.0g were treated with a single dose of the 350μCi ^{131}I-PAM4. The majority of animals having tumors of initial size 0.25g and 0.5g (nine of ten animals in each group) showed tumor regression or growth inhibition for at least sixteen weeks post treatment. In the 1.0g tumor group five out of seven showed no tumor growth for the sixteen week period and in the 2.0g tumor group six out of nine showed no tumor growth for a period of six weeks before progression occurred. Although a single 350μCi dose was not as effective against the larger tumors, a single dose may very well not be the appropriate regimen; toxicity studies indicating the ability to give multiple cycles of radioimunotherapy. Animals bearing CaPan1 tumors averaging 1.0g, were given either a single dose of 350μCi ^{131}I-PAM4, two doses given at times zero and four weeks or were left untreated. The untreated group had a mean survival time of 3.7 +/- 1.0 weeks (survival defined as time for tumor to reach 5 cm^3). Animals died as early as three weeks, with no animal surviving past six weeks. A single dose of 350μCi ^{131}I-PAM4 produced a significant increase in the survival time to 18.8 +/- 4.2 weeks ($p<0.0001$). The range of animal deaths extended from weeks thirteen to twenty five. None of the animals were alive at the end of the study period of twenty six weeks.

[0261] A significant increase in survival time was observed for the two dose group as compared to the single dose

group. Half of the animals were alive at the twenty six week timepoint with tumor sizes from 1.0 - 2.8 cm^3, and a mean tumor growth rate of 1.6 +/- 0.7 fold from initial tumor size. For those animals that were non-survivors at twenty six weeks, the mean survival time (17.7 +/- 5.3 weeks) was similar to the single dose group.

[0262] Therapy studies with PAM4 have also used the orthotopic tumor model. Groups of animals bearing four week old orthotopic tumors (estimated tumor weight of 0.25g) were either left untreated or treated with a single dose of either 350 uCi ^{131}I-PAM4 or 350uCi of ^{131}I-nonspecific Ag8. The untreated animals had a 50% death rate by week ten with no survivors at week fifteen. Animals administered nonspecific ^{131}I-Ag8 at four weeks of tumor growth, showed a 50% death rate at week seven with no survivors at week fourteen. Although statistically (logrank analysis) there were no differences between these two groups, it is possible that radiation toxicity had occurred in about half of the Ag8 treated animals. Radiolabeled PAM4, however, provided a significant survival advantage ($p<0.001$) as compared to the untreated or Ag8 treated animals, with 70% survival at sixteen weeks, the end of the experiment. At this time the surviving animals were sacrificed to determine tumor size. All animals had tumor with an average weight of 1.2g, as well as one or two small (<0.1g) metastases evident in four of the seven animals. At sixteen weeks of growth, these tumors were more representative of an eight week old tumor.

Example 6 - Combined Modality Gemzar Chemotherapy and ^{131}I-PAM4 Radioimmunotherapy

[0263] Initial studies into the combined use of gemcitabine (gemzar) with ^{131}I-PAM4 radioimmunotherapy were performed as a checkerboard array; a single dose of Gemzar (0, 100, 200, 500 mg/kg) versus a single dose of ^{131}I-PAM4 ([MTD = 700μCi] 100%, 75%, 50%, 0% of the MTD). The combined MTD was found to be 500 mg/kg Gemzar with 350μCi ^{131}I-PAM4 (50% MTD). Toxicity, as measured by loss of body weight, went to the maximum considered as nontoxic; that is 20% loss in body weight. Although the combined treatment protocol was significantly more effective than gemzar alone, the treatment was no more effective than radioimmunotherapy alone. The next studies were performed at a low dose of gemzar and radioimmunotherapy to examine if a true synergistic therapeutic effect would be observed. Animals bearing tumors of approximately 1 cm^3 (approximately 5% of body weight) were administered gemzar, 100 mg/kg on days zero, three, six, nine, and twelve, with 100 μCi of ^{131}I-PAM4 given on day zero. A therapeutic effect was observed with statistically significant ($p<0.0001$) regression (two of five tumors less than 0.1 cm^3) and/or growth inhibition of the tumors compared to gemzar alone. Of additional note, in terms of body weight, toxicity was not observed. The combination treatment protocol can, if necessary, be delivered in multiple cycles, with the second treatment cycle beginning in week four as was done with the radioimmunotherapy alone studies described above.

Example 7- Therapy of a Patient with Inoperable Pancreatic Carcinoma

[0264] A 56-year-old male with extensive, inoperable adenocarcinoma of the pancreas, substantial weight loss (30 lbs of weight or more), lethargy and weakness is given ^{90}Y-PAM4 radiolabeled chimerized antibody at a dose of 30 mCi of 90-Y and 50 mg antibody protein, in a two hour i.v. infusion. Five days later, the patient is then given a standard course of gemcitabine chemotherapy. If no evidence after a few months of side effects from therapy, the therapy regimen is repeated. During a follow-up examination a few weeks later, it is predicted that the patient will appear more active and the weight loss will slow. The CT scan of the pancreas is expected to suggest either stable disease or a slight reduction of tumor mass. A repeat examination a few months later should show, by computed tomography, a substantial reduction of tumor mass, and the patient may therefore be considered for resection of the pancreatic tumor mass.

Example8. Pretargeting with Bispecific PAM4 x 734 and ^{99m}Tc-or ^{111}In-Labeled Peptide Haptens

[0265] For imaging of pancreatic cancer using a pretargeted approach we prepared a bispecific $F(ab')_2$ antibody (bsMAb) consisting of a chimeric PAM4 (cPAM4) Fab' and a murine 734 (m734) Fab'. The m734 antibody recognizes an In-DTPA complex. This bsMAb was labeled with ^{125}I and injected (7μCi; 15 μg) into athymic nude mice bearing a human pancreatic cancer xenograft (CaPan1). A non-targeting $F(ab')_2$ bsMAb made from chimeric rituximab (anti-CD20 monoclonal antibody) and m734, was labeled with ^{131}I and co-injected as a control. At various time-points (4, 24, 36, 48, and 72-hours post-injection) mice were necropsied, the tissues removed and counted to determine percent-injected dose per gram (%ID/g). There was significantly greater tumor uptake of bsPAM4 at each time-point in comparison to the control bsRituximab ($p<0.032$ or better). Our past experience with this type of pre-targeting system suggested that a blood level of less than 1% ID/g was necessary to obtain good tumor:non-tumor ratios. At 36-hours post-administration of the bsPAM4 there was 1.10 ± 0.40% ID/g in the blood which fell to 0.56 ± 0.08% ID/g at 48 hours post-injection. Tumor uptake at these two time-points was 6.43 ± 1.50% ID/g and 5.37 ± 2.38% ID/g, respectively. These values were significantly higher than the control bsRituximab which had 0.65 ± 0.33% ID/g and 0.47 ± 0.19% ID/g in the tumor at 36 and 48 hours, respectively ($p<0.018$ and $p<0.0098$). Blood clearance rates, however, were very similar and were not significantly different.

[0266] Based on these data, a pre-targeting experiment was carried out in CaPan1 tumor-bearing mice in which radiolabeled peptide-haptens were injected 40-hours post-bsMAb administration. Two peptides, IMP-192 and IMP-156, were used, each containing divalent DTPA for recognition by the 734 MAb, but one has an additional group specific for binding ^{99m}Tc stably (IMP-192). Tumor-bearing mice (tumor volume -0.30 cm^3) were administered ^{125}I-bsPAM4 (6 μCi; 15 μg) followed 40 hours later by a radiolabeled peptide-hapten (34.5 μCi; $1.5x10^{-11}$ moles; bsMAb:peptide = 10:1). One group of mice received ^{99m}Tc-labeled IMP192 while a second group of mice received "'In-labeled IMP156. Controls for nonspecific targeting included two groups that received ^{125}I-bsRituximab prior to administration of radiolabeled peptide and two other groups that received ^{111}In- or ^{99m}Tc-labeled peptide alone.

[0267] Mice were sacrificed at 3 and 24 hours after the administration of peptides and the %ID/g determined for the tumor and various tissues. Consistent with our previous findings, there was significantly greater bsPAM4 in the tumors in comparison to the non-targeting control bsRituximab, 8.2 ± 3.4% and 0.3 ± 0.08% ID/g, respectively (p<0.0001). This translated into a significantly greatly tumor uptake of ^{111}In-IMP156 (20.2 ± 5.5% ID/g *vs.* 0.9 ± 0.1% ID/g, p<0.0001). There was also significantly greater tumor uptake or ^{99m}Tc-IMP192 in the mice pre-targeted with bsPAM4 than in those pre-targeted with bsRituximab (16.8 ± 4.8% ID/g *vs.* 1.1 ± 0.2% ID/g, p<0.0005). Tumor uptake of each peptide, when administered alone, was significantly less than in those mice that received the bsPAM4 (0.2 ± 0.05% ID/g and 0.1 ± 0.03% ID/g for ^{99m}Tc-IMP192 and ^{111}In-IMP156, p<0.0004 and p<0.0001, respectively).

[0268] As with the 3-hour time-point, there was significantly more bsPAM4 in the tumors at 24 hours post-injection of peptide (64 hours post bsMAb administration) than bsRituximab (6.4 ± 2.2% ID/g *vs.* 0.2 ± 0.09% ID/g, respectively; p<0.0001). At this time-point there was 11.1 ± 3.5% ID/g ^{111}In-IMP156 and 12.9 ± 4.2% ID/g ^{99m}Tc-IMP192 in the tumors of mice pre-targeted with bsPAM4 *versus* 0.5 ± 0.2% ID/g and 0.4 ± 0.03% ID/g in bsRIT pre-targeted tumors (p<0.0008 and p<0.0002, respectively). In the mice that received peptide alone, there was significantly less ^{99m}Tc-IMP192 in the tumors (0.06 ± 0.02% ID/g, p<0.0007) and ^{111}In-IMP156 (0.09 ± 0.02% ID/g, p<0.0002) in comparison to the bsPAM4 pre-targeted peptides.

Table 6. Tumor: Non-Tumor Tissue Ratios at Early Time-Points.

	Pre-targeted ^{111}In-Peptide (3-Hours)		Pre-targeted ^{99m}Tc-Peptide (3-Hours)		^{125}I-bsPAM4 F(ab')$_2$ (4-Hours)	
Tissue	Mean	(±STD)	Mean	(±STD)	Mean	(±STD)
Tumor	1.00	0.00	1.00	0.00	1.00	0.00
Liver	36.07	11.74	16.66	7.19	2.34	0.61
Spleen	33.40	20.62	14.62	9.12	2.15	0.74
Kidney	7.79	2.81	8.13	3.33	1.10	0.20
Lung	44.55	12.99	15.75	5.85	1.58	037
Blood	36.47	8.28	9.93	5.21	0.47	0.11
Bone	123.24	40.00	--	--	--	--
W. Bone	378.00	124.57	--	--	--	--
Pancreas Tumor	155.55	30.07	73.29	32.85	4.65	1.23
Wt. (g) (±STD)	*0.189*	(*0.070*)	*0.174*	(*0.050*)	*0.179*	*(0.139)*

[0269] The table above presents the tumor:non-tumor ratios (T:NT) of various tissues for these groups, each at an early time-point post-administration of radiolabeled product. It is important to note that at 4-hours post-administration of bsPAM4 x m734 F(ab')$_2$, the tumor:blood ratio was less than 2:1. However, at 3-hours post-administration, the pre-targeted ^{111}In-IMP156 and ^{99m}Tc-IMP192 had significantly greater tumor: nontumor ratios for all tissues examined and in particular tumor:blood ratios were equal to 36:1 and 9:1, (p<0.001 and p<0.011, respectively). When we examined tumor:blood ratios at the 24-hour time-point, the pre-targeted ^{111}In-IMP156 and ^{99m}Tc-IMP192 had significantly higher values, 274:1 and 80:1, respectively, *versus* 4:1 for ^{125}I-bsPAM4 alone (p<0.0002). These data strongly suggest the ability to utilize this pretargeted bsPAM4 approach with short half-life, high energy radioisotopes that would then deliver high radiation dose to tumor with minimal radiation dose to non-tumor tissues.

Claims

1. A chimeric PAM4 antibody or fragment thereof, wherein said antibody or fragment thereof comprises the PAM4 V_k polypeptide sequence of Fig. 2A or Fig. 2C, and the PAM4 V_h polypeptide sequence of Fig. 2B or Fig. 2D.

2. A chimeric antibody or fragment thereof, comprising the complementarity-determining regions (CDRs) and framework regions (FR) of a murine PAM4 monoclonal antibody (MAb) and the light and heavy chain constant regions of a human antibody, wherein the CDRs of the light chain variable region of the chimeric PAM4 MAb comprise CDR1 comprising an amino acid sequence of SASSSVSSSYLY; CDR2 comprising an amino acid sequence of STSNLAS; and CDR3 comprising an amino acid sequence of HQWNRYPYT; and the CDRs of the heavy chain variable region of the chimeric PAM4 MAb comprises CDR1 comprising an amino acid sequence of SYVLH; CDR2 comprising an amino acid sequence of YINPYNDGTQYNEKFKG and CDR3 comprising an amino acid sequence of GFGGSYG-FAY.

3. A cancer cell targeting diagnostic or therapeutic conjugate comprising an antibody component that comprises an antibody or fragment thereof of claim 2 that binds to said cell, wherein said antibody component is bound to at least one diagnostic/detection and/or at least one therapeutic agent.

4. The diagnostic conjugate of claim 3, wherein said diagnostic/detection agent is selected from the group comprising a radionuclide, a contrast agent, and a photoactive diagnostic/detection agent.

5. The diagnostic conjugate of claim 4, wherein said diagnostic agent is a radionuclide.

6. The diagnostic conjugate of claim 5, wherein said radionuclide has an energy between 20 and 4,000 keV.

7. The diagnostic conjugate of claim 6, wherein said radionuclide is a gamma-, beta- or a positron-emitting isotope.

8. The diagnostic conjugate of claim 7, wherein said radionuclide is selected from the group consisting of ^{110}In, ^{111}In, ^{177}Lu, ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga , ^{68}Ga, ^{86}Y, ^{90}Y, ^{89}Zr, ^{94m}Tc, ^{94}Tc, ^{99m}Tc ^{120}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154\text{-}158}$Gd, ^{32}P, ^{11}C, ^{13}N, ^{15}O, ^{186}Re, ^{188}Re, ^{51}Mn, ^{52m}Mn, ^{55}Co, ^{72}As, ^{75}Br, ^{76}Br, ^{82m}Rb, ^{83}Sr, and other gamma-, beta-, or positron-emitters.

9. The diagnostic conjugate of claim 4, wherein said diagnostic/detection agent is a contrast agent.

10. The diagnostic conjugate of claim 9 wherein said contrast agent is a paramagnetic ion.

11. The diagnostic conjugate of claim 10, wherein said paramagnetic ion is a metal comprising chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) or erbium (III).

12. The diagnostic conjugate of claim 9, wherein said contrast agent is a metal comprising lanthanum (III), gold (III), lead (II) or bismuth (III).

13. The diagnostic conjugate of claim 9, wherein said contrast agent is an ultrasound enhancing agent.

14. The diagnostic conjugate of claim 13, wherein said ultrasound enhancing agent is a liposome that comprises the chimeric antibody or fragment according to claim 2.

15. The diagnostic conjugate of claim 14, wherein said liposome is gas filled.

16. The diagnostic conjugate of claim 9, wherein said contrast agent is a radiopaque material selected from the group comprising iodine compounds, barium compounds, gallium compounds, and thallium compounds.

17. The diagnostic conjugate of claim 16, wherein said radiopaque material is selected from the group comprising barium, diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide, iodipamide, iodoxamic acid, iogulamide, iohexol, iopamidol, iopanoic acid, ioprocemic acid, iosefamic acid, ioseric acid, iosulamide meglumine, iosemetic acid, iotasul, iotetric acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizoic acid, ipodate, meglumine, metrizamide, metrizoate, propyliodone, and thallous chloride.

18. The diagnotic conjugate of claim 4, wherein said diagnostic/detection agent is a photactive diagnostic/detection agent.

19. The diagnostic conjugate of claim 18, wherein said photoactive diagnostic/detection agent is a fluorescent labeling compound selected from the group comprising fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin,

allophycocyanin, o-phthaldehyde and fluorescamine.

20. The diagnostic conjugate of claim 18, wherein said photoactive diagnostic/detection agent is a chemiluminescent labeling compound selected from the group comprising luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester.

21. The diagnostic conjugate of claim 18, wherein said photoactive diagnostic/detection agent is a bioluminescent compound selected from the group comprising luciferin, luciferase and aequorin.

22. The diagnostic conjugate according to any of claims 4 to 21, wherein said conjugate is for use in intraoperative, endoscopic, or intravascular tumor diagnosis.

23. The therapeutic conjugate of claim 3, wherein said therapeutic agent is selected from the group consisting of a radionuclide, an immunomodulator, a hormone, a hormone antagonist, an enzyme, an enzyme inhibitor, an oligonucleotide, a photoactive therapeutic agent, a cytotoxic agent, an antibody, an angiogenesis inhibitor, and a combination thereof.

24. The therapeutic conjugate of claim 23, wherein said oligonucleotide is an antisense oligonucleotide.

25. The therapeutic conjugate of claim 24, wherein said oligonucleotide is an antisense oligonucleotide against an oncogene.

26. The therapeutic conjugate of claim 25, wherein said oncogene is bcl-2 or p53.

27. The therapeutic conjugate of claim 23, wherein said therapeutic agent is a cytotoxic agent.

28. The therapeutic conjugate of claim 27, wherein said cytotoxic agent is a drug or a toxin.

29. The therapeutic conjugate of claim 28, wherein said drug possesses the pharmaceutical property selected from the group consisting of antimitotic, alkylating, antimetabolite, antiangiogenic, apoptotic, alkaloid, and antibiotic agents and combinations thereof.

30. The therapeutic conjugate of claim 28, wherein said drug is selected from the group consisting of nitrogen mustards, gemcitabine, ethylenimine derivatives, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, anthracyclines, SN-38, taxanes, COX-2 inhibitors, pyrimidine analogs, purine analogs, antibiotics, enzymes, enzyme inhibitors, epipodophyllotoxins, platinum coordination complexes, vinca alkaloids, substituted ureas, methyl hydrazine derivatives, adrenocortical suppressants, hormone antagonists, endostatin, taxols, camptothecins, doxorubicins and their analogs, antimetabolites, alkylating agents, antimitotics, antiangiogenic, apoptotoic agents, methotrexate, CPT-11, and a combination thereof.

31. The therapeutic conjugate of claim 28, wherein said toxin derived from a source selected from the group comprising an animal, a plant, and a microbial source.

32. The therapeutic conjugate of claim 28, wherein said toxin is selected from the group consisting of ricin, abrin, alpha toxin, saporin, ribonuclease (RNase), DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtherin toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin.

33. The therapeutic conjugate of claim 23, wherein said therapeutic agent is an immunomodulator.

34. The therapeutic conjugate of claim 33, wherein said immunomodulator is selected from the group consisting of a cytokine, a stem cell growth factor, a lymphotoxin, a hematopoietic factor, a colony stimulating factor (CSF), an interferon (IFN), erythropoietin, thrombopoietin and a combination thereof.

35. The therapeutic conjugate of claim 34, wherein said lymphotoxin is tumor necrosis factor (TNF), said hematopoietic factor is an interleukin (IL), said colony stimulating factor is granulocyte-colony stimulating factor (G-CSF) or granulocyte macrophage-colony stimulating factor (GM-CSF)), said interferon is interferons-α, -β or - y, and said stem cell growth factor is designated "S1 factor".

36. The therapeutic conjugate of claim 33, wherein said immunomodulator comprises IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, IL-21, interferon- y, TNF- a or a combination thereof.

37. The therapeutic conjugate of claim 23, wherein said therapeutic agent is a radionuclide.

38. The therapeutic conjugate of claim 37 wherein said radionuclide has an energy between 60 and 700 keV.

39. The therapeutic conjugate of claim 38, wherein said radionuclide is selected from the group consisting of ^{32}P, ^{33}P, ^{47}Sc, , ^{64}Cu , ^{67}Cu, ^{67}Ga, ^{86}Y, ^{90}Y, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{211}At, ^{223}Ra and ^{225}Ac, and combinations thereof.

40. The therapeutic conjugate of claim 23, wherein said therapeutic agent is a photoactive therapeutic agent.

41. The therapeutic conjugate of claim 40, wherein said photoactive therapeutic agent is selected from the group comprising chromogens and dyes.

42. The therapeutic conjugate of claim 27, wherein said therapeutic agent is an enzyme.

43. The therapeutic conjugate of claim 42, wherein said enzyme is selected from the group comprising malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, α-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, ß-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

44. A multivalent, multispecific antibody or fragment thereof comprising one or more antigen binding sites having affinity towards the PAM4 target epitope recognized by PAM4 antibody, wherein the CDRs of the light chain variable region of the PAM4 antibody comprise CDR1 comprising an amino acid sequence of SASSSVSSSYLY; CDR2 comprising an amino acid sequence of STSNLAS; and CDR3 comprising an amino acid sequence of HQWNRYPYT; and the CDRs of the heavy chain variable region of the PAM4 MAb comprises CDR1 comprising an amino acid sequence of SYVLH; CDR2 comprising an amino acid sequence of YINPYNDGTQYNEKFKG and CDR3 comprising an amino acid sequence of GFGGSYGFAY, preferably the antibody according to claim 2, or fragment thereof, and one or more hapten binding sites having affinity towards hapten molecules.

45. The antibody or fragment thereof of claim 44, further comprising a diagnostic or therapeutic agent.

46. An antibody fusion protein or fragment thereof comprising at least two chimeric PAM4 MAbs or fragments thereof of claim 1 or 2.

47. The multivalent, multispecific antibody or fragment thereof according to claim 44 for use in a method for diagnosing or treating cancer comprising: (a) administering to a subject in need thereof the antibody or fragment thereof of the multivalent, multispecific antibody or fragment thereof; (b) waiting a sufficient amount of time for an amount of the non- antibody to clear the subject's blood stream; and (c) administering to said subject a carrier molecule comprising a diagnostic/detection agent, a therapeutic agent, or a combination thereof, that binds to a binding site of the antibody.

48. The multivalent, multispecific antibody or fragment thereof for use as in claim 47, wherein said method can be used for intraoperative identification of diseased tissues, endoscopic identification of diseased tissues, or intravascular identification of diseased tissues.

49. The chimeric antibody or fragment thereof according to claim 2 or an antibody fusion protein and fragment thereof comprising PAM4 antibody, wherein said PAM4 antibody is the antibody according to claim 2, and wherein said antibody or fragment thereof or antibody fusion protein of fragment thereof is bound to at least one therapeutic agent, for use in the treatment of a malignancy in a subject wherein said antibody or fragment is optionally formulated in a pharmaceutically suitable excipient.

50. The chimeric antibody or fragment thereof and the antibody fusion protein or fragment thereof for use as in claim 49, further comprising a second MAb or fragment thereof that is not a PAM4 MAb or fragment thereof.

51. The chimeric antibody or fragment thereof and the antibody fusion protein or fragment thereof for use as in claim

50, wherein said second MAb or fragment thereof is a naked MAb or fragment thereof.

52. The chimeric antibody or fragment thereof and the antibody fusion protein or fragment thereof for use as in claim 51, wherein said second MAb or fragment thereof is selected from the group consisting of CAI9.9, SUPAN2, SPAN1, Nd2, B72.3, CC49, CEA, aLe^a, antibodies defined by the Lewis antigen Le(y), CSAp, MUC-2, MUC-3, MUC-4, TAG-72, EGFR, CD40, angiogenesis factors (e.g. VEGF), products of oncogenes, insulin like growth factor (IGF), tenascin, platelet derived growth factor, IL-6, and HER2/neu.

53. The chimeric antibody or fragment thereof and the antibody fusion protein or fragment thereof for use as in claim 51, wherein said second MAb is conjugated to a therapeutic or diagnostic/detection agent.

54. The chimeric antibody or fragment thereof and the antibody fusion protein and fragment thereof for use as in claim 49, further comprising a second PAM4 MAb or fragment thereof.

55. The chimeric antibody or fragment thereof and the antibody fusion protein or fragment thereof for use as in claim 49, wherein said PAM4 antibody is administered parentally.

56. The chimeric antibody or fragment thereof and the antibody fusion protein or fragment thereof for use as in claim 49, wherein said PAM4 antibody is administered in a dosage of 20 to 2000 milligrams protein per dose.

57. The chimeric antibody or fragment thereof and the antibody fusion protein or fragment thereof for use as in claim 56, wherein said dosage is repeatedly administered.

58. The chimeric antibody or fragment thereof and the antibody fusion protein or fragment thereof for use as in claim 49, wherein said chimerized PAM4 antibody constant and hinge regions comprise constant and hinge regions of a human IgG.

59. The chimeric antibody or fragment thereof and the antibody fusion protein or fragment thereof for use as in claim 49, wherein said PAM4 antibody is administered before, in conjunction with, or after a second naked or conjugated antibody reactive with a second tumor marker expressed by said malignancy is administered to said subject.

60. The chimeric antibody or fragment thereof and the antibody fusion protein or fragment thereof for use as in claim 49, wherein said PAM4 antibody is administered before, concurrently, or after at least one therapeutic or diagnostic/detection agent is administered to said subject.

61. A diagnostic conjugate comprising a PAM4 MAb or fragment thereof or a PAM4 antibody fusion protein or fragment thereof, wherein said PAM4 MAb or fragment thereof or antibody fusion protein or fragment thereof is conjugated to at least one diagnostic/detection agent and wherein the PAM4 antibody is the antibody according to claim 2, for use in a method of diagnosing a malignancy in a subject comprising:

administering to said subject a diagnostically effective amount of the diagnostic conjugate, and optionally formulating said PAM4 antibody or fragment thereof or said fusion protein or fragment thereof in a pharmaceutically suitable excipient.

62. The chimeric antibody or fragment thereof of claim 2, wherein the chimeric antibody or fragment thereof is a naked chimeric antibody or fragment thereof, or a PAM4 MAb antibody fusion protein or fragment thereof comprising naked PAM4 MAb wherein the PAM4 MAb is the antibody according to claim 2, wherein the naked chimeric antibody or fragment thereof or the naked antibody fusion protein or fragment thereof is comprised in a composition, for use in a method for treating a cancer cell in a subject, wherein such method comprises administering to said subject a therapeutically effective amount of the composition, wherein said PAM4 antibody or fragment is optionally formulated in a pharmaceutically suitable excipient.

63. The chimeric antibody or fragment thereof and the PAM4 MAb antibody fusion protein or fragment thereof for use as in claim 62, wherein said composition further comprises a second naked antibody or fragment thereof.

64. The chimeric antibody or fragment thereof and the PAM4 MAb antibody fusion protein or fragment thereof for use as in claim 63, wherein said second antibody or fragment thereof is not a PAM4 MAb or fragment thereof.

65. The chimeric antibody or fragment thereof and the PAM4 MAb antibody fusion protein or fragment thereof for use as in claim 64, wherein said second antibody or fragment thereof is selected from the group consisting of CA19.9, DUPAN2, SPAN1, Nd2, B72.3, CC49, CEA, aLe^{a} , antibodies defined by the Lewis antigen Le(y), CSAp, MUC-2, MUC-3, MUC-4, TAG-72, EGFR, CD40, angiogenesis factors (e.g. VEGF), insulin-like growth factor (IGF), tenasin, platelet derived growth factor, IL-6, products of oncogenes and HER2/neu.

66. The chimeric antibody or fragment thereof and the PAM4 MAb antibody fusion protein or fragment thereof for use as in claim 62, wherein said naked PAM4 antibody is administered parentally.

67. The chimeric antibody or fragment thereof and the PAM4 MAb antibody fusion protein or fragment thereof for use as in claim 66, wherein said naked PAM4 antibody is administered in a dosage of 20 to 2000 milligrams protein per dose.

68. The chimeric antibody or fragment thereof and the PAM4 MAb antibody fusion protein or fragment thereof for use as in claim 67, wherein said dosage is repeatedly administered.

69. The chimeric antibody or fragment thereof and the PAM4 MAb antibody fusion protein or fragment thereof for use as in claim 62, wherein said naked PAM4 antibody constant and hinge regions comprise constant and hinge regions of a human IgG.

70. The chimeric antibody or fragment thereof and the PAM4 MAb antibody fusion protein or fragment thereof for use as in claim 69, wherein said second naked PAM4 antibody is administered before, in conjunction with, or after a naked antibody is administered to said subject.

71. The chimeric antibody or fragment thereof and the PAM4 MAb antibody fusion protein or fragment thereof for use as in claim 62, wherein said naked PAM4 antibody is administered before, concurrently or after a therapeutic and/or diagnostic/detection agent.

72. A method of diagnosing a pancreatic cancer in a subject comprising performing an *in vitro* diagnosis assay on a specimen from said subject with a composition comprising a naked PAM4 MAb or fragment thereof or a naked PAM4 MAb antibody fusion protein or fragment thereof wherein the PAM4 MAb antibody is the antibody according to claim 2.

73. The method of claim 72, wherein said in vitro diagnosis is selected from the group consisting of immunoassays and immunohistochemistry.

74. The method of claim 73, wherein said in vitro diagnosis assay is immunoassays.

75. The method of claim 72, wherein said specimen is body fluid or a tissue.

76. The method of claim 73, wherein said diagnosis assay is immunohistochemistry.

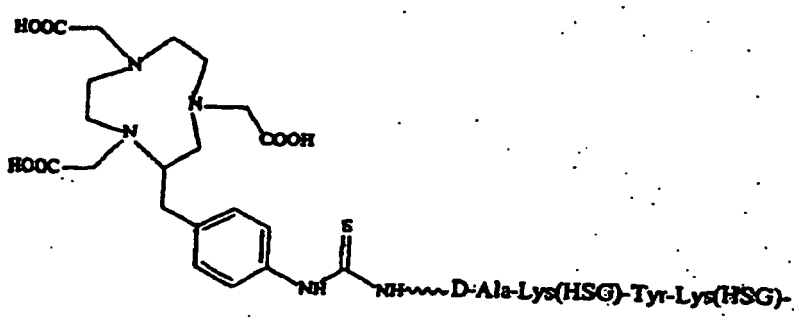
77. The method of claim 75, wherein said specimen is a population of cells or a tissue.

78. A bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue expressing PAM4 antigen and at least one other arm that specifically binds a targetable conjugate, wherein said one arm that specifically binds a targeted tissue is a cPAM4 antibody or fragment thereof, wherein the CDRs of the light chain variable region of the cPAM4 antibody comprises CDR1 comprising an amino acid sequence of SASSS-VSSSYLY; CDR2 comprising an amino acid sequence of STSNLAS; and CDR3 comprising an amino acid sequence of HQWNRYPYT; and the CDRs of the heavy chain variable region of the cPAM4 MAb comprises CDR1 comprising an amino acid sequence of SYVLH; CDR2 comprising an amino acid sequence of YINPYNDGTQYNEKFKG and CDR3 comprising an amino acid sequence of GFGGSYGFAY, for use in a method of identifying diseased tissues expressing a PAM4 antigen, in a subject, comprising:

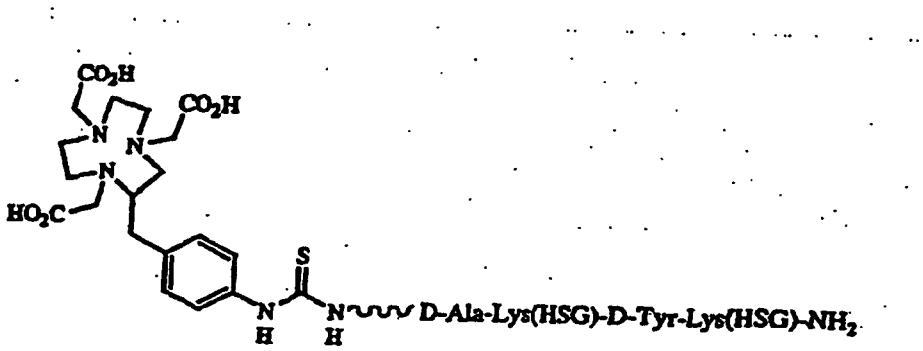
(a) administering an effective amount of the bispecific antibody or antibody fragment; and
(b) administering a targetable conjugate selected from the group consisting of

(i) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2

(ii) DOTA-Phe-Lye(HSG)-Tyr-Lys-(HSG)-NH_2
(iii) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(HSG)-Lys(Tscg-Cys)-NH_2
(iv)



and
(v)



wherein the method of identifying diseased tissues expressing a PAM4 antigen is a method of intraoperatively identifying diseased tissues, a method for endoscopical identification of diseased tissues or a method for intravascular identification of diseased tissues.

79. A bispecific antibody F(ab)2 or F(ab')2 fragment, diabody, triabody, or tetrabody, wherein the bispecific antibody or fragment has a first antibody binding site which specifically binds to a PAM4 antigen, wherein the antibody is a PAM4 antibody and wherein the CDRs of the light chain variable region of the PAM4 antibody comprises CDR1 comprising an amino acid sequence of SASSSVSSSYLY; CDR2 comprising an amino acid sequence of STSNLAS; and CDR3 comprising an amino acid sequence of HQWNRYPYT; and the CDRs of the heavy chain variable region of the PAM4 antibody comprises CDR1 comprising an amino acid sequence of SYVLH; CDR2 comprising an amino acid sequence of YINPYNDGTQYNEKFKG and CDR3 comprising an amino acid sequence of GFGGSYGFAY, and has a second antibody binding site which specifically binds to a hapten for use in a method of detection of lesions during an endoscopic, intravascular catheter, or surgical procedure, wherein the method comprises:

injecting a subject who is to undergo such a procedure with the bispecific antibody F(ab)2 or F(ab')2 fragment, diabody, triabody, or tetrabody, and permitting the antibody fragment to accrete at target sites; optionally clearing non-targeted antibody fragments using a galactosylated anti-idiotype clearing agent if the bispecific fragment is not largely cleared from circulation within about 24 hours of injection, and injecting a bivalent labeled hapten, which quickly localizes at the target site and clears through the kidneys; detecting the presence of the hapten by close-range detection of elevated levels of accreted label at the target sites with detection means, within 48 hours of the first injection, and conducting said procedure, wherein said detection is performed without the use of a contrast agent or subtraction agent.

80. A cPAM4 immunoconjugate or fragment thereof binding with the same antigen that is recognized by the full-length

cPAM4 antibody for use in a method for close-range lesion detection, during an operative, intravascular, or endoscopic procedure, wherein the method comprises:

injecting a subject to such a procedure parentally with an effective amount of the cPAM4 immunoconjugate or fragment thereof;
conducting the procedure within 48 hours of the injection;
scanning the accessed interior of the subject at close range with a detection means for detecting the presence of said labelled antibody or fragment thereof; and locating the sites of accretion of said labelled antibody or fragment thereof by detecting elevated levels of said labelled antibody or fragment thereof at such sites with the detection means,
wherein the immunoconjugate comprises a cPAM4 antibody, wherein the CDRs of the light chain variable region of the PAM4 antibody comprises CDR1 comprising an amino acid sequence of SASSSVSSSYLY; CDR2 comprising an amino acid sequence of STSNLAS; and CDR3 comprising an amino acid sequence of HQWNRYPYT; and the CDRs of the heavy chain variable region of the PAM4 MAb comprises CDR1 comprising an amino acid sequence of SYVLH; CDR2 comprising an amino acid sequence of YINPYNDGTQYNEKFKG and CDR3 comprising an amino acid sequence of GFGGSYGFAY.

Patentansprüche

1. Chimärer PAM4-Antikörper oder Fragment davon, wobei der Antikörper oder Fragment davon die PAM4 V_k-Polypeptid-Sequenz von Fig. 2A oder Fig. 2C und die PAM4 V_h-Polypeptid-Sequenz von Fig. 2B oder Fig. 2D umfasst.

2. Chimärer Antikörper oder Fragment davon, umfassend die komplementaritätsbestimmenden Regionen (CDRs) und Gerüstregionen (FR) eines murinen PAM4 monoklonalen Antikörpers (MAb) und die konstanten Regionen der leichten und schweren Kette eines humanen Antikörpers, wobei die CDRs der variablen Region der leichten Kette des chimären PAM4-MAb CDR1 umfassend eine Aminosäuresequenz aus SASSSVSSSYLY; CDR2 umfassend eine Aminosäuresequenz aus STSNLAS; und CDR3 umfassend eine Aminosäuresequenz aus HQWNRYPYT; und die CDRs der variablen Region der schweren Kette des chimären PAM4 MAb CDR1 umfassend eine Aminosäuresequenz aus SYVLH; CDR2 umfassend eine Aminosäuresequenz aus YINPYNDGTQYNEKFKG und CDR3 umfassend eine Aminosäuresequenz aus GFGGSYGFAY umfassen.

3. Diagnostisches oder therapeutisches Konjugat, das eine Krebszelle targetiert und einen Antikörperbestandteil umfasst, der einen Antikörper oder Fragment davon nach Anspruch 2 umfasst, der/das an die Zelle bindet, wobei der Antikörperbestandteil an wenigstens ein diagnostisches Agens/Nachweisagens und/oder wenigstens ein therapeutisches Agens gebunden ist.

4. Diagnostisches Konjugat nach Anspruch 3, wobei das diagnostische/Nachweisagens ausgewählt ist aus der Gruppe umfassend ein Radionuklid, ein Kontrastmittel und ein photoaktives diagnostisches Agens/Nachweisagens.

5. Diagnostisches Konjugat nach Anspruch 4, wobei das diagnostische Agens ein Radionuklid ist.

6. Diagnostisches Konjugat nach Anspruch 5, wobei das Radionuklid eine Energie zwischen 20 und 4.000 keV aufweist.

7. Diagnostisches Konjugat nach Anspruch 6, wobei das Radionuklid ein gamma-, beta-oder ein Positronen-emittierendes Isotop ist.

8. Diagnostisches Konjugat nach Anspruch 7, wobei das Radionuklid ausgewählt ist aus der Gruppe bestehend aus ^{110}In, ^{111}In, ^{177}Lu, ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{99}Y, ^{89}Zr, ^{94m}Tc ^{94}Tc, ^{99m}Tc, ^{120}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154\text{-}158}Gd$, ^{32}P, ^{11}C, ^{13}N, ^{15}O, ^{186}Re, ^{188}Re, ^{51}Mn, ^{52m}Mn, ^{55}Co, ^{72}AS, ^{75}Br, ^{76}Br, ^{82m}Rb, ^{83}Sr, und anderen gamma-, beta- oder Positron-Emittenten.

9. Diagnostisches Konjugat nach Anspruch 4, wobei das diagnostische Agens/Nachweisagens ein Kontrastmittel ist.

10. Diagnostisches Konjugat nach Anspruch 9, wobei das Kontrastmittel ein paramagnetisches Ion ist.

11. Diagnostisches Konjugat nach Anspruch 10, wobei das paramagnetische Ion ein Metall ist umfassend Chrom (III), Mangan (II), Eisen (III), Eisen (II), Kobalt (II), Nickel (II), Kupfer (II), Neodym (III), Samarium (III), Ytterbium (III),

Gadolinium (III), Vanadium (II), Terbium (III), Dysprosium (III), Holmium (III) oder Erbium (III).

12. Diagnostisches Konjugat nach Anspruch 9, wobei das Kontrastmittel ein Metall umfassend Lanthan (III), Gold (III), Blei (II) oder Wismut (III) ist.

13. Diagnostisches Konjugat nach Anspruch 9, wobei das Kontrastmittel ein ultraschallverstärkendes Mittel ist.

14. Diagnostisches Konjugat nach Anspruch 13, wobei das ultraschallverstärkende Agens ein Liposom ist, das den chimären Antikörper oder Fragment davon nach Anspruch 2 umfasst.

15. Diagnostisches Konjugat nach Anspruch 14, wobei das Liposom gasgefüllt ist.

16. Diagnostisches Konjugat nach Anspruch 9, wobei das Kontrastmittel ein strahlenundurchlässiges Material ist, das ausgewählt ist aus der Gruppe umfassend Jodverbindungen, Bariumverbindungen, Galliumverbindungen und Thalliumverbindungen.

17. Diagnostisches Konjugat nach Anspruch 16, wobei das strahlenundurchlässige Material ausgewählt ist aus der Gruppe umfassend Barium, Diatrizoat, ethiodisiertes Öl, Galliumcitrat, Iocarminsäure, Iocetamsäure, Iodamid, Iodipamid, Iodoxaminsäure, Iogulamid, Iohexol, Iopamidol, Iopansäure, Ioprocemsäure, Iosefamsäure, Iosersäure, Iosulamidmeglumin, Iosemetsäure, Iotasul, iotetrische Säure, Iothalamsäure, iotroxische Säure, ioxaglische Säure, ioxotrizoische Säure, Ipodat, Meglumin, Metrizamid, Metrizoat, Propyliodon und Thalliumchlorid.

18. Diagnostisches Konjugat nach Anspruch 4, wobei das diagnostische Agens/Nachweisagens ein photoaktives diagnostisches Agens/photoaktives Nachweisagens ist.

19. Diagnostisches Konjugat nach Anspruch 18, wobei das photoaktive diagnostische Agens/Nachweisagens eine fluoreszierende Markierungsverbindung ist, die ausgewählt ist aus der Gruppe umfassend Fluoreszein-Isothiocyanat, Rhodamin, Phycoerytherin, Phycocyanin, Allophycocyanin, o-Phthaldehyd und Fluoreszamin.

20. Diagnostisches Konjugat nach Anspruch 18, wobei das photoaktive diagnostische Agens/Nachweisagens eine chemilumineszierende Markierungsverbindung ist, die ausgewählt ist aus der Gruppe umfassend Luminol, Isoluminol, einen aromatischen Acridinester, ein Imidazol, ein Acridinsalz und einen Oxalatester.

21. Diagnostisches Konjugat nach Anspruch 18, wobei das photoaktive diagnostische Agens/Nachweisagens eine biolumineszierende Verbindung ist, die ausgewählt ist aus der Gruppe umfassend Luciferin, Luciferase und Aequorin.

22. Diagnostisches Konjugat nach einem der Ansprüche 4 bis 21, wobei das Konjugat für die Verwendung bei intraoperativer, endoskopischer oder intravaskularer Tumordiagnose ist.

23. Therapeutisches Konjugat nach Anspruch 3, wobei das therapeutische Agens ausgewählt ist aus der Gruppe bestehend aus einem Radionuklid, einem Immunmodulator, einem Hormon, einem Hormonantagonisten, einem Enzym, einem Enzyminhibitor, einem Oligonukleotid, einem photoaktiven therapeutischen Agens, einem zytotoxischen Agens, einem Antikörper, einem Angiogeneseinhibitor und einer Kombination davon.

24. Therapeutisches Konjugat nach Anspruch 23, wobei das Oligonukleotid ein Antisense-Oligonukleotid ist.

25. Therapeutisches Konjugat nach Anspruch 24, wobei das Oligonukleotid ein Antisense-Oligonukleotid gegen ein Onkogen ist.

26. Therapeutisches Konjugat nach Anspruch 25, wobei das Onkogen bcl-2 oder p53 ist.

27. Therapeutisches Konjugat nach Anspruch 23, wobei das therapeutische Agens ein zytotoxisches Agens ist.

28. Therapeutisches Konjugat nach Anspruch 27, wobei das zytotoxische Agens ein Wirkstoff oder ein Toxin ist.

29. Therapeutisches Konjugat nach Anspruch 28, wobei der Wirkstoff die pharmazeutische Eigenschaft aufweist, die ausgewählt ist aus der Gruppe bestehend aus antimitotisch, alkylierend, antimetabolisch, antiangiogen, apoptotisch, alkaloid und antibiotische Agenzien und Kombinationen davon.

30. Therapeutisches Konjugat nach Anspruch 28, wobei der Wirkstoff ausgewählt ist aus der Gruppe bestehend aus Stickstoffsenfgasen, Gemcitabin, Ethylenimin-Derivativen, Alkylsulfonaten, Stickstoffharnstoffen, Triazenen, Folsäureanaloga, Anthracycline, SN-38, Taxanen, COX-2-Inhibitoren, Pyrimidin-Analogen, Purin-Analogen, Antibiotika, Enzymen, Enzyminhibitoren, Epipodophyllotoxinen, Platinkoordinationskomplexen, Vincaalkaloiden, substituierten Harnstoffen, Methylhydrazinderivativen, Adrenocorticalsuppresoren, Hormonantagonisten, Endostatin, Taxolen, Camptothecinen, Doxorubicinen und deren Analogen, Antimetaboliten, alkylierenden Agenzien, Antimitotica, Antiangiogene, apoptotische Agenzien, Methotrexat, CPT-11, und eine Kombination davon.

31. Therapeutisches Konjugat nach Anspruch 28, wobei das Toxin aus einer Quelle stammt, die ausgewählt ist aus der Gruppe umfassend eine tierische, eine pflanzliche und eine mikrobielle Quelle.

32. Therapeutisches Konjugat nach Anspruch 28, wobei das Toxin ausgewählt ist aus der Gruppe bestehend aus Rizin, Abrin, Alphatoxin, Saporin, Ribonuklease (RNase), DNase I, Staphylokokken-Enterotoxin-A, Pokeweed-antivirales Protein, Gelonin, Diphtherintoxin, Pseudomonas-Exotoxin und Pseudomonas-Endotoxin.

33. Therapeutischen Konjugat nach Anspruch 23, wobei das therapeutische Agens ein Immunmodulator ist.

34. Therapeutisches Konjugat nach Anspruch 33, wobei der Immunmodulator ausgewählt ist aus der Gruppe bestehend aus einem Cytokin, einem Stammzellwachstumsfaktor, einem Lymphotoxin, einem hämatopoetischen Faktor, einem Kolonie-stimulierenden Faktor (CSF), einem Interferon (IFN), Erythropoietin, Thrombopoietin und einer Kombination davon.

35. Therapeutisches Konjugat nach Anspruch 34, wobei das Lymphotoxin Tumornekrosefaktor (TNF) ist, der hämatopoietische Faktor ein Interleukin (IL) ist, der Kolonie-stimulierende Faktor Granulozyten-Kolonie-stimulierender Faktor (G-CSF) oder Granulozyten-Makrophagen-Kolonie-stimulierender Faktor (GM-CSF) ist, das Interferon Interferon-α, -β oder -γ ist, und der Stammzellwachstumsfaktor mit "S1-Faktor" bezeichnet ist.

36. Therapeutisches Konjugat nach Anspruch 33, wobei der Immunmodulator IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, IL-21, interferon-γ, TNF-α oder eine Kombination davon umfasst.

37. Therapeutisches Konjugat nach Anspruch 23, wobei das therapeutische Agens ein Radionuklid ist.

38. Therapeutisches Konjugat nach Anspruch 37, wobei das Radionuklid eine Energie zwischen 60 und 700 keV aufweist.

39. Therapeutisches Konjugat nach Anspruch 38, wobei das Radionuklid ausgewählt ist aus der Gruppe bestehend aus ^{32}P, ^{33}P, ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{86}Y, ^{90}Y, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{211}At, ^{223}Ra und ^{225}Ac, und Kombinationen davon.

40. Therapeutisches Konjugat nach Anspruch 23, wobei das therapeutische Agens ein photoaktives therapeutisches Agens ist.

41. Therapeutisches Konjugat nach Anspruch 40, wobei das photoaktive therapeutische Agens ausgewählt ist aus der Gruppe umfassend Chromogene und Farbstoffe.

42. Therapeutisches Konjugat nach Anspruch 27, wobei das therapeutische Agens ein Enzym ist.

43. Therapeutisches Konjugat nach Anspruch 42, wobei das Enzym ausgewählt ist aus der Gruppe umfassend Malatedehydrogenase, Staphylokokken-Nuklease, Delta-V-Steroid-Isomerase, Alkoholdehydrogenase von Hefe, α-Glycerophosphat-Dehydrogenase, Triosephosphat-Isomerase, Meerrettich-Peroxidase, alkalische Phosphatase, Asparaginase, Glucoseoxidase, ß-Galactosidase, Ribonuclease, Urease, Katalase, Glucose-6-Phosphat-Dehydrogenase, Glucoamylase und Acetylcholinesterase.

44. Multivalenter, multispezifischer Antikörper oder Fragment davon, umfassend eine oder mehrere Antigenbindungsstellen mit Affinität zu dem PAM4-Zielepitop, das von PAM4-Antikörper erkannt wird, wobei die CDRs der variablen Region der leichten Kette des PAM4-Antikörpers CDR1 umfassend eine Aminosäuresequenz aus SASSSVSSSYLY; CDR2 umfassend eine Aminosäuresequenz aus STSNLAS; und CDR3 umfassend eine Aminosäuresequenz aus HQWNRYPYT und die CDRs der variablen Region der schweren Kette des PAM4 MAb CDR1 umfassend eine

Aminosäuresequenz aus SYVLH; CDR2 umfassend eine Aminosäuresequenz aus YINPYNDGTQYNEKFKG und CDR3 umfassend eine Aminosäuresequenz aus GFGGSYGFAY umfassen, bevorzugterweise den Antikörper gemäß Anspruch 2, oder ein Fragment davon, und eine oder mehrere Haptenbindungsstellen mit Affinität für Haptenmoleküle.

45. Antikörper oder Fragment davon nach Anspruch 44, weiter umfassend ein diagnostisches oder therapeutisches Agens.

46. Antikörperfusionsprotein oder Fragment davon umfassend wenigstens zwei chimäre PAM4 MAbs oder Fragmente davon nach Anspruch 1 oder 2.

47. Multivalenter, multispezifischer Antikörper oder Fragment davon nach Anspruch 44 zur Verwendung in einem Verfahren zum Diagnostizieren oder Behandeln von Krebs, umfassend: (a) Verabreichen an ein Lebewesen, das dessen bedarf, des Antikörpers oder Fragmentes davon des multivalenten, multispezifischen Antikörpers oder Fragmentes davon; (b) Warten für eine ausreichend lange Zeit, damit eine Menge des nicht-Antikörpers den Blutstrom des Lebewesens verlässt; (c) Verabreichen an das Lebewesens eines Trägermoleküls umfassend ein diagnostisches Agens/Nachweisagnes, ein therapeutisches Agens, oder eine Kombination davon, das an eine Bindungsstelle des Antikörpers bindet.

48. Multivalenter, multispezifischer Antikörper oder Fragment davon zur Verwendung nach Anspruch 47, wobei das Verfahren verwendet werden kann für das intraoperative Identifizieren von erkrankten Geweben, endoskopische Identifizieren von erkrankten Geweben oder intravaskuläre Identifizieren von erkrankten Geweben.

49. Chimärer Antikörper oder Fragment davon nach Anspruch 2 oder ein Antikörperfusionsprotein und Fragment davon umfassend PAM4-Antikörper, wobei der PAM4-Antikörper der Antikörper nach Anspruch 2 ist, und wobei der Antikörper oder Fragment davon oder das Antikörperfusionsprotein oder Fragment davon an wenigstens ein therapeutisches Agens gebunden ist, zur Verwendung bei der Behandlung einer Malignität in einem Lebewesen, wobei der Antikörper oder Fragment optional in einem pharmazeutisch geeigneten Bindemittel formuliert ist.

50. Chimärer Antikörper oder Fragment davon und Antikörperfusionsprotein der Fragment davon zur Verwendung nach Anspruch 49, weiter umfassend einen zweiten MAb oder Fragment davon, der kein PAM4-MAb oder Fragment davon ist.

51. Chimärer Antikörper oder Fragment davon und Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 50, wobei der zweite MAb oder Fragment davon ein nackter MAb oder Fragment davon ist.

52. Chimärer Antikörper oder Fragment davon und Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 51, wobei der zweite MAb oder Fragment davon ausgewählt ist aus der Gruppe bestehend aus CAI9.9, SUPAN2, SPAN1, Nd2, B72.3, CC49, CEA, aLe^a, durch das Lewis-Antigen Le(y) definierter Antikörper, CSAp, MUC-2, MUC-3, MUC-4, TAG-72, EGFR, CD40, angiogene Faktoren (z. B. VEGF), Produkten von Onkogenen, Insulin-ähnlichem Wachstumsfaktor (IGF), Tenascin, von Plättchen gewonnenem Wachstumsfaktor, IL-6 und HER2/neu.

53. Chimärer Antikörper oder Fragment davon und Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 51, wobei der zweite MAb konjugiert ist mit einem therapeutischen oder diagnostischen Agens/Nachweisagens.

54. Chimärer Antikörper oder Fragment davon und Antikörperfusionsprotein und Fragment davon zur Verwendung nach Anspruch 49, weiter umfassend einen zweiten PAM4-MAb oder Fragment davon.

55. Chimärer Antikörper oder Fragment davon und Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 49, wobei der PAM4-Antikörper parenteral verabreicht wird.

56. Chimärer Antikörper oder Fragment davon und Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 49, wobei der PAM4-Antikörper in einer Dosierung von 20 bis 2000 Milligramm Protein pro Dosis verabreicht wird.

57. Chimärer Antikörper oder Fragment davon und Antikörperfusionsprotein oder Fragment davon zur Verwendung

nach Anspruch 56, wobei die Dosierung wiederholt verabreicht wird.

58. Chimärer Antikörper oder Fragment davon und Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 49, wobei die konstanten und Gelenksregionen des chimärisierten PAM4-Antikörpers konstante und Gelenksregionen eines humanen IgG umfassen.

59. Chimärer Antikörper oder Fragment davon und Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 49, wobei der PAM4-Antikörper verabreicht wird, bevor, zusammen mit oder nachdem ein zweiter nackter oder konjugierter Antikörper, der mit einem zweiten Tumormarker reaktiv ist, der durch die Malignität exprimiert ist, an das Lebewesen verabreicht wird.

60. Chimärer Antikörper oder Fragment davon und Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 49, wobei der PAM4-Antikörper verabreicht wird, bevor zusammen mit oder nachdem wenigstens ein therapeutisches oder diagnostisches/Nachweisagens dem an das Lebewesen verabreicht wird..

61. Diagnostisches Konjugat umfassend einen PAM4-MAb oder Fragment davon oder ein PAM4-Antikörperfusionsprotein oder Fragment davon, wobei der PAM4-MAb oder Fragment davon oder Antikörperfusionsprotein oder Fragment davon konjugiert ist mit wenigstens einem diagnostischen Agens/Nachweisagens und wobei der PAM4-Antikörper der Antikörper gemäß Anspruch 2 ist, zur Verwendung in einem Verfahren zum Diagnostizieren einer Malignität in einem Lebewesen, umfassend:

> Verabreichen an das Lebewesen einer diagnostisch wirksamen Menge des diagnostischen Konjugates und optional Formulieren des PAM4-Antikörpers oder Fragmentes davon oder des Fusionsproteins oder Fragments davon in einem pharmazeutisch geeigneten Bindemittel.

62. Chimärer Antikörper oder Fragment davon nach Anspruch 2, wobei der chimäre Antikörper oder Fragment davon ein nackter chimärer Antikörper oder Fragment davon ist, oder ein PAM4-MAb-Antikörperfusionsprotein oder Fragment davon umfassend nackten PAM4-MAb, wobei der PAM4-MAb der Antikörper nach Anspruch 2 ist, wobei der nackte chimäre Antikörper oder Fragment davon oder das nackte Antikörperfusionsprotein oder Fragment davon in einer Zusammensetzung enthalten ist, zur Verwendung in einem Verfahren zum Behandeln einer Krebszelle in einem Lebewesen, wobei das Verfahren umfasst, eine therapeutisch wirksame Menge der Zusammensetzung an das Lebewesen zu verabreichen, wobei der PAM4-Antikörper oder Fragment optional in einem pharmazeutisch geeigneten Bindemittel formuliert ist.

63. Chimärer Antikörper oder Fragment davon und PAM4-MAb-Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 62, wobei die Zusammensetzung weiter einen zweiten nackten Antikörper oder Fragment davon umfasst.

64. Chimärer Antikörper oder Fragment davon und PAM4-MAb-Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 63, wobei der zweite Antikörper oder Fragment davon kein PAM4-MAb oder Fragment davon ist.

65. Chimärer Antikörper oder Fragment davon und PAM4-MAb-Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 64, wobei der zweite Antikörper oder Fragment davon ausgewählt ist aus der Gruppe bestehend aus CA19.9, DUPAN2, SPAN1, Nd2, B72.3, CC49, CEA, aLe^a, durch das Lewis-Antigen Le(y) definierte Antikörper, CSAp, MUC-2, MUC-3, MUC-4, TAG-72, EGFR, CD40, Angiogenesefaktoren (z. B. VEGF), Insulinähnlichem Wachstumsfaktor (IGF), Tenascin, von Plättchen gewonnenem Wachstumsfaktor, IL-6, Produkten von Onkogenen und HER3/neu.

66. Chimärer Antikörper oder Fragment davon und PAM4-MAb-Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 62, wobei der nackte PAM4-Antikörper parenteral verabreicht wird.

67. Chimärer Antikörper oder Fragment davon und PAM4-MAb-Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 66, wobei der nackte PAM4-Antikörper in einer Dosierung von 20 bis 2000 Milligramm Protein pro Dosis verabreicht wird.

68. Chimärer Antikörper oder Fragment davon und PAM4-MAb-Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 67, wobei die Dosierung wiederholt verabreicht wird.

69. Chimärer Antikörper oder Fragment davon und PAM4-MAb-Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 62, wobei die konstanten und Gelenksregionen des nackten PAM4-Antikörpers konstante und Gelenksregionen eines menschlichen IgG umfassen.

70. Chimärer Antikörper oder Fragment davon und PAM4-MAb-Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 69, wobei der zweite nackte PAM4-Antikörper verabreicht wird bevor, zusammen mit oder nachdem ein nackter Antikörper dem Lebewesen verabreicht wird.

71. Chimärer Antikörper oder Fragment davon und PAM4-MAb-Antikörperfusionsprotein oder Fragment davon zur Verwendung nach Anspruch 62, wobei der nackte PAM4-Antikörper verabreicht wird vor, zusammen mit oder nach einem therapeutischen und/oder diagnostischen Agens/Nachweisagens.

72. Verfahren zum Diagnostizieren von Pankreaskrebs in einem Lebewesen, umfassend Durchführen eines *in vitro* Diagnosetests an einer Probe von dem Lebewesen mit einer Zusammensetzung umfassend einen nackten PAM4-MAb oder Fragment davon oder ein nacktes PAM4-MAb-Antikörperfusionsprotein oder Fragment davon, wobei der PAM4-MAb-Antikörper der Antikörper gemäß Anspruch 2 ist.

73. Verfahren nach Anspruch 72, wobei die in vitro Diagnose ausgewählt ist aus der Gruppe bestehend aus Immunoassays und Immunhistochemie.

74. Verfahren nach Anspruch 73, wobei der in vitro Diagnosetest Immunoassays ist.

75. Verfahren nach Anspruch 72, wobei die Probe Körperflüssigkeit oder ein Gewebe ist.

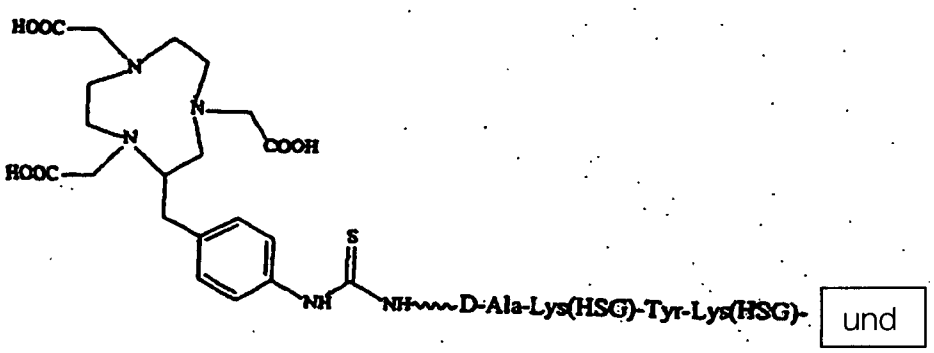
76. Verfahren nach Anspruch 73, wobei der Diagnosetes Immunhistochemie ist.

77. Verfahren nach Anspruch 75, wobei die Probe eine Zellpopulation oder ein Gewebe ist.

78. Bispezifischer Antikörper oder Antikörperfragment umfassend wenigstens einen Arm, der spezifisch an ein targetiertes Gewebe bindet, das PAM4-Antigen exprimiert, und wenigstens einen anderen Arm, der spezifisch ein targetierbares Konjugat bindet, wobei der eine Arm, der spezifisch ein targetiertes Gewebe bindet, ein cPAM4-Antikörper oder Fragmenten davon ist, wobei die CDRs der variablen Region der leichten Kette des cPMA4-Antikörpers CDR1 umfassend eine Aminosäuresequenz aus SASSSVSSSYLY; CDR2 umfassend eine Aminosäuresequenz aus STSNLAS und CDR3 umfassend eine Aminosäuresequenz aus HQWNRYPYT, und die CDRs der variablen Region der schweren Kette des cPAM4 Mab umfasst CDR1 umfassend eine Aminosäuresequenz aus SYVLH; CDR2 umfassend eine Aminosäuresequenz aus YINPYNDGTQYNEKFKG und CDR3 umfassend eine Aminosäuresequenz aus GFGGSYGFAY umfassen, zur Verwendung in einem Verfahren zum Identifizieren von erkrankten Geweben, die ein PAM4-Antigen exprimieren, in einem Lebewesen, umfassend:

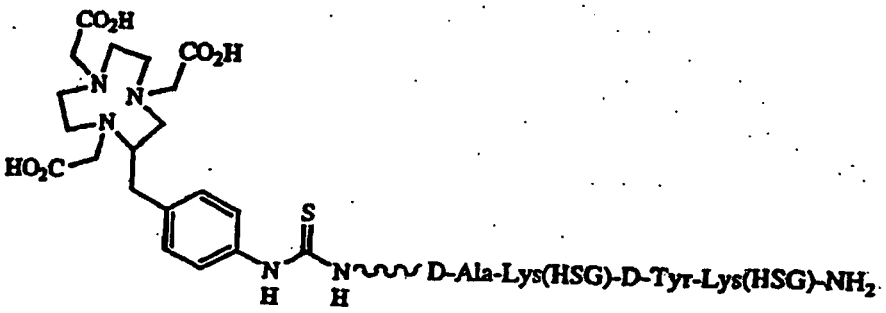
(a) Verabreichen einer wirksamen Menge des bispezifischen Antikörpers oder Antikörperfragment; und
(b) Verabreichen eines targetierbaren Konjugates, das ausgewählt ist aus der Gruppe bestehend aus:

(i) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2
(ii) DOTA-Phe-Lye(HSG)-Tyr-Lys-(HSG)-NH_2
(iii) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(HSG)-Lys(Tscg-Cys)-NH_2
(iv)



und

(v)



wobei das Verfahren zum Identifizieren von erkrankten Geweben, die PAM4-Antigen exprimieren, ein Verfahren zum intraoperativen Identifizieren von erkrankten Geweben, ein Verfahren zum endoskopischen Identifizieren von erkrankten Geweben oder ein Verfahren zum intravaskulären Identifizieren von erkrankten Geweben ist.

79. Bispezifische Antikörper F(ab)2 oder F(ab')2 Fragment, Diabody, Triabody oder Tetrabody, wobei der bispezifische Antikörper oder Fragment eine erste Antikörperbindungsstelle aufweist, die spezifisch an ein PAM4-Antigen bindet, wobei der Antikörper ein PAM4-Antikörper ist, und wobei die CDRs der variablen Region der leichten Kette des PAM4-Antikörpers CDR1 umfassend eine Aminosäuresequenz aus SASSSVSSSYLY; CDR2 umfassend eine Aminosäuresequenz aus STSNLAS; und CDR3 umfassend eine Aminosäuresequenz aus HQWNRYPYT; und die CDRs der variablen Region der schweren Kette des PAM4-Antikörpers CDR1 umfassend eine Aminosäuresequenz aus SYVLH; CDR2 umfassend eine Aminosäuresequenz aus YINPYNDGTQYNEKFKG und CDR3 umfassend eine Aminosäuresequenz aus GFGGSYGFAY umfassen, und eine zweite Antikörperbindungsstelle aufweist, die spezifisch an ein Hapten bindet, zur Verwendung in einem Verfahren zum Nachweis von Läsionen während endoskopischer, intravaskularer Katheter- oder chirurgischer Eingriffe, wobei das Verfahren umfasst:

Injizieren einem Lebewesen, das einem derartigen Verfahren unterzogen wird, des bispezifischen Antikörpers F(ab)2 oder F(ab')2-Fragmentes, Diabody, Traibody oder Tetrabody und Erlauben, dass sich das Antikörperfragment an Zielstellen ansammelt, optional Klären nicht-targetierter Antikörperfragmente unter Verwendung eines galactosylierten anti-Idiotyp-Klärungsmittels, wenn das bispezifische Fragment nicht größtenteils innerhalb von etwa 24 Stunden nach Injektion aus dem Blutkreislauf geklärt ist, und Injizieren eines bivalenten markierten Haptens, welches sich schnell an der Zielstelle lokalisiert und durch die Nieren geklärt wird; Nachweisen der Anwesenheit des Haptens durch Nahbereichsnachweis von erhöhten Titern von angesammelter Markierung an den Zielstellen mit Nachweismitteln innerhalb von 48 Stunden nach der ersten Injektion, und Durchführen des Verfahrens, wobei der Nachweis ohne die Verwendung eines Kontrastmittels oder Subtraktionsagens durchgeführt wird.

80. cPAM4-Immunkonjugat oder Fragment davon, das mit dem gleichen Antigen bindet, der durch den Vollängen-cPAM4-Antikörper erkannt wird, zur Verwendung in einem Verfahren zum Nahbereichsläsionennachweis während

eines operativen, intravaskulären oder endoskopischen Eingriffes, wobei das Verfahren umfasst:

parenterales Injizieren einem Lebewesen, das sich einem derartigen Eingriff unterzieht, einer wirksamen Menge des cPAM4-Immunkonjugates oder Fragmentes davon;
Durchführen des Eingriffes innerhalb von 48 Stunden nach der Injektion;
Abtasten des zugänglichen Inneren des Lebewesens im Nahbereich mit einem Nachweismittel zum Nachweisen der Gegenwart des markierten Antikörpers oder Fragmentes davon; und Lokalisieren der Stellen des Ansammelns des markierten Antikörpers oder Fragmentes davon durch Nachweisen erhöhter Titern des markierten Antikörpers oder Fragmentes davon an derartigen Stellen mit dem Nachweismittel,
wobei das Immunkonjugat einen cPAM4-Antikörper umfasst, wobei die CDRs der variablen Region der leichten Kette des PAM4-Antikörpers CDR1 umfassend eine Aminosäuresequenz aus SASSSVSSSYLY; CDR2 umfassend eine Aminosäuresequenz aus STSNLAS; und CDR3 umfassend eine Aminosäuresequenz aus HQWNRYPYT; und die CDRs der variablen Region der schweren Kette des PAM4-Antikörpers umfassend CDR1 umfassend eine Aminosäuresequenz aus SYVLH; CDR2 umfassend eine Aminosäuresequenz aus YINPYNDGTQYNEKFKG und CDR3 umfassend eine Aminosäuresequenz aus GFGGSYGFAY umfassen.

Revendications

1. Anticorps chimérique PAM4 ou fragment de ce dernier, ledit anticorps ou fragment de ce dernier comprenant la séquence polypeptidique V_k de PAM4 de la Figure 2A ou de la Figure 2C et la séquence polypeptidique V_h de PAM4 de la Figure 2B ou de la Figure 2D.

2. Anticorps chimérique ou fragment de ce dernier, comprenant les régions déterminant la complémentarité (RDC) et les régions charpentes (RC) d'un anticorps monoclonal (AcM) murin PAM4 et les régions constantes de la chaîne légère et de la chaîne lourde d'un anticorps humain, dans lequel les RDC de la région variable de la chaîne légère du AcM PAM4 chimérique comprennent RDC1 comprenant une séquence d'acides aminés SASSSVSSSYLY ; RDC2 comprenant une séquence d'acides aminés STSNLAS ; et RDC3 comprenant une séquence d'acides aminés HQWNRYPYT ; et les RDC de la région variable de la chaîne lourde du AcM PAM4 chimérique comprennent RDC1 comprenant une séquence d'acides aminés SYVLH ; RDC2 comprenant une séquence d'acides aminés YINPYNDGTQYNEKFKG, et RDC3 comprenant une séquence d'acides aminés GFGGSYGFAY.

3. Conjugué de diagnostic ou thérapeutique ciblant de cellule cancéreuse comprenant un composant anticorps qui comprend un anticorps ou un fragment de ce dernier selon la revendication 2, qui se lie à ladite cellule, ledit composant anticorps étant lié à au moins un agent de diagnostic/de détection et/ou à au moins un agent thérapeutique.

4. Conjugué de diagnostic selon la revendication 3, dans lequel ledit agent de diagnostic/de détection est choisi dans le groupe comprenant un radionucléide, un produit de contraste et un agent de diagnostic/de détection photoactif.

5. Conjugué de diagnostic selon la revendication 4, dans lequel ledit agent de diagnostic est un radionucléide.

6. Conjugué de diagnostic selon la revendication 5, dans lequel ledit radionucléide a une énergie comprise entre 20 et 4 000 keV.

7. Conjugué de diagnostic selon la revendication 6, dans lequel ledit radionucléide est un isotope émettant des rayons gamma, bêta ou des positrons.

8. Conjugué de diagnostic selon la revendication 7, dans lequel ledit radionucléide est choisi dans le groupe consistant en ^{110}In, ^{111}In, ^{177}Lu, ^{18}F, ^{52}Fe ^{62}Cu, ^{64}Cu, ^{67}Cu ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{89}Zr, ^{94m}Tc, ^{94}Tc, ^{99m}Tc ^{120}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154\text{-}158}Gd$, ^{32}P ^{11}C, ^{13}N, ^{15}O, ^{186}Re, ^{188}Re, ^{51}Mn, ^{52m}Mn, ^{55}Co, ^{72}As, ^{75}Br, ^{76}Br, ^{82m}Rb, ^{83}Sr, et d'autres émetteurs de rayons gamma, bêta ou de positrons.

9. Conjugué de diagnostic selon la revendication 4, dans lequel ledit agent de diagnostic/de détection est un produit de contraste.

10. Conjugué de diagnostic selon la revendication 9, dans lequel ledit produit de contraste est un ion paramagnétique.

11. Conjugué de diagnostic selon la revendication 10, dans lequel ledit ion paramagnétique est un métal comprenant le chrome(III), le manganèse(II), le fer(III), le fer(II), le cobalt(II), le nickel(II), le cuivre(II), le néodyme(III), le samarium(III), l'ytterbium(III), le gadolinium(III), le vanadium(II), le terbium(III), le dysprosium(III), l'holmium(III) ou l'erbium(III).

12. Conjugué de diagnostic selon la revendication 9, dans lequel ledit produit de contraste est un métal comprenant le lanthane(III), l'or(III), le plomb(II) ou le bismuth(III).

13. Conjugué de diagnostic selon la revendication 9, dans lequel ledit produit de contraste est un agent améliorateur de contraste aux ultrasons.

14. Conjugué de diagnostic selon la revendication 13, dans lequel ledit agent améliorateur de contraste aux ultrasons est un liposome qui comprend l'anticorps chimérique ou le fragment selon la revendication 2.

15. Conjugué de diagnostic selon la revendication 14, dans lequel ledit liposome est rempli d'un gaz.

16. Conjugué de diagnostic selon la revendication 9, dans lequel ledit produit de contraste est un matériau radio-opaque choisi dans le groupe comprenant les composés de l'iode, les composés du baryum, les composés du gallium et les composés du thallium.

17. Conjugué de diagnostic selon la revendication 16, dans lequel ledit matériau radio-opaque est choisi dans le groupe comprenant le baryum, le diatrizoate, l'huile éthiodisée, le citrate de gallium, l'acide iocarmique, l'acide iocétamique, l'iodamide, l'iodipamide, l'acide iodoxamique, l'iogulamide, l'iohexol, l'iopamidol, l'acide iopanoïque, l'acide ioprocémique, l'acide ioséfamique, l'acide iosérique, l'iosulamide méglumine, l'acide iosémétique, l'iotasul, l'acide iotétrique, l'acide iothalamique, l'acide iotroxique, l'acide ioxaglique, l'acide ioxotrizoïque, l'ipodate, la méglumine, le métrizamide, le métrizoate, la propyliodone et le chlorure thalleux.

18. Conjugué de diagnostic selon la revendication 4, dans lequel ledit agent de diagnostic/de détection est un agent de diagnostic/de détection photoactif.

19. Conjugué de diagnostic selon la revendication 18, dans lequel ledit agent de diagnostic/de détection photoactif est un composé marqueur fluorescent choisi dans le groupe comprenant l'isothiocyanate de fluorescéine, la rhodamine, la phycoérythrine, la phycocyanine, l'allophycocyanine, l'ophtaldéhyde et la fluorescamine.

20. Conjugué de diagnostic selon la revendication 18, dans lequel ledit agent de diagnostic/de détection photoactif est un composé marqueur chimioluminescent choisi dans le groupe comprenant le luminol, l'isoluminol, un ester d'acridinium aromatique, un imidazole, un sel d'acridinium et un ester oxalate.

21. Conjugué de diagnostic selon la revendication 18, dans lequel ledit agent de diagnostic/de détection photoactif est un composé bioluminescent choisi dans le groupe comprenant la luciférine, la luciférase et l'aequorine.

22. Conjugué de diagnostic selon l'une quelconque des revendications 4 à 21, ledit conjugué étant destiné à une utilisation pour le diagnostic intraopératoire, endoscopique ou intravasculaire d'une tumeur.

23. Conjugué thérapeutique selon la revendication 3, dans lequel ledit agent thérapeutique est choisi dans le groupe consistant en un radionucléide, un immunomodulateur, une hormone, un antagoniste d'hormone, une enzyme, un inhibiteur d'enzyme, un oligonucléotide, un agent thérapeutique photoactif, un agent cytotoxique, un anticorps, un inhibiteur de l'angiogenèse et une combinaison de ceux-ci.

24. Conjugué thérapeutique selon la revendication 23, dans lequel ledit oligonucléotide est un oligonucléotide antisens.

25. Conjugué thérapeutique selon la revendication 24, dans lequel ledit oligonucléotide est un oligonucléotide antisens dirigé contre un oncogène.

26. Conjugué thérapeutique selon la revendication 25, dans lequel ledit oncogène est bcl-2 ou p53.

27. Conjugué thérapeutique selon la revendication 23, dans lequel ledit agent thérapeutique est un agent cytotoxique.

28. Conjugué thérapeutique selon la revendication 27, dans lequel ledit agent cytotoxique est un médicament ou une toxine.

29. Conjugué thérapeutique selon la revendication 28, dans lequel ledit médicament possède la propriété pharmaceutique choisie dans le groupe consistant en antimitotique, d'alkylation, antimétabolite, antiangiogénique, apoptotique, alcaloïdes, et antibiotique, et les combinaisons de celles-ci.

30. Conjugué thérapeutique selon la revendication 28, dans lequel ledit médicament est choisi dans le groupe consistant en les moutardes azotées, la gemcitabine, les dérivés de l'éthylène-imine, les alkylsulfonates, les nitrosourées, les triazènes, les analogues de l'acide folique, les anthracyclines, le SN-38, les taxanes, les inhibiteurs de COX-2, les analogues de la pyrimidine, les analogues de la purine, les antibiotiques, les enzymes, les inhibiteurs d'enzymes, les épipodophyllotoxines, les complexes de coordination du platine, les vinca-alcaloïdes, les urées substituées, les dérivés de la méthylhydrazine, les suppresseurs adénocorticaux, les antagonistes d'hormones, l'endostatine, les taxols, les camptothécines, les doxorubicines et leurs analogues, les antimétabolites, les agents d'alkylation, les antimitotiques, les antiangiogéniques, les agents apoptotiques, le méthotrexate, le CPT-11 et une combinaison de ceux-ci.

31. Conjugué thérapeutique selon la revendication 28, dans lequel ladite toxine a été obtenue à partir d'une source choisie dans le groupe consistant en un animal, une plante et une source microbienne.

32. Conjugué thérapeutique selon la revendication 28, dans lequel ladite toxine est choisie dans le groupe consistant en la ricine, l'abrine, l'alpha-toxine, la saporine, la ribonucléase (ARN-ase), l'ADN-ase I, l'entérotoxine staphylococcique A, la protéine antivirale de phytolaque, la gélonine, la toxine diphtérique, l'exotoxine de Pseudomonas et l'endotoxine de Pseudomonas.

33. Conjugué thérapeutique selon la revendication 23, dans lequel ledit agent thérapeutique est un immunomodulateur.

34. Conjugué thérapeutique selon la revendication 33, dans lequel ledit immunomodulateur est choisi dans le groupe consistant en une cytokine, un facteur de croissance des cellules souches, une lymphotoxine, un facteur hématopoïétique, un facteur de stimulation des colonies (CSF), un interféron (IFN), l'érythropoïétine, la thrombopoïétine et une combinaison de ceux-ci.

35. Conjugué thérapeutique selon la revendication 34, dans lequel ladite lymphotoxine est un facteur de nécrose tumorale (TNF), ledit facteur hématopoïétique est une interleukine (IL), ledit facteur de stimulation des colonies est le facteur de stimulation des colonies de granulocytes (G-CSF) ou un facteur de stimulation des colonies de gralunocytes-macrophages (GM-CSF), ledit interféron est les interférons α, β ou γ, et ledit facteur de croissance des cellules souches est appelé "facteur S1".

36. Conjugué thérapeutique selon la revendication 33, dans lequel ledit immunomodulateur comprend l'IL-1, l'IL-2, l'IL-3, l'IL-6, l'IL-10, l'IL-12, l'IL-18, l'IL-21, l'interféron y, le TNF α ou une combinaison de ceux-ci.

37. Conjugué thérapeutique selon la revendication 23, dans lequel ledit agent thérapeutique est un radionucléide.

38. Conjugué thérapeutique selon la revendication 37, dans lequel ledit radionucléide a une énergie comprise entre 60 et 700 keV.

39. Conjugué thérapeutique selon la revendication 38, dans lequel ledit radionucléide est choisi dans le groupe consistant en ^{32}P, ^{33}P, ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{86}Y, ^{90}Y, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{211}At, ^{223}Ra et ^{225}Ac, et les combinaisons de ceux-ci.

40. Conjugué thérapeutique selon la revendication 23, dans lequel ledit agent thérapeutique est un agent thérapeutique photoactif.

41. Conjugué thérapeutique selon la revendication 40, dans lequel ledit agent thérapeutique photoactif est choisi dans le groupe comprenant les chromogènes et les colorants.

42. Conjugué thérapeutique selon la revendication 27, dans lequel ledit agent thérapeutique est une enzyme.

43. Conjugué thérapeutique selon la revendication 42, dans lequel ladite enzyme est choisie dans le groupe comprenant la malate déshydrogénase, la nucléase staphylococcique, la delta-V-stéroïde isomérase, l'alcool déshydrogénase de levure, l'α-glycérophosphate déshydrogénase, la triose phosphate isomérase, la peroxydase du raifort, la phosphatase alcaline, l'asparaginase, la glucose oxydase, la β-galactosidase, la ribonucléase, l'uréase, la catalase, la glucose-6-phosphate déshydrogénase, la glucoamylase et l'acétylcholinestérase.

44. Anticorps multispécifique multivalent ou fragment de ce dernier comprenant un ou plusieurs sites de liaison à l'antigène ayant une affinité pour l'épitope cible de PAM4 reconnu par l'anticorps PAM4, dans lequel les RDC de la région variable de la chaîne légère de l'anticorps PAM4 comprennent RDC1 comprenant une séquence d'acides aminés SASSSVSSSYLY ; RDC2 comprenant une séquence d'acides aminés STSNLAS ; et RDC3 comprenant une séquence d'acides aminés HQWNRYPYT ; et les RDC de la région variable de la chaîne lourde du AcM PAM4 comprennent RDC1 comprenant une séquence d'acides aminés SYVLH ; RDC2 comprenant une séquence d'acides aminés YINPYNDGTQYNEKFKG et RDC3 comprenant une séquence d'acides aminés GFGGSYGFAY, de préférence l'anticorps selon la revendication 2, ou un fragment de ce dernier, et un ou plusieurs sites de liaison aux haptènes, ayant une affinité pour les molécules d'haptènes.

45. Anticorps ou fragment de ce dernier selon la revendication 44, comprenant en outre un agent de diagnostic ou thérapeutique.

46. Protéine de fusion à un anticorps ou fragment de cette dernière comprenant au moins deux AcM PAM4 chimériques ou fragments de ces derniers selon la revendication 1 ou 2.

47. Anticorps multispécifique multivalent ou fragment de ce dernier selon la revendication 44 pour une utilisation dans un procédé pour le diagnostic ou le traitement du cancer, comprenant : (a) l'administration à un sujet qui en a besoin de l'anticorps ou du fragment de ce dernier de l'anticorps multispécifique multivalent ou du fragment de ce dernier ; (b) l'attente d'un laps de temps suffisant pendant lequel on attend qu'une certaine quantité du non-anticorps purifie la circulation sanguine du sujet ; et (c) l'administration audit sujet d'une molécule support comprenant un agent de diagnostic/de détection, un agent thérapeutique ou une combinaison de ceux-ci, qui se lie à un site de liaison de l'anticorps.

48. Anticorps multispécifique multivalent ou fragment de ce dernier pour une utilisation comme dans la revendication 47, pour lequel ledit procédé peut être utilisé pour une identification intra-opératoire de tissus pathologiques, l'identification endoscopique de tissus pathologiques ou l'identification intravasculaire de tissus pathologiques.

49. Anticorps chimérique ou fragment de ce dernier selon la revendication 2 ou protéine de fusion d'anticorps ou fragment de cette dernière comprenant l'anticorps PAM4, dans lequel ledit l'anticorps PAM4 est l'anticorps selon la revendication 2, et dans lequel ledit anticorps ou fragment de ce dernier, ou ladite protéine de fusion d'anticorps ou le fragment de cette dernière est lié à au moins un agent thérapeutique, pour une utilisation dans le traitement d'une tumeur maligne chez un sujet, ledit anticorps ou fragment étant optionnellement formulé dans un excipient pharmaceutiquement acceptable.

50. Anticorps chimérique ou fragment de ce dernier et protéine de fusion d'anticorps ou fragment de cette dernière pour une utilisation comme dans la revendication 49, comprenant en outre un deuxième AcM ou fragment de ce dernier, qui n'est pas un AcM PAM4 ou fragment de ce dernier.

51. Anticorps chimérique ou fragment de ce dernier et protéine de fusion d'anticorps ou fragment de cette dernière pour une utilisation comme dans la revendication 50, dans lequel ledit deuxième AcM ou fragment de ce dernier est un AcM non conjugué ou un fragment de ce dernier.

52. Anticorps chimérique ou fragment de ce dernier et protéine de fusion d'anticorps ou fragment de cette dernière pour une utilisation comme dans la revendication 51, dans lequel ledit deuxième AcM ou fragment de ce dernier est choisi dans le groupe consistant en CAI9.9, SUPAN2, SPAN1, Nd2, B72.3, CC49, CEA, aLe^a, les anticorps définis par l'antigène Lewis Le(y), CSAp, MUC-2, MUC-3, MUC-4, TAG-72, EGFR, CD40, les facteurs d'angiogenèse (par exemple VEGF), les produits d'oncogènes, le facteur de croissance de type insulinique (IGF), la tenascine, le facteur de croissance dérivé des plaquettes, IL-6 et HER2/neu.

53. Anticorps chimérique ou fragment de ce dernier et protéine de fusion d'anticorps ou fragment de cette dernière pour une utilisation comme dans la revendication 51, dans lequel ledit deuxième AcM est conjugué à un agent thérapeu-

tique ou de diagnostic/de détection.

54. Anticorps chimérique ou fragment de ce dernier et protéine de fusion d'anticorps et fragment de cette dernière pour une utilisation comme dans la revendication 49, comprenant en outre un deuxième AcM PAM4 ou fragment de ce dernier.

55. Anticorps chimérique ou fragment de ce dernier et protéine de fusion d'anticorps ou fragment de cette dernière pour une utilisation comme dans la revendication 49, dans lequel ledit anticorps PAM4 est administré par voie parentérale.

56. Anticorps chimérique ou fragment de ce dernier et protéine de fusion d'anticorps ou fragment de cette dernière pour une utilisation comme dans la revendication 49, dans lequel ledit anticorps PAM4 est administré à une posologie de 20 à 2 000 milligrammes de protéine par dose.

57. Anticorps chimérique ou fragment de ce dernier et protéine de fusion d'anticorps ou fragment de cette dernière pour une utilisation comme dans la revendication 56, dans lequel ladite posologie est administrée d'une manière répétée.

58. Anticorps chimérique ou fragment de ce dernier et protéine de fusion d'anticorps ou fragment de cette dernière pour une utilisation comme dans la revendication 49, dans lequel lesdites régions constantes et charnières de l'anticorps PAM4 chimérisé comprennent des régions constantes et charnières d'un IgG humain.

59. Anticorps chimérique ou fragment de ce dernier et protéine de fusion d'anticorps ou fragment de cette dernière pour une utilisation comme dans la revendication 49, ledit anticorps PAM4 étant administré avant, en même temps que ou après qu'un deuxième anticorps non conjugué ou conjugué pouvant réagir avec un deuxième marqueur tumoral exprimé par ladite tumeur maligne a été administré audit sujet.

60. Anticorps chimérique ou fragment de ce dernier et protéine de fusion d'anticorps ou fragment de cette dernière pour une utilisation comme dans la revendication 49, ledit anticorps PAM4 étant administré avant, en même temps que ou après qu'au moins un agent thérapeutique ou de diagnostic/de détection a été administré audit sujet.

61. Conjugué de diagnostic comprenant un AcM PAM4 ou un fragment de ce dernier ou une protéine de fusion de l'anticorps PAM4 ou un fragment de cette dernière, ledit AcM PAM4 ou fragment de ce dernier ou ladite protéine de fusion d'anticorps ou ledit fragment de cette dernière étant conjugué à au moins un agent de diagnostic/de détection, et l'anticorps PAM4 étant l'anticorps selon la revendication 2, pour une utilisation dans un procédé pour diagnostiquer une tumeur maligne chez un sujet, comprenant l'administration audit sujet d'une quantité efficace d'un point de vue diagnostic du conjugué de diagnostic, et optionnellement la formulation dudit anticorps PAM4 ou fragment de ce dernier ou de ladite protéine de fusion ou dudit fragment de cette dernière dans un excipient pharmaceutiquement acceptable.

62. Anticorps chimérique ou fragment de ce dernier selon la revendication 2, l'anticorps chimérique ou le fragment de ce dernier étant un anticorps chimérique non conjugué ou un fragment de ce dernier, ou une protéine de fusion de l'anticorps AcM PAM4 ou un fragment de cette dernière comprenant le AcM PAM4 non conjugué, le AcM PAM4 étant l'anticorps selon la revendication 2, l'anticorps chimérique non conjugué ou le fragment de ce dernier, ou la protéine de fusion de l'anticorps non conjugué ou le fragment de cette dernière, étant compris dans une composition, pour une utilisation dans un procédé pour le traitement d'une cellule cancéreuse chez un sujet, ledit procédé comprenant l'administration audit sujet d'une quantité thérapeutiquement efficace de la composition, ledit anticorps PAM4 ou ledit fragment étant optionnellement formulé dans un excipient pharmaceutiquement acceptable.

63. Anticorps chimérique ou fragment de ce dernier et protéine de fusion de l'anticorps AcM PAM4 ou fragment de cette dernière pour une utilisation comme dans la revendication 62, ladite composition comprenant en outre un deuxième anticorps non conjugué, ou fragment de ce dernier.

64. Anticorps chimérique ou fragment de ce dernier et protéine de fusion de l'anticorps AcM PAM4 ou fragment de cette dernière pour une utilisation comme dans la revendication 63, ledit deuxième anticorps ou fragment de ce dernier n'étant pas un AcM PAM4 ou fragment de ce dernier.

65. Anticorps chimérique ou fragment de ce dernier et protéine de fusion de l'anticorps AcM PAM4 ou fragment de cette dernière pour une utilisation comme dans la revendication 64, ledit deuxième anticorps ou fragment de ce dernier étant choisi dans le groupe consistant en CA19.9, DUPAN2, SPAN1, Nd2, B72.3, CC49, CEA, aLe^a, les anticorps

définis par l'anticorps Lewis Le(y), CSAp, MUC-2, MUC-3, MUC-4, TAG-72, EGFR, CD40, les facteurs d'angiogenèse (par exemple VEGF), le facteur de croissance de type insuline (IGF), la ténasine, le facteur de croissance dérivé des plaquettes, IL-6, les produits d'oncogènes et HER2/neu.

66. Anticorps chimérique ou fragment de ce dernier et protéine de fusion de l'anticorps AcM PAM4 ou fragment de cette dernière pour une utilisation comme dans la revendication 62, ledit anticorps PAM4 non conjugué étant administré par voie parentérale.

67. Anticorps chimérique ou fragment de ce dernier et protéine de fusion de l'anticorps AcM PAM4 ou fragment de cette dernière pour une utilisation comme dans la revendication 66, ledit anticorps PAM4 non conjugué étant administré à une posologie de 20 à 2 000 milligrammes de protéine par dose.

68. Anticorps chimérique ou fragment de ce dernier et protéine de fusion de l'anticorps AcM PAM4 ou fragment de cette dernière pour une utilisation comme dans la revendication 67, ladite posologie étant administrée d'une manière répétée.

69. Anticorps chimérique ou fragment de ce dernier et protéine de fusion de l'anticorps AcM PAM4 ou fragment de cette dernière pour une utilisation comme dans la revendication 62, les régions constantes et charnières dudit anticorps PAM4 non conjugué comprenant des régions constantes et charnières d'un IgG humain.

70. Anticorps chimérique ou fragment de ce dernier et protéine de fusion de l'anticorps AcM PAM4 ou fragment de cette dernière pour une utilisation comme dans la revendication 69, ledit deuxième anticorps PAM4 non conjugué étant administré avant, en même temps ou après qu'un anticorps non conjugué est administré audit sujet.

71. Anticorps chimérique ou fragment de ce dernier et protéine de fusion de l'anticorps AcM PAM4 ou fragment de cette dernière pour une utilisation comme dans la revendication 62, ledit anticorps PAM4 non conjugué étant administré avant, en même temps que ou après un agent thérapeutique et/ou de diagnostic/de détection.

72. Procédé pour diagnostiquer un cancer du pancréas chez un sujet, comprenant la mise en oeuvre d'un essai de diagnostic *in vitro* sur un spécimen provenant dudit sujet, avec une composition comprenant un AcM PAM4 non conjugué ou un fragment de ce dernier ou une protéine de fusion de l'anticorps AcM PAM4 non conjugué ou un fragment de cette dernière, ledit anticorps AcM PAM4 étant l'anticorps selon la revendication 2.

73. Procédé selon la revendication 72, dans lequel le diagnostic *in vitro* est choisi dans le groupe consistant en les essais immunologiques et une immunohistochimie.

74. Procédé selon la revendication 73, dans lequel ledit essai de diagnostic *in vitro* est un essai immunologique.

75. Procédé selon la revendication 72, dans lequel ledit spécimen est un fluide corporel ou un tissu.

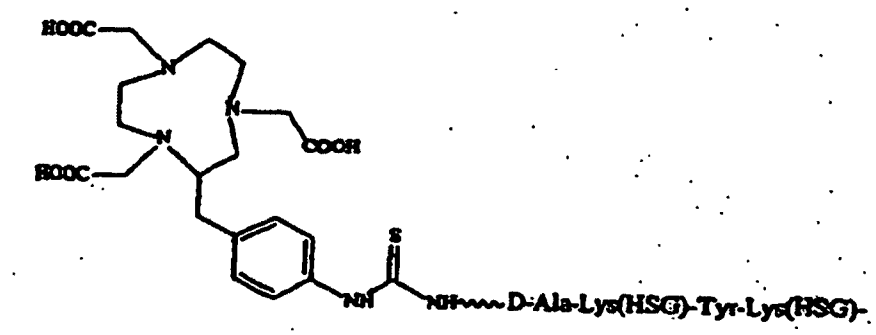
76. Procédé selon la revendication 73, dans lequel ledit essai de diagnostic est un essai immunohistochimique.

77. Procédé selon la revendication 75, dans lequel ledit spécimen est une population de cellules ou un tissu.

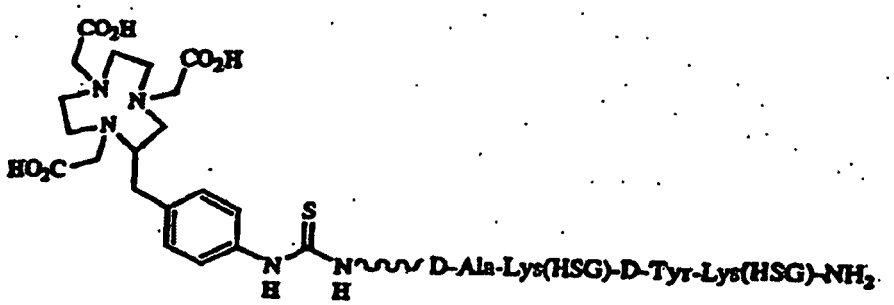
78. Anticorps ou fragment d'anticorps bispécifique comprenant au moins un bras qui se lie spécifiquement à un tissu ciblé exprimant l'antigène PAM4 et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable, dans lequel ledit un bras qui se lie spécifiquement à un tissu ciblé est un anticorps cPAM4 ou un fragment de ce dernier, dans lequel les RDC de la région variable de la chaîne légère de l'anticorps cPAM4 comprennent RDC1 comprenant une séquence d'acides aminés SASSSVSSSYLY ; RDC2 comprenant une séquence d'acides aminés STSNLAS ; et RDC3 comprenant une séquence d'acides aminés HQWNRYPYT ; et les RDC de la région variable de la chaîne lourde du AcM cPAM4 comprennent RDC1 comprenant une séquence d'acides aminés SYVLH ; RDC2 comprenant une séquence d'acides aminés YINPYNDGTQYNEKFKG, et RDC3 comprenant une séquence d'acides aminés GFGGSYGFAY, pour une utilisation dans un procédé d'identification de tissus pathologiques exprimant un antigène PAM4, chez un sujet, comprenant :

(a) l'administration d'une quantité efficace de l'anticorps ou du fragment d'anticorps bispécifique ; et
(b) l'administration d'un conjugué ciblable choisi dans le groupe consistant en

(i) **DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2**
(ii) **DOTA-Phe-Lye(HSG)-Tyr-Lys-(HSG)-NH_2**
(iii) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(HSG)-Lys(Tscg-Cys)-NH_2
(iv)



et
(v)



le procédé pour l'identification des tissus pathologiques exprimant un antigène PAM4 étant un procédé d'identification intra-opératoire de tissus pathologiques, un procédé pour l'identification endoscopique de tissus pathologiques ou un procédé pour l'identification intravasculaire de tissus pathologiques.

79. Fragment F(ab)2 ou F(ab')2 d'anticorps bispécifique, diabody, triabody ou tétrabody, l'anticorps ou le fragment bispécifique ayant un premier site de liaison d'anticorps qui se lie spécifiquement à un antigène PAM4, l'antigène étant un anticorps PAM4, et dans lequel les RDC de la région variable de la chaîne légère de l'anticorps PAM4 comprennent RDC1 comprenant une séquence d'acides aminés SASSSVSSSYLY ; RDC2 comprenant une séquence d'acides aminés STSNLAS ; et RDC3 comprenant une séquence d'acides aminés HQWNRYPYT ; et les RDC de la région variable de la chaîne lourde de l'anticorps PAM4 comprennent RDC1 comprenant une séquence d'acides aminés SYVLH ; RDC2 comprenant une séquence d'acides aminés YINPYNDGTQYNEKFKG, et RDC3 comprenant une séquence d'acides aminés GFGGSYGFAY, et ayant un deuxième site de liaison d'anticorps qui se lie spécifiquement à un haptène pour une utilisation dans un procédé de détection de lésions pendant une intervention endoscopique, par sonde intravasculaire, ou chirurgicale, le procédé comprenant :

l'injection à un sujet destiné à subir une intervention de ce type, du fragment F(ab)2 ou F(ab')2 de l'anticorps bispécifique, diabody, triabody ou tétrabody, et le fait de permettre au fragment d'anticorps de subir une accrétion au niveau de sites cibles ; optionnellement, purification des fragments d'anticorps non ciblés par utilisation d'un agent d'épuration anti-idiotype galactosylé si le fragment bispécifique n'est pas fortement éliminé de la circulation approximativement dans les 24 heures suivant l'injection, et l'injection d'un haptène marqué bivalent, qui se localise rapidement au niveau du site cible et s'élimine par les reins ; la détection de la présente de l'haptène par détection rapprochée de niveaux élevés d'un marqueur ayant subi une accrétion au niveau des sites cibles avec des moyens de détection, dans les 48 heures suivant la première injection, et la mise en oeuvre dudit mode opératoire, ladite détection étant réalisée sans utilisation d'un produit de contraste ou d'un agent de différenciation.

80. Immunoconjugué de cPAM4 ou fragment de ce dernier se liant au même antigène que celui qui est reconnu par l'anticorps cPAM4 complet pour une utilisation dans un procédé de détection des lésions proches, pendant une intervention opératoire, intravasculaire ou endoscopique, le procédé comprenant :

l'injection à un sujet, conformément à une procédure parentérale, d'une quantité efficace de l'immunoconjugué de cPAM4 ou du fragment de ce dernier ;
la conduite de la procédure dans les 48 heures suivant l'injection ;
le balayage de l'intérieur du sujet auquel on peut avoir accès, en rapproché avec un moyen de détection pour détecter la présence dudit anticorps marqué ou dudit fragment de ce dernier ; et la localisation des sites d'accrétion dudit anticorps marqué ou dudit fragment, par détection de niveaux élevés dudit anticorps marqué ou du fragment de ce dernier au niveau de ces sites, avec les moyens de détection,
dans lequel l'immunoconjugué comprend un anticorps cPAM4, dans lequel les RDC de la région variable de la chaîne légère de l'anticorps PAM4 comprend RDC1 comprenant une séquence d'acides aminés SASSSVSSSYLY ; RDC2 comprenant une séquence d'acides aminés STSNLAS ; et RDC3 comprenant une séquence d'acides aminés HQWNRYPYT ; et les RDC de la région variable de la chaîne lourde du AcM PAM4 comprennent RDC1 comprenant une séquence d'acides aminés SYVLH ; RDC2 comprenant une séquence d'acides aminés YINPYNDGTQYNEKFKG, et RDC3 comprenant une séquence d'acides aminés GFGGSYGFAY.

PAM4 V_k

```
GATATTGTGATGACCCAGTCTCCAGCAATCATGTCTGCATCTCCTGGGGAGAAGGTCACCATGACCTGCAGTGCCAGCTCAAGTGTAAGT  90
 1                          10                                  20                  27 A
 D  I  V  M  T  Q  S  P  A  I  M  S  A  S  P  G  E  K  V  T  M  T  C  S  A  S  S  S  V  S
                                                                                  CDR1

TCCAGCTACTTGTACTGGTACCAGCAGAAGCCAGGATCCTCCCCCAAACTCTGGATTTATAGCACATCCAACCTGGCTTCTGGAGTCCCT 180
30                             40                                  50
 S  S  Y  L  Y  W  Y  Q  Q  K  P  G  S  S  P  K  L  W  I  Y  S  T  S  N  L  A  S  G  V  P
                                                                  CDR2

GCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCTCTTATTTCTGCCAT 270
60                             70                                  80
 A  R  F  S  G  S  G  S  G  T  S  Y  S  L  T  I  S  S  M  E  A  E  D  A  A  S  Y  F  C  H

CAGTGGAATAGGTACCCGTACACGTTCGGAGGGGGGACCAAGCTGGAAATAAAA                                    324
90                             100                 107
 Q  W  N  R  Y  P  Y  T  F  G  G  G  T  K  L  E  I  K
       CDR3
```

Nucleotide and Amino Acid Sequences of Murine PAM4 V_K

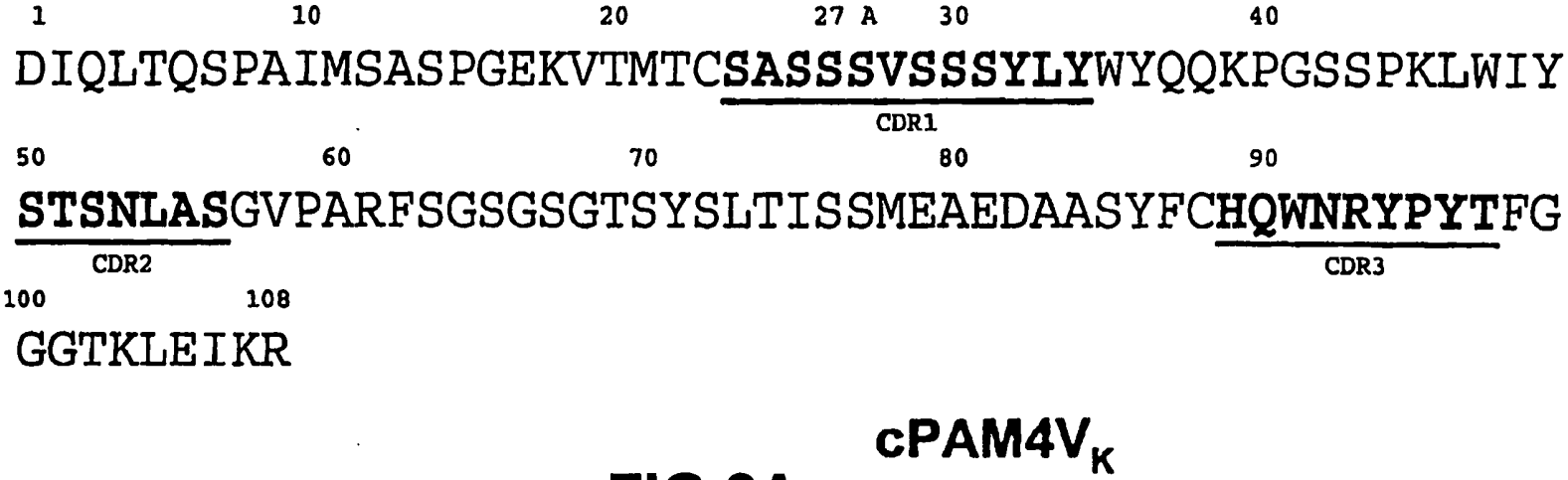
FIG.1A.

PAM4 V_H

```
GAGGTTCAGCTGCAGGAGTCTGGACCTGAGCTGGTAAAGCCTGGGGCTTCAGTGAAGATGTCCTGCAAGGCTTCTGGATACACATTCCCT  90
1                         10                        20                        30
E  V  Q  L  Q  E  S  G  P  E  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  Y  T  F  P

AGCTATGTTTTGCACTGGGTGAAGCAGAAGCCTGGGCAGGGCCTTGAGTGGATTGGATATATTAATCCTTACAATGATGGTACTCAGTAC 180
                          40                        50    52 A
S  Y  V  L  H  W  V  K  Q  K  P  G  Q  G  L  E  W  I  G  Y  I  N  P  Y  N  D  G  T  Q  Y
      CDR1                                                                  CDR 2

AATGAGAAGTTCAAAGGCAAGGCCACACTGACTTCAGACAAATCGTCCAGCACAGCCTACATGGAGCTCAGCCGCCTGACCTCTGAGGAC 270
60                        70                        80    82 A B C
N  E  K  F  K  G  K  A  T  L  T  S  D  K  S  S  S  T  A  Y  M  E  L  S  R  L  T  S  E  D

TCTGCGGTCTATTACTGTGCAAGAGGCTTCGGTGGTAGCTACGGATTTGCTTACTGGGGCCAAGGGACTCTGATCACTGTCTCTGCA  357
        90                        100 A B                       110       113
S  A  V  Y  Y  C  A  R  G  F  G  G  S  Y  G  F  A  Y  W  G  Q  G  T  L  I  T  V  S  A
                          CDR3
```

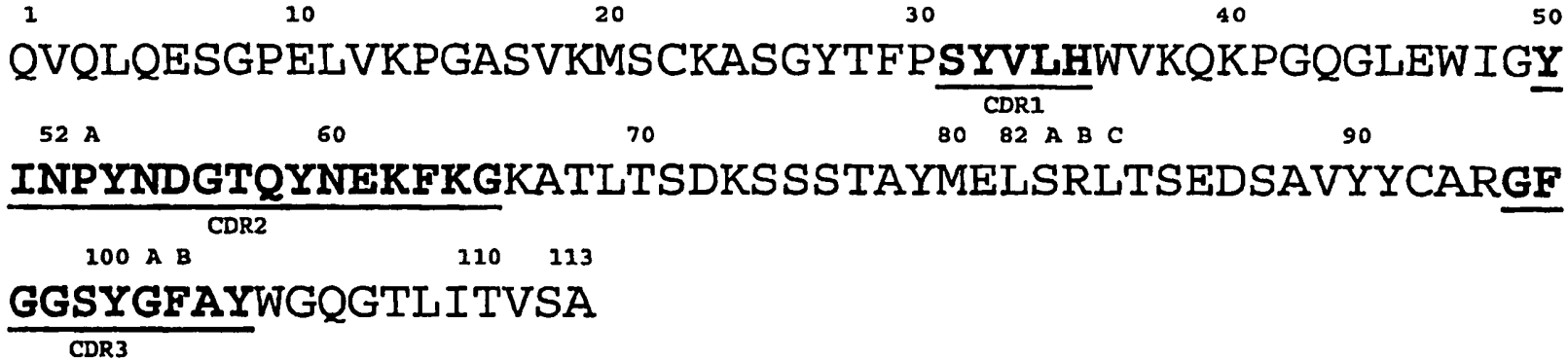
Nucleotide and Amino Acid Sequences of Murine PAM4 V_H

FIG.1B.



cPAM4V$_K$

FIG.2A.



cPAM4V$_H$

FIG.2B.

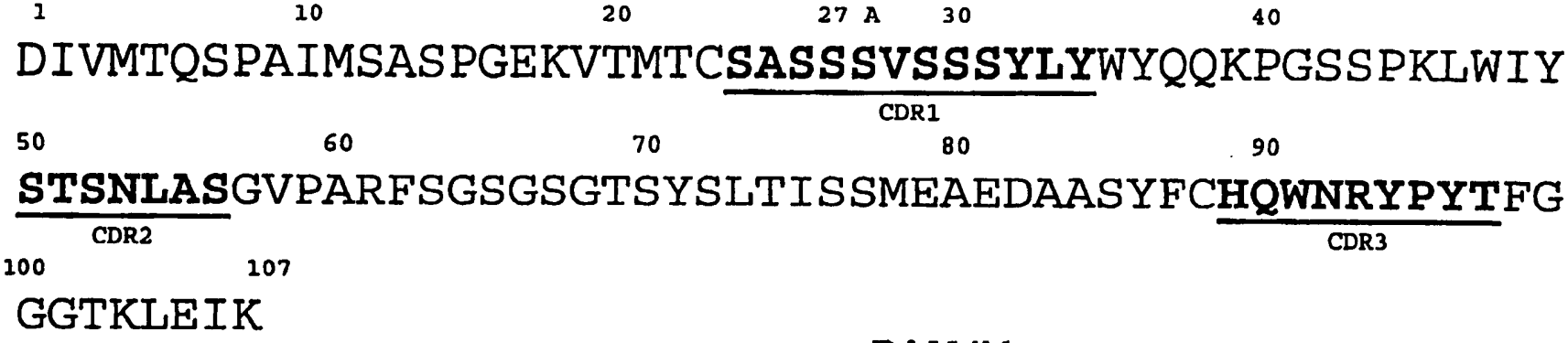


FIG.2C. $cPAM4V_K$

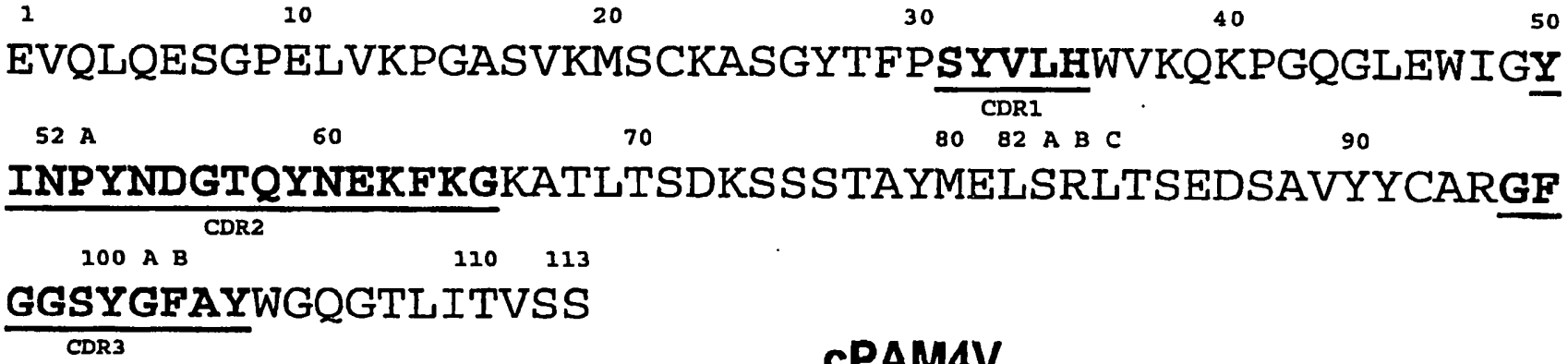
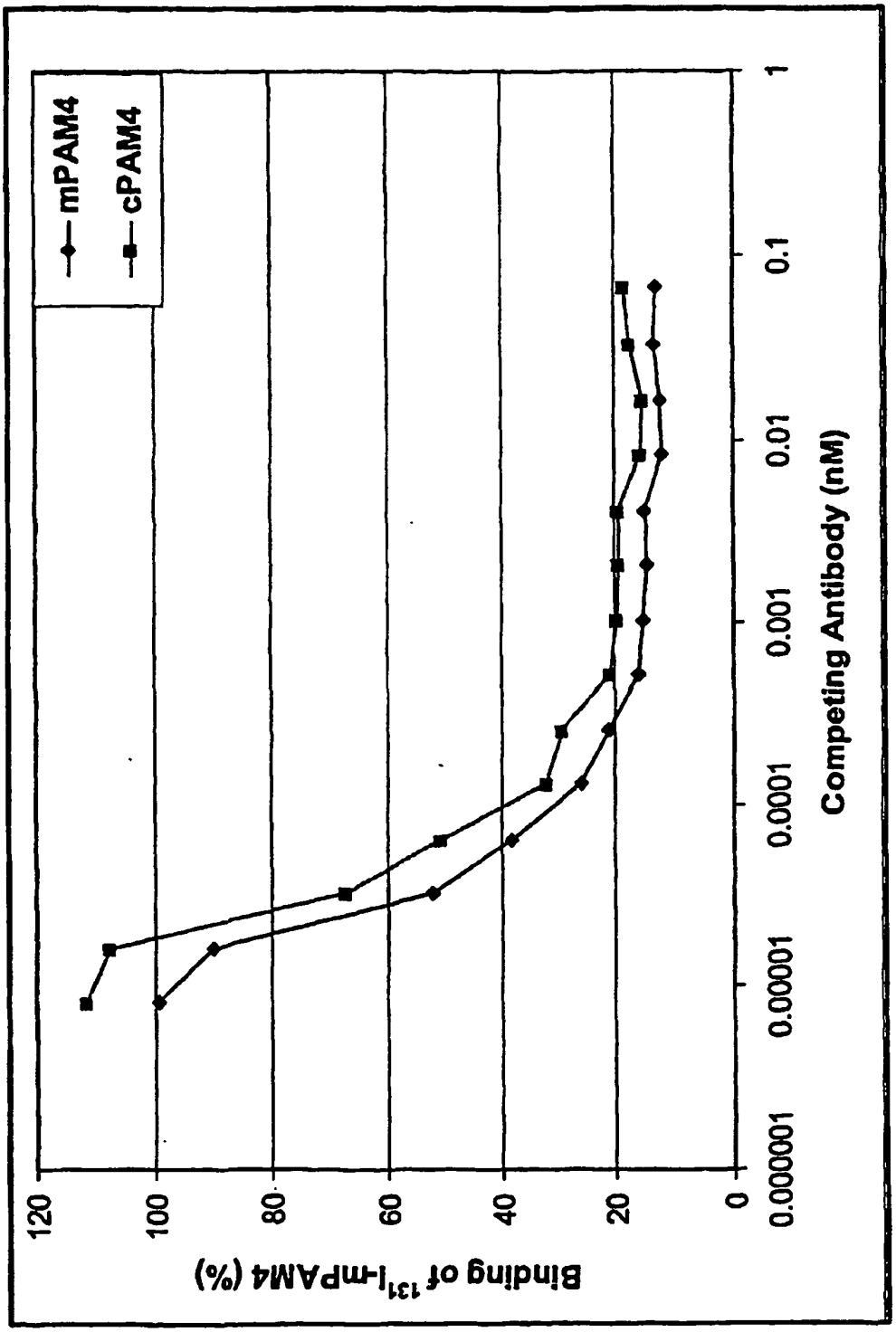


FIG.2D. $cPAM4V_H$



Binding Activity of Chimerized PAM4 and Murine PAM4

FIG.3.

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摘要(译)

本发明涉及单价和多价单特异性抗体以及单价和多价多特异性抗体。这些抗体的一个实施方案具有一个或多个相同的结合位点，其中每个结合位点与靶抗原或靶抗原上的表位结合。这些抗体的另一个实施方案具有两个或更多个结合位点，其中这些结合位点对靶抗原或不同靶抗原上的不同表位具有亲和力，或对靶抗原和半抗原具有亲和力。本发明进一步涉及可用于在宿主中表达这些功能性抗体的重组载体。更具体地，本发明涉及命名为PAM4的肿瘤和害羞相关抗体。本发明还涉及嵌合PAM4抗体，以及此类抗体在诊断和治疗中的用途。