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(54) **PRODUCTION AND USE OF NOVEL PEPTIDE-BASED AGENTS FOR USE WITH BI-SPECIFIC ANTIBODIES**

HERSTELLUNG UND VERWENDUNG NEUER MITTEL AUF PEPTIDBASIS ZUR VERWENDUNG MIT BISPEZIFISCHEN ANTIKÖRPERN

PRODUCTION ET UTILISATION DE NOUVEAUX AGENTS A BASE DE PEPTIDES EN VUE D'UNE UTILISATION AVEC DES ANTICORPS BISPECIFIQUES

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(56) References cited:
WO-A-99/66951 **WO-A2-99/66951**
US-A- 5 274 076 **US-A- 6 096 289**
US-A- 6 096 289 **US-A1- 2002 006 379**

- **KARACAY H ET AL: "Pretargeting studies with a murine anti-colon-specific antigen-p (CSAp) X chimeric anti-(indium-DTPA) bispecific antibody and technetium-99m-labeled peptide" CANCER BIOTHERAPY AND RADIOPHARMACEUTICALS, vol. 15, no. 4, 2000, page 412, XP008065412 & EIGHTH CONFERENCE ON RADIOIMMUNODETECTION AND RADIOIMMUNOTHERAPY OF CANCER; PRINCETON, NEW JERSEY, USA; OCTOBER 12-14, 2000 ISSN: 1084-9785**

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- KARACAY H ET AL: "Experimental pretargeting studies of cancer with a humanized anti-CEA x murine anti-[In-DTPA] bispecific antibody construct and a (99m)Tc-/(188)Re-labeled peptide." BIOCONJUGATE CHEMISTRY. 2000 NOV-DEC, vol. 11, no. 6, November 2000 (2000-11), pages 842-854, XP002230983 ISSN: 1043-1802
- BLUMENTHAL R D ET AL: "TARGETED THERAPY OF ATHYMIC MICE BEARING GW-39 HUMAN COLONIC CANCER MICROMETASTASES WITH 131I-LABELED MONOCLONAL ANTIBODIES", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD., US, vol. 52, no. 21, 1 November 1992 (1992-11-01), pages 6036-6044, XP008071618, ISSN: 0008-5472
- GOLD D V ET AL: "Murine monoclonal antibodies to colon-specific antigen p.", CANCER RESEARCH 1 OCT 1990 LNKD- PUBMED: 2169346, vol. 50, no. 19, 1 October 1990 (1990-10-01), pages 6405-6409, ISSN: 0008-5472
- ROBERT M. SHARKEY ET AL: 'Phase I clinical evaluation of a new murine monoclonal antibody (mu-9) against colon-specific antigen-p for targeting gastrointestinal carcinomas' CANCER vol. 73, no. S3, 01 February 1994, pages 864 - 877, XP055005840 ISSN: 0008-543X
- SHARKEY ET AL: "Selection of radioimmunoconjugates for the therapy of well-established or micrometastatic colon carcinoma.", INTERNATIONAL JOURNAL OF CANCER, vol. 72, 29 July 1997 (1997-07-29), pages 477-485, USA
- KRISHNAN I S ET AL: "CHIMERIZATION OF MU-9 A COLON-SPECIFIC ANTIGEN-P ANTIBODY REACTIVE WITH GASTROINTESTINAL CARCINOMAS", CANCER, AMERICAN CANCER SOCIETY, PHILADELPHIA, PA, US, vol. 80, 1 January 1997 (1997-01-01), pages 2667-2674, XP002920741, ISSN: 0008-543X, DOI: 10.1002/(SICI)1097-0142(19971215)80:12+<26 67::AID-CNCR44>3.0.CO;2-7

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The file contains technical information submitted after the application was filed and not included in this specification

Description

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application is a Continuation in part of US Application 09/337,756, filed 06/22/1999,

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0002] The disclosure relates to immunological reagents for therapeutic use, for example, in radioimmunotherapy (RAIT) and chemoimmunotherapy, and detection and/or diagnostic uses, for example, in radioimmunodetection (RAID), ultrasonography, and magnetic resonance imaging (MRI). In particular, the disclosure relates to naked antibodies (unconjugated) and directly-conjugated antibodies, as well as bi-specific antibodies (bsAbs) and bi-specific antibody fragments (bsFabs) which have at least one arm which is reactive against a targeted tissue and at least one other arm which is reactive against a linker moiety. Further, the disclosure relates to monoclonal antibodies that have been raised against specific immunogens, being human, humanized and chimeric monoclonal antibodies, as well as human, humanized and chimeric bi-specific antibodies and antibody fragments having at least one arm which is reactive against a targeted tissue and at least one other arm which is reactive against a linker moiety, DNAs that encode such antibodies and antibody fragments, and vectors for expressing the DNAs.

[0003] The present disclosure also relates to humanized, chimeric and human anti-CSAp antibodies, particularly monoclonal antibodies (mAbs), therapeutic and detection/diagnostic conjugates of humanized, chimeric and human anti-CSAp antibodies and methods of diagnosing/detection or treating a malignancy using humanized, chimeric and human anti-CSAp antibodies. The present disclosure also relates to antibody fusion proteins or fragments thereof comprising at least two anti-CSAp mAbs or fragments thereof or at least one anti-CSAp mAb or fragment thereof and at least one second mAb or fragment thereof, other than the anti-CSAp mAb or fragment thereof. The humanized, chimeric and human anti-CSAp mAbs, fragments thereof, antibody fusion proteins thereof, or fragments thereof may be administered alone, as a therapeutic conjugate or in combination with a therapeutic immunoconjugate, with other naked antibodies, or with other therapeutic agents or as a diagnostic/detection conjugate. The present disclosure also provides DNA sequences encoding humanized, chimeric and human anti-CSAp antibodies, and antibody fusion proteins, vectors and host cells containing the DNA sequences, and methods of making the humanized, chimeric and human anti-CSAp antibodies.

2. Related Art

[0004] An approach to cancer therapy and detection/diagnosis involves directing antibodies or antibody fragments to disease tissues, wherein the antibody or antibody fragment can target a detection/diagnostic agent or therapeutic agent to the disease site. One approach to this methodology that has been under investigation involves the use of bi-specific monoclonal antibodies (bsAbs) having at least one arm that is reactive against a targeted diseased tissue and at least one other arm that is reactive against a low molecular weight hapten. In this methodology, a bsAb is administered and allowed to localize to target, and to clear normal tissue. Some time later, a radiolabeled low molecular weight hapten is given, which being recognized by the second specificity of the bsAb, also localizes to the original target. The same technology can be used to target therapeutic isotopes, drugs and toxins selectively to diseased tissues, particularly cancers against which the bsAb is targeted, or non-radioactive diagnostic agents for improved diagnosis and detection of pathological lesions expressing the target antigen.

[0005] Although low MW haptens used in combination with bsAbs possess a large number of specific imaging and therapy uses, it is impractical to prepare individual bsAbs for each possible application. Further, the application of a bsAb/low MW hapten system has to contend with several other issues. First, the arm of the bsAb that binds to the low MW hapten must bind with high affinity, since a low MW hapten is designed to clear the living system rapidly, when not bound by bsAb. Second, the non-bsAb-bound low MW hapten actually needs to clear the living system rapidly to avoid non-target tissue uptake and retention. Third, the detection and/or therapy agent must remain associated with the low MW hapten throughout its application within the bsAb protocol employed.

[0006] Of interest with this approach are bsAbs that direct chelators and metal chelate complexes to cancers using Abs of appropriate dual specificity. The chelators and metal chelate complexes used are often radioactive, using radionuclides such as cobalt-57 (Goodwin et al., U.S. Patent No. 4,863,713), indium-111 (Barbet et al., U.S. Patent No. 5,256,395 and U.S. Patent No. 5,274,076, Goodwin et al., J. Nucl. Med. 33:1366-1372 (1992), and Kranenborg et al. Cancer Res (suppl.) 55:5864s-5867s (1995) and Cancer (suppl.) 80:2390-2397 (1997)) and gallium-68 (Boden et al., Bioconjugate Chem. 6:373-379, (1995) and Schuhmacher et al. Cancer Res. 55:115-123 (1995)) for radioimmuno-

imaging. Because the Abs were raised against the chelators and metal chelate complexes, they have remarkable specificity for the complex against which they were originally raised. Indeed, the bsAbs of Boden *et al.* have specificity for single enantiomers of enantiomeric mixtures of chelators and metal-chelate complexes. This great specificity has proven to be a disadvantage in one respect, in that other nuclides such as yttrium-90 and bismuth-213, useful for radioimmunotherapy (RAIT), and gadolinium, useful for MRI, cannot be readily substituted into available reagents for alternative uses. As a result, iodine-131, a non-metal, has been adopted for RAIT purposes by using an I-131-labeled indium-metal-chelate complex in the second targeting step. A second disadvantage to this methodology requires that antibodies be raised against every agent desired for diagnostic or therapeutic use.

[0007] Thus, there is a continuing need for an immunological agent which can be directed to diseased tissue and is reactive with a subsequently administered linker moiety which is bonded to or associated with a therapeutic or diagnostic/detection metal chelate complex or a therapeutic or diagnostic/detection chelator.

[0008] The present disclosure relates to recombinantly produced chimeric, humanized and human monoclonal antibodies directed against cancers, including colorectal, pancreatic, and ovarian cancers. Chimeric, humanized and human monoclonal antibodies cause less production of human anti-mouse antibodies than completely murine antibodies. Additionally, when the antibodies are covalently conjugated to a diagnostic or therapeutic reagent, they retain their binding characteristics. Further, if the human, humanized or chimeric antibodies have human constant regions that can be immunologically functional in patients, such as is the case for IgG_1, then these can also be active against such tumors as naked, or unconjugated, antibodies, and as such may also potentiate the antitumor effects of other therapeutic modalities, such as chemotherapy and radiation.

[0009] Colorectal, pancreatic, and ovarian cancers remain important contributors to cancer mortality. Their response to traditional chemotherapy and radiation therapy is mixed, however. Furthermore, these conventional forms of therapy have toxic side effects that limit their utility.

[0010] The use of monoclonal antibodies offers an alternative to traditional chemotherapy and radiation therapy. Tumor-specific and tumor-associated monoclonal antibodies can function alone (naked antibody therapy) or as conjugates in treatment regimes. The use of targeting monoclonal antibodies conjugated to radionuclides or other cytotoxic agents offers the possibility of delivering such agents directly to the tumor site, thereby limiting the exposure of normal tissues to toxic agents (Goldenberg, Semin. Nucl. Med., 19: 332 (1989); Goldenberg, DM, Radioimmunotherapy, in: Nuclear Medicine Annual 2001, L. Freeman, ed., Lippincott, William & Wilkins, Philadelphia, 2001, pp.167-206). In recent years, the potential of antibody-based therapy and its accuracy in the localization of tumor-associated antigens have been demonstrated both in the laboratory and clinical studies (see, e.g., Thorpe, TIBTECH, 11: 42 (1993); Goldenberg, Scientific American, Science & Medicine, 1: 64 (1994); Baldwin et al., U.S. Pat. Nos. 4,923,922 and 4.916,213; Young, U.S. Pat. No. 4,918,163; U.S. Pat. No. 5,204,095; Irie et al., U.S. Pat. No. 5,196,337; Hellstrom et al., U.S. Pat. Nos. 5,134,075 and 5,171,665; Thorpe et al., U.S. Pat. No. 6, 342,221, and Epstein et al., U.S. Pat. Nos. 5,965,132, 6,004554, 6,071,491, 6,017,514, 5,882,626 and 5,019,368. In general, the use of radiolabeled antibodies or antibody fragments against tumor-associated markers for localization of tumors has been more successful than for therapy, in part because antibody uptake by the tumor is generally low, ranging from only 0.01 % to 0.001 % of the total dose injected (Vaughan et al., Brit. J. Radiol., 60: 567 (1987)). Increasing the concentration of the radiolabel to increase the dosage to the tumor is generally counterproductive, as this also increases exposure of healthy tissue to radioactivity.

[0011] Mu-9 is a murine monoclonal antibody of the IgG_1 subtype, directed against the colon-specific antigen-p mucin (CSAp). CSAp is a tumor-associated antigen that is present in a high percentage of colorectal, as well as pancreatic and ovarian cancers. (Gold et al., Cancer Res., 50: 6405 (1990), and references cited therein). In pre-clinical and clinical testing, the antibody has shown excellent tumor targeting ability (Blumenthal et al., Int. J. Cancer, 22: 292 (1989); Sharkey et al., Cancer, 73(suppl): 864 (1994)). Mu-9 has an advantage over other antibodies that target tumor antigens because it recognizes an epitope which is not present in the circulation (Pant et al., Cancer, 50: 919 (1982)). Circulating antigen can alter the delivery of antibody therapy because the antibody forms circulating immune complexes, which in turn could affect tumor targeting and antibody pharmacokinetics and biodistribution.

[0012] As with most other promising non-human antibodies, the clinical use of murine Mu-9 may be limited by the development in humans of an anti-mouse antibody (HAMA) responses. This can limit the diagnostic/detection and therapeutic usefulness of the antibodies, not only because of the potential anaphylactic problem, but also because a major portion of the circulating antibody may be complexed to and sequestered by the circulating anti-mouse antibodies. The production of HAMA may also affect the accuracy of murine antibody-based immunoassays. Thus, HAMA responses in general pose a potential obstacle to realizing the full diagnostic and therapeutic potential of the Mu-9 antibody.

[0013] In order to maximize the value of the Mu-9 antibody as a therapeutic or diagnostic/detection modality and to increase its utility in multiple and continuous administration modalities and settings, an object of this invention is to provide a mouse-human chimeric mAb (cMu-9), a fully human, and a humanized mAb (hMu-9) that relate to Mu-9 by retaining the antigen-binding specificity of Mu-9, but that elicit reduced HAMA or other immune responses in a subject receiving the same.

[0014] Another object of this disclosure is to provide DNA sequences that encode the amino acid sequences of the

variable regions of the light and heavy chains of the cMu-9, human Mu-9, and hMu-9 mAbs, including the complementarity-determining regions (CDRs).

[0015] A further object of this disclosure is to provide conjugates of the hMu-9, human Mu-9, and cMu-9 mAbs containing therapeutic or diagnostic/detection modalities.

[0016] Another object of this disclosure is to provide combinations of antibodies with CSAp antibody or antibodies with other carcinoma-targeting antibodies, wherein said antibodies can be used as naked immunoglobulins or as conjugates with drugs, toxins, isotopes, cytokines, enzymes, enzyme-inhibitors, hormones, hormone antagonists, and other therapy-enhancing moieties.

[0017] Yet another object of this disclosure is to provide methods of therapy and diagnosis/detection that utilize the humanized, chimeric and fully human MAbs of the invention.

SUMMARY OF THE INVENTION

[0018] The present disclosure provides a monoclonal (MAb) antibody or fragment thereof that binds to a colon-specific antigen-p mucin (CSAp) antigen. Preferably, the monoclonal antibody or fragment thereof binds the Mu-9 epitope. Still preferred, the monoclonal antibody or fragment thereof is humanized, chimerized or fully human.

[0019] The invention also provides a humanized Mu-9 (hMu-9) monoclonal antibody (mAb) or a fragment thereof. The hMu-9 antibody or fragment contains the complementarity-determining regions (CDRs) of the light and heavy chain variable regions of a non-human Mu-9 antibody, which are joined to the framework (FR) regions of the light and heavy chain variable regions of a human antibody, which are subsequently joined to the light and heavy chain constant regions of a human antibody. This humanized antibody or fragment retains the CSAp antigen specificity of the parental Mu-9 antibody, but is less immunogenic in a human subject.

[0020] Disclosed is herein a chimeric Mu-9 (cMu-9) monoclonal antibody or fragment thereof. The cMu-9 antibody or fragment contains the light and heavy chain variable regions of a non-human Mu-9 antibody, which are joined to the light and heavy chain constant regions of a human antibody. This chimeric antibody retains the CSAp antigen specificity of the parental Mu-9 antibody, but is less immunogenic in a human subject.

[0021] Also contemplated in the present disclosure is a fully human Mu-9 antibody and fragments thereof.

[0022] Also contemplated in the present disclosure is a humanized antibody or fragment thereof comprising the complementarity-determining regions (CDRs) of a murine anti-CSAp MAb and the framework (FR) regions of the light and heavy chain variable regions of a human antibody and the light and heavy chain constant regions of a human antibody, wherein the CDRs of the light chain variable region of the humanized anti-CSAp MAb comprises CDR1 comprising an amino acid sequence of RSSQSIVHSNGNTYLE; CDR2 comprising an amino acid sequence of KVSNRFS and CDR3 comprising an amino acid sequence of FQGSRVPYT; and the CDRs of the heavy chain variable region of the humanized anti-CSAp MAb comprises CDR1 comprising an amino acid sequence of EYVIT; CDR2 comprising an amino acid sequence of EIYPGSGSTSYNEKFK and CDR3 comprising an amino acid sequence of EDL.

[0023] The present invention further provides a CDR-grafted humanized heavy chain comprising the complementarity determining regions (CDRs) of a murine anti-CSAp MAb and the framework region of the heavy chain variable region of a human antibody and the heavy chain constant region of a human antibody, wherein the CDRs of the heavy chain variable region of the humanized anti-CSAp MAb comprises CDR1 comprising an amino acid sequence of EYVIT; CDR2 comprising an amino acid sequence of EIYPGSGSTSYNEKFK and CDR3 comprising an amino acid sequence of EDL.

[0024] In a related vein, the present invention provides a CDR-grafted humanized light chain comprising the complementarity determining regions (CDRs) of a murine anti-CSAp MAb and the framework region of the light chain variable region of a human antibody and the light chain constant region of a human antibody, wherein the CDRs of the light chain variable region of the humanized anti-CSAp MAb comprises CDR1 comprising an amino acid sequence of RSSQSIVHSNGNTYLE; CDR2 comprising an amino acid sequence of KVSNRFS and CDR3 comprising an amino acid sequence of FQGSRVPYT.

[0025] The disclosure further relates to a diagnostic/detection immunoconjugate comprising an antibody component comprising an anti-CSAp MAb or fragment thereof or an antibody fusion protein or fragment thereof of any one of anti-CSAp antibodies described herein, wherein the antibody component is bound to at least one diagnostic/detection agent. Preferably, the diagnostic/detection immunoconjugate comprises at least one photoactive diagnostic/detection agent. More preferably, the photoactive diagnostic/detection agent comprises a chromagen or dye. Still preferred, the diagnostic/detection agent is a radionuclide with an energy between 20 and 2,000 keV.

[0026] In a related vein, the disclosure further provides a therapeutic immunoconjugate comprising an antibody component comprising an anti-CSAp MAb or fragment thereof or an antibody fusion protein or fragment thereof of any one of anti-CSAp antibodies described herein, wherein the antibody component is bound to at least one therapeutic agent. In a preferred embodiment, the therapeutic agent is a radionuclide, boron, gadolinium or uranium atoms, an immunomodulator, a cytokine, a hormone, a hormone antagonist, an enzyme, an enzyme inhibitor, a photoactive therapeutic agent, a cytotoxic drug, a toxin, an angiogenesis inhibitor, a second, different antibody, and a combination thereof. When

the therapeutic immunoconjugate is a radionuclide, the energy is preferably between 20 and 10,000 keV.

[0027] The disclosure further provides multivalent, multispecific antibody or fragment thereof comprising one or more antigen binding sites having affinity toward a CSAp target antigen and one or more hapten binding sites having affinity towards hapten molecules.

[0028] The disclosure also relates to an antibody fusion protein or fragment thereof comprising at least two anti-CSAp MAbs or fragments thereof, as described herein. Similarly, the invention contemplates an antibody fusion protein or fragment thereof that comprises at least one first anti-CSAp MAb or fragment thereof, as described herein, and at least one second MAb or fragment thereof, other than the anti-CSAp antibodies of the present disclosure.

[0029] The disclosure also provides a method of treating a malignancy in a subject, comprising the step of administering to said subject a therapeutically effective amount of an anti-CSAp antibody or fragment thereof, formulated in a pharmaceutically acceptable vehicle.

[0030] Similarly, the present disclosure provides for a method of treating or diagnosing/detecting a malignancy in a subject, comprising (i) administering to a subject in need thereof the antibody or fragments thereof of the present invention; (ii) waiting a sufficient amount of time for an amount of the non-binding protein to clear the subject's bloodstream; and (iii) administering to said subject a carrier molecule comprising a diagnostic agent, a therapeutic agent, or a combination thereof, that binds to a binding site of the antibody.

[0031] The disclosure also provides for a DNA sequence comprising a nucleic acid encoding a anti-CSAp MAb or fragment thereof selected from the group consisting

(a) an anti-CSAp MAb or fragment thereof as described herein;

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

(c) an antibody fusion protein or fragment thereof comprising at least one first anti-CSAp MAb or fragment thereof comprising said MAb or fragment thereof of any one of the anti-CSAp antibodies of the present disclosure and at least one second MAb or fragment thereof, other than the MAb or fragment thereof of the present disclosure 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 claims 1-47 and at least one second MAb or fragment thereof, other than the MAb or fragment thereof of any one of claims 1-47 wherein said second MAb is selected from the group consisting of CEA, EGP-1, EGP-2, MUC-1, MUC-2, MUC-3, MUC-4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, BrE3, Le-Y, A3, KS-1, CD40, VEGF antibody, and the antibody A33, and a combination thereof.

[0032] The disclosure also relates to a method of delivering a diagnostic/detection agent, a therapeutic agent, or a combination thereof to a target, comprising: (i) administering to a subject the antibody or fragments thereof of any one the anti-CSAp antibodies of the present disclosure; (ii) waiting a sufficient amount of time for an amount of the non-binding protein to clear the subject's blood stream; and (iii) 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 said antibody.

[0033] Described herein is also a method of treating a malignancy in a subject comprising administering to said subject a therapeutically effective amount of an antibody or fragment thereof or an antibody fusion protein or fragment thereof comprising at least two MAbs or fragments thereof, wherein at least one anti-CSAp MAb or fragment thereof or fusion proteins or fragments thereof as desribed herein, formulated in a pharmaceutically suitable excipient.

[0034] The present disclosure further relates to a method of treating a cancer cell in a subject comprising (i) administering to said subject a therapeutically effective amount of a composition comprising a naked anti-CSAp MAb or fragment thereof or a naked antibody fusion protein or fragment thereof of any one of the anti-CSAp antibodies of the present disclosure (ii) formulating the naked CSAp MAb or fragment thereof or antibody fusion protein or fragment thereof in a pharmaceutically suitable excipient.

[0035] In an additional aspect, the disclosure provides conjugates in which the hMu-9, human Mu-9, or cMu-9 is bonded to a diagnostic/detection or therapeutic reagent.

[0036] In a further aspect, the disclosure provides that unconjugated (naked) hMu-9, human Mu-9, or cMu-9 is administered in combination with other traditional as well as experimental therapy modalities, such as radiation, chemotherapy and surgery, or even with conjugates involving other, non-CSAp antibodies, and that the combination(s) may be simulataneously or at different times in the therapy cycle.

[0037] In still another aspect, the disclosure provides methods of diagnosing/detecting or treating a malignancy that include administering an effective amount of the aforementioned antibodies or conjugates. The antibodies or conjugates may be formulated in a pharmaceutically acceptable vehicle.

[0038] In a further aspect, the disclosure provides isolated polynucleotides that comprise DNA sequences encoding the amino acid sequences of the CDRs of the light and heavy chain variable regions of the hMu-9, human Mu-9, or cMu-

9 mAbs. Similarly, the disclosure provides isolated polynucleotides that comprise DNA sequences encoding the amino acid sequence of the light and heavy chain variable regions of the hMu-9, human Mu-9, or cMu-9 mAbs.

[0039] In yet another aspect, the disclosure provides amino acid sequences of the CDRs of the light and heavy chain variable regions of a Mu-9 antibody.

[0040] The present disclosure also seeks to provide *inter alia* a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate that can be modified for use in a wide variety of diagnostic and therapeutic applications.

[0041] The present inventors have discovered that it is advantageous to raise bsAbs against a targetable conjugate that is capable of carrying one or more diagnostic/detection or therapeutic agents. By utilizing this technique, the characteristics of the chelator, metal chelate complex, therapeutic agent or diagnostic/detection agent can be varied to accommodate differing applications, without raising new bsAbs for each new application. Further, by using this approach, two or more distinct chelators, metal chelate complexes or therapeutic agents can be used with the inventive bsAb.

[0042] Provided in the present disclosure is a method of treating or identifying diseased tissues in a subject, comprising:

(A) administering to a subject a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, wherein the arm that specifically binds a targeted tissue is a Mu-9 antibody;

(B) optionally, administering to the subject a clearing composition, and allowing said composition to clear non-localized antibodies or antibody fragments from circulation;

(C) administering to the subject a first targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents; and

(D) when said therapeutic agent is an enzyme, further administering to the subject

1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site; or

2) a drug which is capable of being detoxified in said subject
to form an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or

3) a prodrug which is activated in said subject through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or

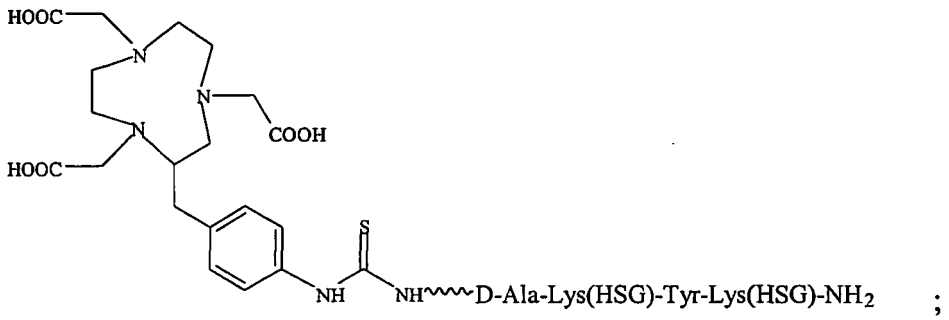
4) a second targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site.

[0043] The disclosure further provides a targetable conjugate that comprises at least two HSG haptens.

[0044] Also contemplated herein is a method for detecting or treating tumors expressing CSAp in a mammal, comprising:

(A) administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, wherein the one arm that specifically binds a targeted tissue is a Mu-9 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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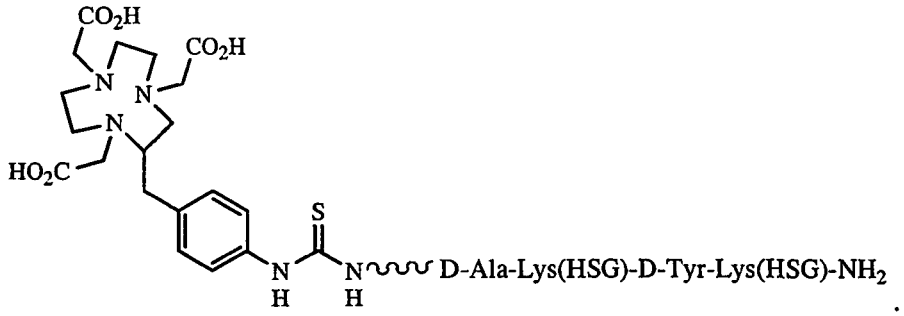

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[0045] This method optionally comprises administering to the subject a clearing composition, and allowing the composition to clear non-localized antibodies or antibody fragments from circulation.

[0046] Further, the disclosure provides a kit useful for treating or identifying diseased tissues in a subject comprising:

(A) a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, wherein said one arm that specifically binds a targeted tissue is a Mu-9 antibody or fragment thereof;

(B) a first targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents; and

(C) optionally, a clearing composition useful for clearing non-localized antibodies and antibody fragments; and

(D) optionally, when the therapeutic agent conjugated to said first targetable conjugate is an enzyme,

1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site; or

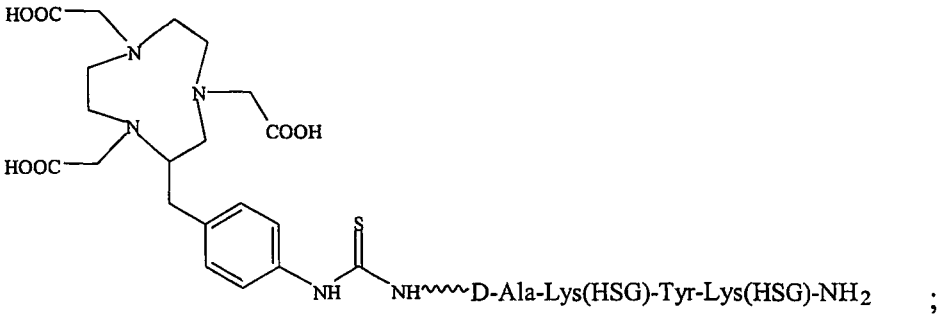
2) a drug which is capable of being detoxified in said subject to form an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or

3) a prodrug which is activated in said subject through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or

4) a second targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site.

[0047] As described herein, the targetable conjugate may consist 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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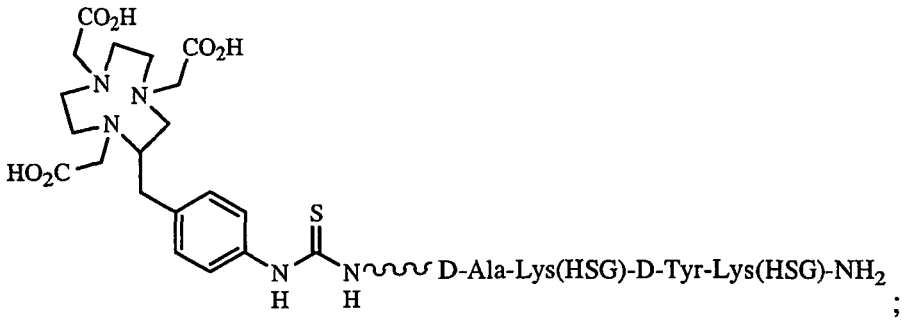

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[0048] The disclosure also relates to a method of screening for a targetable conjugate comprising:

(A) contacting the targetable construct with a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds said targetable conjugate to give a mixture, wherein the one arm that specifically binds a targeted tissue is a Mu-9 antibody or fragment thereof; and

(B) optionally incubating said mixture; and

(C) analyzing said mixture.

[0049] The disclosure further provides a method for imaging malignant tissue or cells in a mammal expressing CSAp, comprising:

(A) administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, wherein the one arm that specifically binds a targeted tissue is a Mu-9 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;
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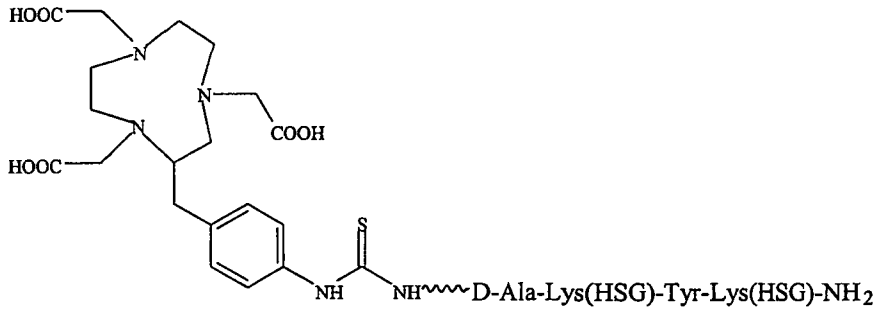

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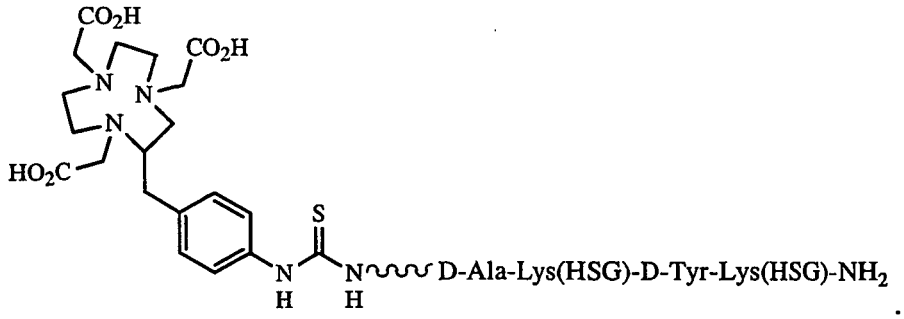

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[0050] The disclosure also provides a method of intraoperatively, endoscopically and intravascularly identifying/disclosing diseased tissues expressing CSAp in a subject, comprising the administration of a detectable amount of a CSAp-labeled antibody, preferably a fragment or subfragment, whereby the label is detected by a suitable probe or miniature camera within 48 hours of said labeled CSAp antibody/fragment administration, without the need of a clearing agent for non-targeted, labeled antibody or fragment.

[0051] In a related vein, disclosure provides a method of intraoperatively identifying/disclosing diseased tissues expressing CSAp, in a subject, comprising:

(A) administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue expressing CSAp and at least one other arm that specifically binds a targetable conjugate, wherein the one arm that specifically binds a targeted tissue is a Mu-9 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;
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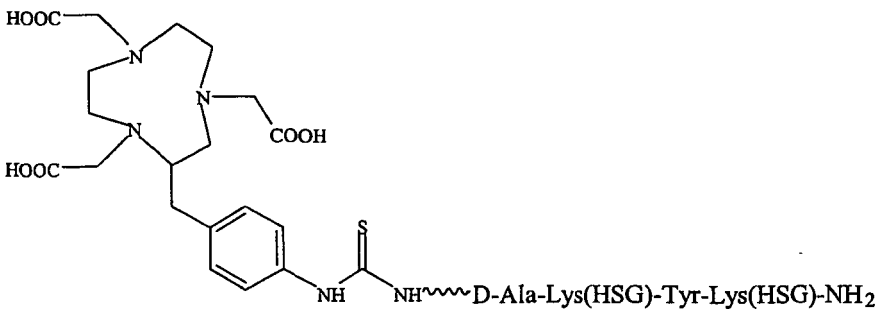

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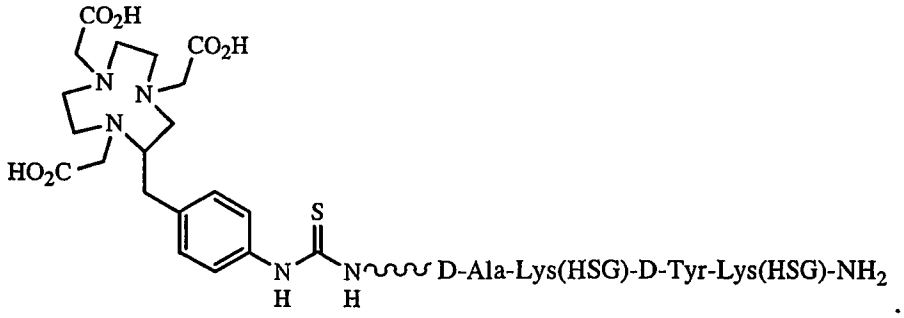

[0052] The disclosure further relates to a method for the endoscopic identification of diseased tissues expressing CSAp, in a subject, comprising:

(A) administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue expressing CSAp and at least one other arm that specifically binds a targetable conjugate wherein the one arm that specifically binds a targeted tissue is a Mu-9 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;
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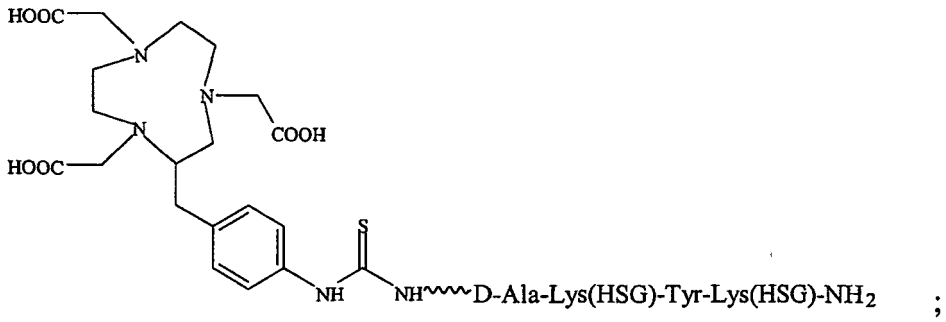

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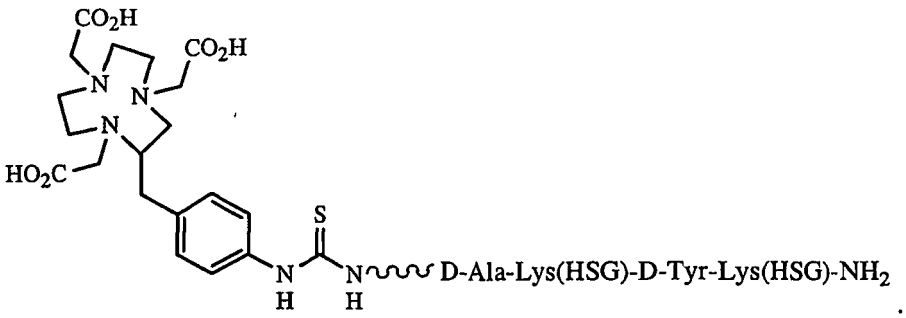

[0053] Also provided herein is a method for the intravascular identification of diseased tissues expressing CSAp, in a subject, comprising:

(A) administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue expressing CSAp and at least one other arm that specifically binds a targetable conjugate wherein the one arm that specifically binds a targeted tissue is a Mu-9 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;
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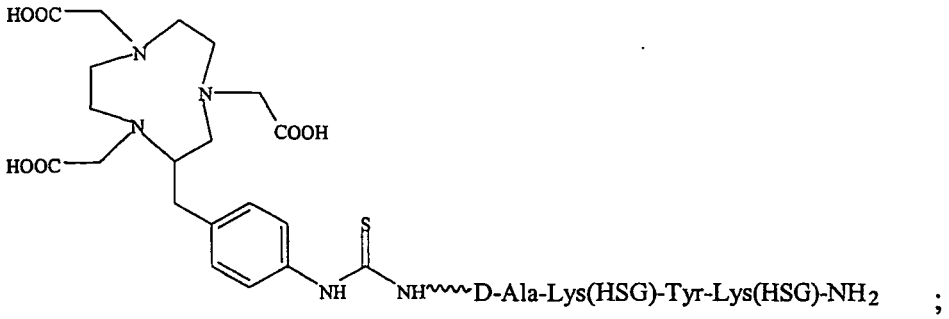

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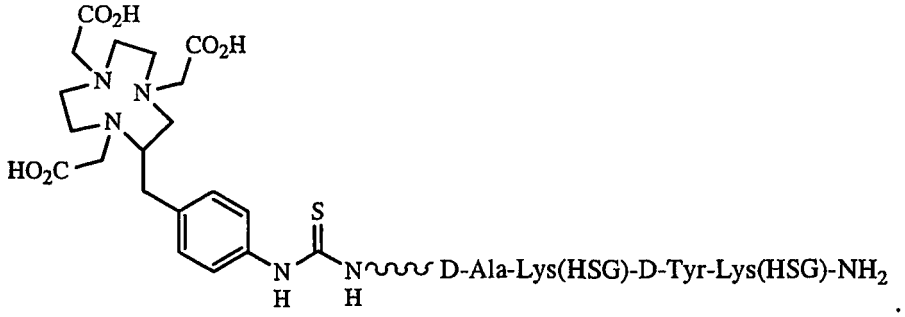

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[0054] The disclosure also relates to a method of detection of lesions during an endoscopic, laparoscopic, intravascular catheter, or surgical procedure, wherein the method comprises:

(a) injecting a subject who is to undergo such a procedure with a bispecific antibody $F(ab)_2$ or $F(ab')_2$ fragment, wherein the bispecific antibody or fragment has a first antibody binding site which specifically binds to a CSAp 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.

[0055] In a preferred embodiment, the hapten is labeled with a diagnostic radioisotope, a MRI image enhancing agent or a fluorescent label.

[0056] The disclosure further relates to a method for close-range lesion detection, during an operative, intravascular, laparoscopic, or endoscopic procedure, wherein the method comprises:

(a) injecting a subject to such a procedure parenterally with an effective amount of a Mu-9 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

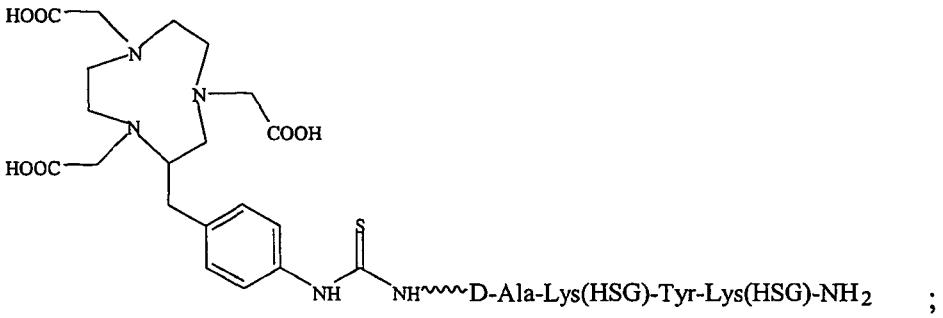
(d) locating the sites of accretion of said labeled antibody or fragment thereof by detecting elevated levels of said labeled antibody or fragment thereof at such sites with the detection means.

[0057] In the above examples of intraoperative, endoscopic and intravascular uses, the label attached to the diagnostic compound is capable of being detected by a suitable instrument or probe, including miniature cameras, which are made for said label detection (e.g., a gamma-detecting probe when a gamma-emitting isotope is the diagnostic/detection conjugate) (see Goldenberg, US Patents U.S. Pat. Nos. 5,716,595, 6, 096,289 and U.S. Patent Application publication number US 2001006618. .

[0058] The present disclosure also seeks to provide *inter alia* a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate that can be modified for use in a wide variety of diagnostic and therapeutic applications.

[0059] Further, the disclosure provides pre-targeting methods of diagnosis and therapy using the combination of bi-specific antibody and the targetable conjugates:

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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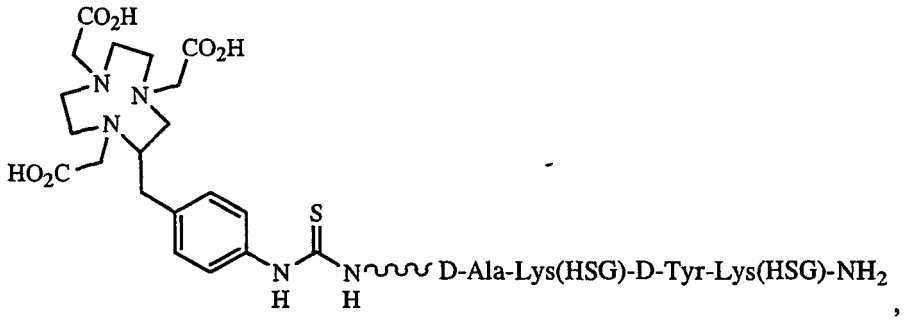

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as well as methods of making the bi-specifics, and kits for use in such methods.

[0060] The present inventors have discovered that it is advantageous to raise bsAbs against a targetable conjugate that is capable of carrying one or more diagnostic or therapeutic agents. By utilizing this technique, the characteristics of the chelator, metal chelate complex, therapeutic agent or diagnostic agent can be varied to accommodate differing

applications, without raising new bsAbs for each new application. Further, by using this approach, two or more distinct chelators, metal chelate complexes or therapeutic agents can be used with the bsAb.

[0061] The disclosure relates to a method of treating or identifying diseased tissues in a subject, comprising:

(A) administering to said subject a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate comprising at least two HSG haptens;

(B) optionally, administering to said subject a clearing composition, and allowing said composition to clear non-localized antibodies or antibody fragments from circulation;

(C) administering to said subject a targetable conjugate which comprises a carrier portion which comprises or bears at least two HSG haptens and may comprise a diagnostic or therapeutic cation, and/or one or more chelated or chemically bound therapeutic or diagnostic agents, or enzymes; and

(D) when said targetable conjugate comprises an enzyme, further administering to said subject

1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site; or
2) a drug which is capable of being detoxified in said subject to form an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or
3) a prodrug which is activated in said subject through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site.

[0062] The disclosure further relates to a method for detecting or treating target cells, tissues or pathogens in a mammal, comprising:

administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith; and
administering a targetable conjugate selected from the group consisting of

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
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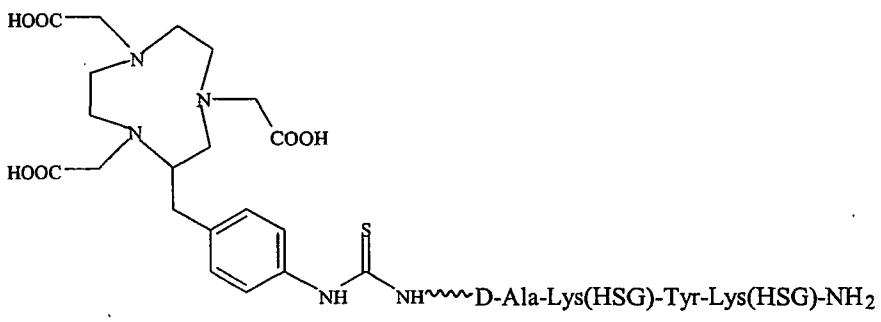

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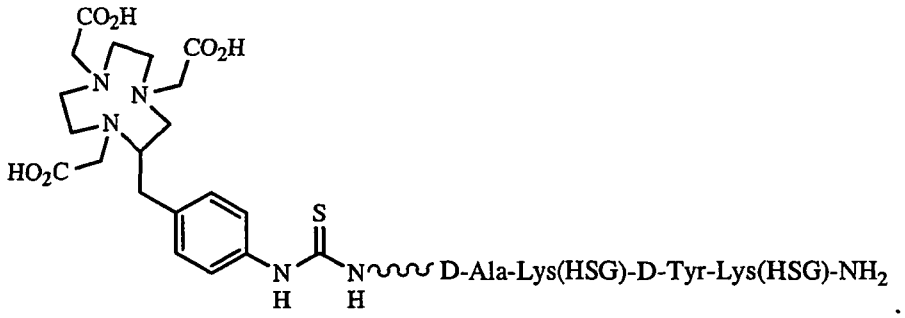

[0063] The disclosure further relates to a method of treating or identifying diseased tissues in a subject, comprising:

administering to said subject a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;
optionally, administering to said subject a clearing composition, and allowing said composition to clear non-localized antibodies or antibody fragments from circulation; and
administering to said subject a targetable conjugate selected from the group consisting of:

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
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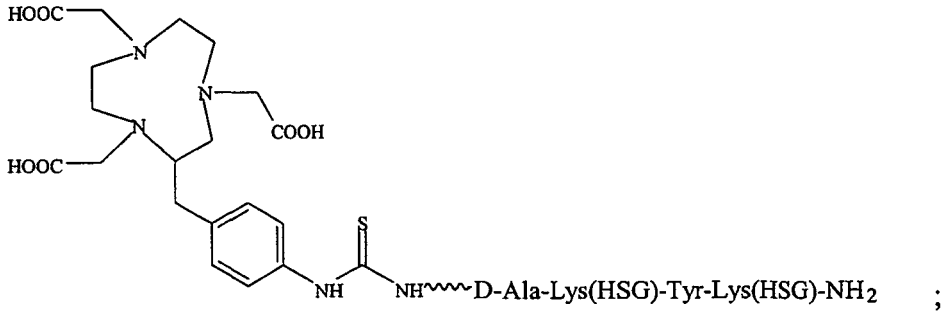

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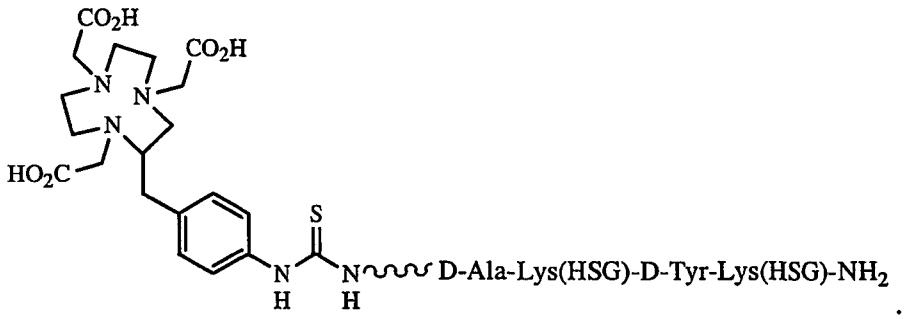

[0064] The disclosure further relates to a kit useful for treating or identifying diseased tissues in a subject comprising:

(A) a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, wherein said conjugate is selected from the group consisting of

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
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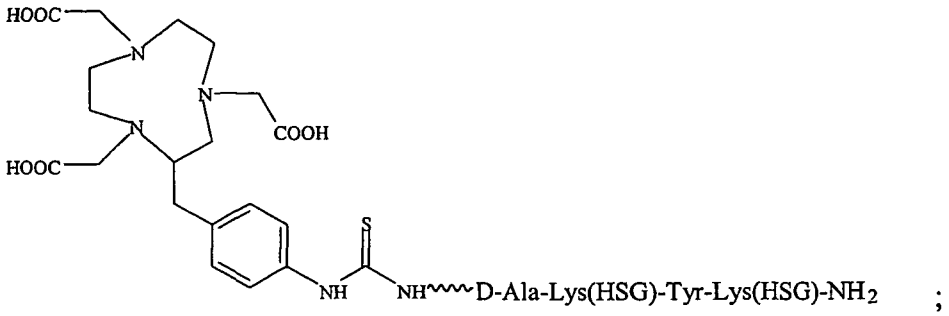

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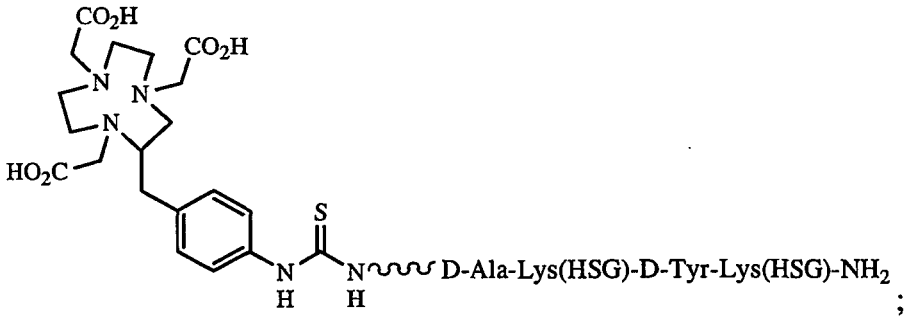

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(B) a targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents, or enzymes; and

(C) optionally, a clearing composition useful for clearing non-localized antibodies and antibody fragments; and

(D) optionally, when said first targetable conjugate comprises an enzyme,

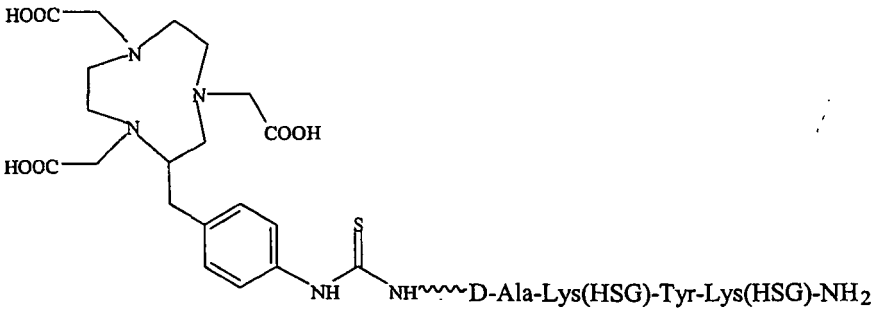
1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site; or

2) a drug which is capable of being detoxified in said subject to form an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or

3) a prodrug which is activated in said subject through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site.

[0065] The disclosure further relates to a targetable conjugate selected from the group consisting of:

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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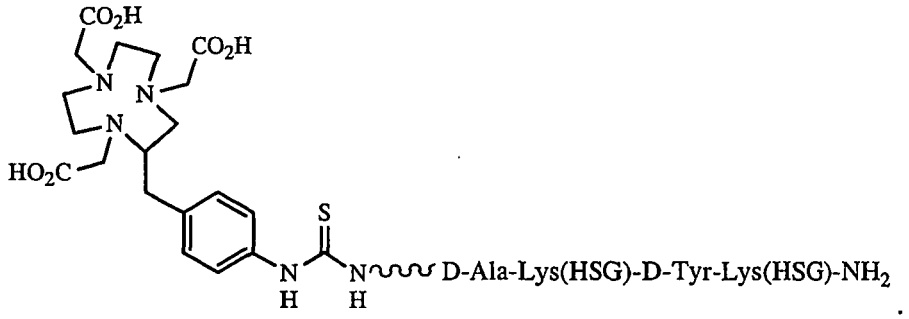

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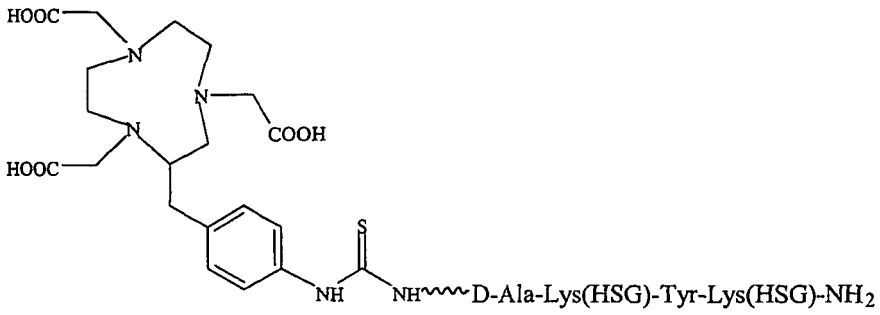
[0066] The disclosure further relates to a method of screening for a targetable conjugate comprising:

contacting said targetable construct with a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds said targetable conjugate to give a mixture;
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith; and
optionally incubating said mixture; and
analyzing said mixture.

[0067] The disclosure further relates to a method for imaging normal tissue in a mammal, comprising:

administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith; and
administering a targetable conjugate selected from the group consisting of

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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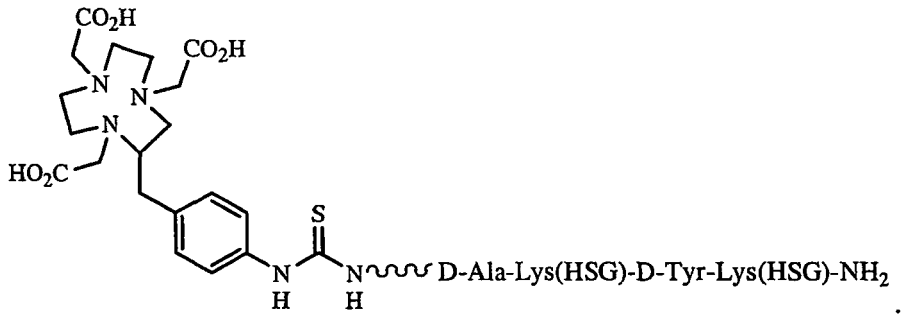

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[0068] The disclosure further relates to a method of intraoperatively identifying diseased tissues, in a subject, comprising:

administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate; wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith; and

administering a targetable conjugate selected from the group consisting of

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

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

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

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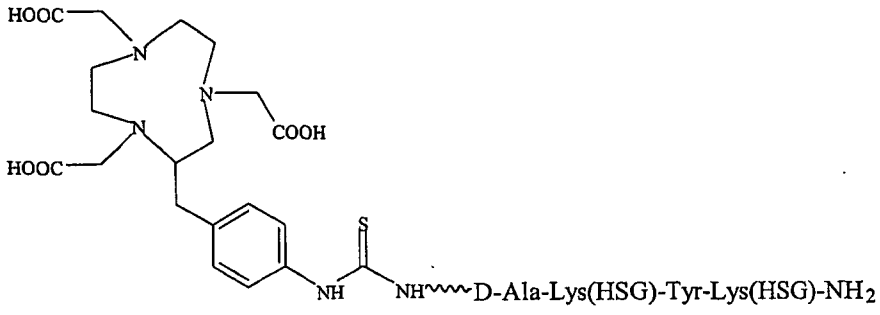

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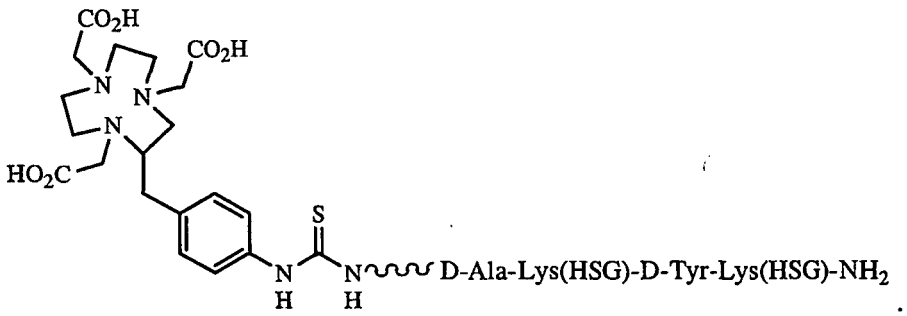

[0069] The disclosure further relates to a method for the endoscopic identification of diseased tissues, in a subject, comprising:

administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate; wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith; and
administering a targetable conjugate selected from the group consisting of

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
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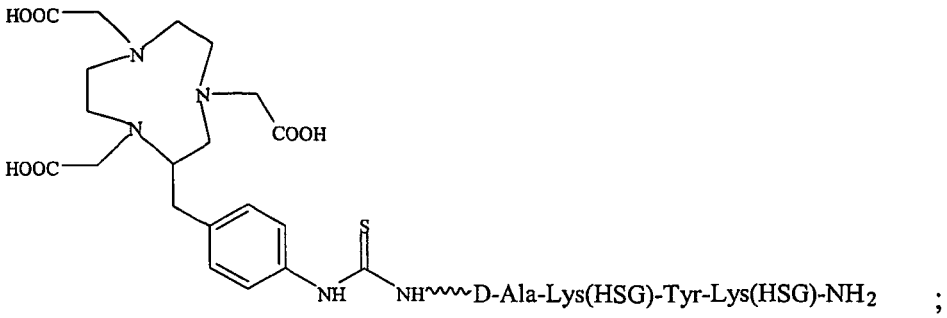

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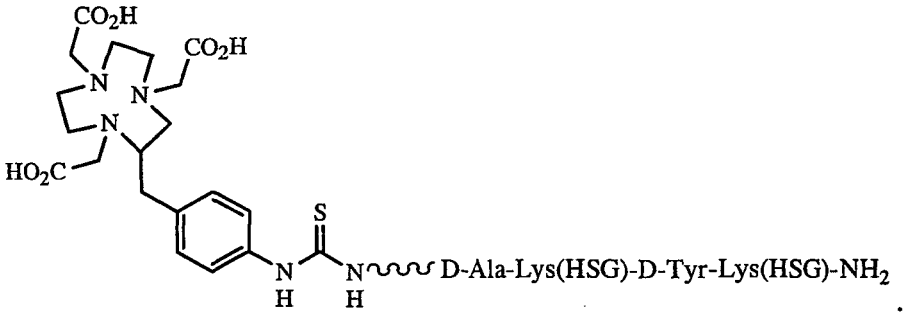


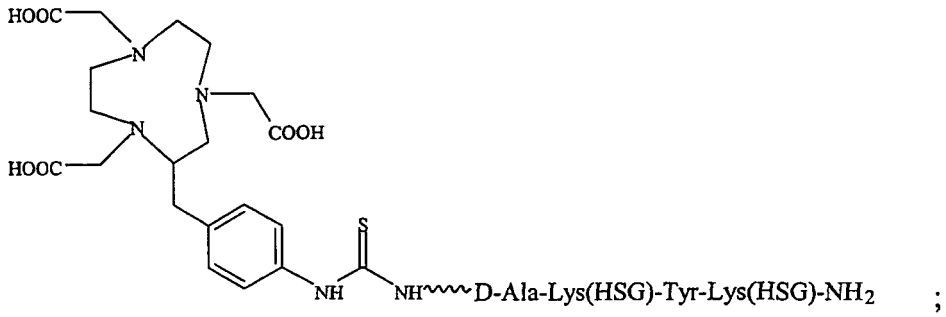
[0070] The disclosure further relates to a method for the intravascular identification of diseased tissues, in a subject, comprising:

administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;

wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith; and

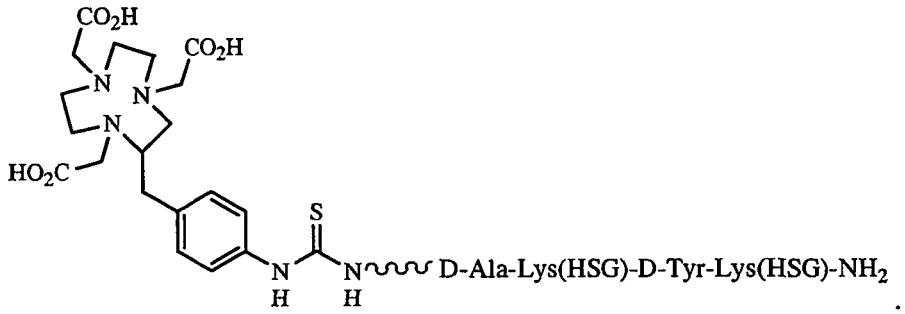
administering a targetable conjugate selected from the group consisting of

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



;

and
(e)



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[0071] These and other aspects and embodiments of the invention will become apparent by reference to the following specification and appended claims.

BRIEF DESCRIPTION OF THE FIGURES

[0072]

Figure 1 shows the DNA and amino acid sequences of the murine 679 V_k obtained by 5'-RACE and determined by DNA sequencing. Amino acid sequences encoded by the corresponding DNA sequences are given as one letter codes below the nucleotide sequence. The nucleotide and amino acid residues are numbered sequentially and indicated on the right side. The amino acid residues in the CDR regions are shown in bold and underlined.

Figure 2 shows the DNA and amino acid sequences of the murine 679 V_H obtained by RT-PCR and determined by DNA sequencing. Amino acid sequences encoded by the corresponding DNA sequences are given as one letter codes below the nucleotide sequence. The nucleotide and amino acid residues are numbered sequentially and indicated on the right side. The amino acid residues in the CDR regions are shown in bold and underlined.

Figure 3 shows the DNA and amino acid sequences of a single chain Fv fragment (scFv) of the MAb 679. Amino acid sequences encoded by the corresponding DNA sequences are given as one letter codes below the nucleotide sequence. The nucleotide and amino acid residues are numbered sequentially and indicated on the right side. The amino acid residues in the CDR regions are shown in bold and underlined. The amino acid residues serving as the

linkage between the V and VH are also underlined and indicated.

Figure 4 shows the DNA and amino acid sequences of the functional Mu-9V obtained by cDNA screening. Amino acid residues encoded by the corresponding DNA sequences are given as one letter codes below the nucleotide sequence. Both nucleotide and amino acid residues are numbered sequentially and indicated on the right side. The amino acid residues in the CDR regions are shown in bold and underlined.

Figure 5 shows the DNA and amino acid coding sequences of the Mu-9VH obtained by RT-PCR. Only the VH coding sequence is shown. Amino acid sequences encoded by the corresponding DNA sequences are given as one letter codes below the nucleotide sequence. Both nucleotide and amino acid residues are numbered sequentially and indicated on the right side. The amino acid residues in the CDR regions are shown in bold and underlined.

Figure 6 shows the DNA sequence coding for the humanized Mu-9 (hMu-9) light chain variable region as generated by a combination of long oligonucleotide synthesis and PCR as described in Example 11. The amino acid residues encoded by the DNA are given as one letter codes and shown under the corresponding codons. The amino acid residues in the CDR regions are shown in bold and underlined.

Figure 7 shows the DNA and amino acid sequences of the hMu-9 heavy chain variable region as generated by a combination of long oligonucleotide synthesis and PCR as described in Example 11. The amino acid residues encoded by the DNA are given as one letter codes and shown under the corresponding codons. The amino acid residues in the CDR regions are shown in bold and underlined.

Figure 8 shows the DNA and amino acid sequences of the Mu-9VH obtained by RT-PCR. The sequence arrow-underlined represents the 5'-end PCR primer sequence. The VH region was amplified using primers VH1BACK (5' AGG T(C/G)(A/C) A(A/G)C TGC AG(C/G) AGT C(A/T)G G 3') and CH1B. 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 residues numbered with a letter only are the insertion residues defined by Kabat numbering scheme and have the same preceeding digits as the previous one. For example, residues 82, 82A, 82B, and 82C in Figure 8 are indicated as 82, A, B, and C, respectively.

Figure 9 shows the DNA and amino acid sequences of the chimeric Mu-9 (cMu-9) heavy and light chain variable regions expressed in Sp2/0 cells. Figure 9A shows the DNA and amino acid sequences of the cMu-9VH. Figure 9B shows the double-stranded DNA and amino acid sequences of the cMu-9V . Amino acid sequences encoded by the corresponding DNA sequences are given as one letter codes. The amino acid residues in the CDR regions are shown in bold and underlined. Numbering of the nucleotide sequence is on the right side. The numbering of amno acids is same as that in Figure 8. The restriction sites used for constructing the cMu9 are underlined and indicated.

Figure 10 shows the alignment of the amino acid sequences of heavy and light chain variable regions of a human antibody, Mu-9 and hMu-9. Figure 10A shows the VH amino acid sequence alignment of the human antibody EU (FR1-3) and NEWM (FR4) with Mu-9 and hMu9-9 and Figure 10B shows the V amino acid sequence alignment of the human antibody WOL with Mu-9 and hMu-9. Dots indicate the residues in Mu-9 that are identical to the corresponding residues in the human antibodies. Boxed regions represent the CDR regions. Both N- and C-terminal residues (underlined) of hMu-9 are fixed by the staging vectors used and not compared with the human antibodies. Kabat's Ig molecule number scheme is used to number the residues as in Fig. 8.

Figure 11 shows the DNA and amino acid sequences of the hMu-9 heavy and light chain variable regions expressed in Sp2/0 cells. Figure 11A shows the DNA and amino acid sequences of the hMu-9VH. Figure 11B shows the DNA and amino acid sequences of the hMu-9V_{κ}. Numbering of the nucleotide sequence is on the right side. Amino acid sequences encoded by the corresponding DNA sequences are given as one letter codes. The amino acid residues in the CDR regions are shown in bold and underlined. Kabat's Ig molecule numbering scheme is used for amino acid residues as in Fig. 8.

Figure 12 shows a comparison of mMu-9 and hMu-9 in competitive binding assays. Varying concentrations of competeing Abs were used to compete with the binding of a constant amount of HRP-mMu-9 to the antigen coated wells. hMu-9 showed comparable blocking activity as that of mMu-9. A comparison of mMu-9 and cMu-9 can be found in Krishnan et al. (Cancer, 80:2667-2674 (1997)).

DETAILED DESCRIPTION

I. Overview

[0073] The present disclosure encompasses antibodies and antibody fragments. The antibody fragments are antigen binding portions of an antibody, such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, and the like. The antibody fragments bind to the same antigen that is recognized by the intact antibody. For example, an anti-CD22 or anti-CSAp monoclonal antibody fragment binds to an epitope of CD22 or CSAp, respectively.

[0074] 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, "Fv" fragments, consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy chain variable regions are connected by a peptide linker ("sFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the "hypervariable region." Three of these so-called "hypervariable" regions or "complementarity-determining regions" (CDR) are found in each variable region of the light or heavy chain. Each CDR is flanked by relatively conserved framework regions (FR). The FR are thought to maintain the structural integrity of the variable region. The CDRs of a light chain and the CDRs of a corresponding heavy chain form the antigen-binding site. The "hypervariability" of the CDRs accounts for the diversity of specificity of antibodies.

[0075] Also contemplated in the present disclosure are human, chimeric and humanized anti-CSAp antibodies and fragments thereof. The fully human anti-CSAp antibody of the present invention is preferably against the Mu-9 antigen. A human antibody is an antibody obtained, for example, 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 locus 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 Opinion in Structural Biology 3:5564-571 (1993).

[0076] Human antibodies may also be generated by *in vitro* activated B cells. See U.S. Patent Nos. 5,567,610 and 5,229,275.

[0077] The present disclosure also provides a chimeric anti-CSAp monoclonal antibody or fragment thereof. The chimeric anti-CSAp antibody or fragment contains a light and heavy chain variable region of a non-human anti-CSAp antibody, which are joined to the light and heavy chain constant regions of a human antibody. Preferably, the light and heavy chain variable regions come from a murine anti-CSAp antibody. In a preferred embodiment, the anti-CSAp antibody binds a Mu-9 epitope on the CSAp antigen. Accordingly, a Mu-9 antibody is an anti-CSAp antibody that binds to the Mu-9 epitope.

[0078] The process for making cMu-9 is described in detail below. Briefly, cDNAs encoding the $V\kappa$ and V_H regions of the Mu-9 mAb have been isolated and separately recombinantly subcloned into mammalian expression vectors that contain the genes a human κ light chain constant region sequence and a human $\gamma 1$ chain sequence, respectively. Cotransfection of mammalian cells with these two recombinant DNAs resulted in expression of a cMu-9 mAb that, like the parent Mu-9 mAb, bound avidly to the CSAp antigen.

[0079] In a preferred embodiment, the light chain variable region of the cMu-9 antibody comprises the amino acids of SEQ. ID NO: (figure 9B) or the heavy chain variable region of the cMu-9 antibody comprises the amino acids of SEQ. ID NO: (figure 9A). Still preferred, the light chain variable region of the cMu-9 antibody comprises the amino acids of SEQ. ID NO: (figure 9B) and the heavy chain variable region of the cMu-9 antibody comprises the amino acids of SEQ. ID NO: (figure 9A).

[0080] The present invention further provides a humanized Mu-9 (hMu-9) monoclonal antibody (mAb) or a fragment thereof. The hMu-9 antibody or fragment contains the complementarity-determining regions (CDRs) of the light and heavy chain variable regions of a non-human Mu-9 antibody, which are joined to the framework (FR) regions of the light and heavy chain variable regions of a human antibody, which are subsequently joined to the light and heavy chain constant regions of a human antibody. This humanized antibody or fragment retains the CSAp antigen specificity of the

parental Mu-9 antibody, but is less immunogenic in a human subject.

[0081] Methods for making hMu-9 are described in detail below. Briefly, however, to make hMu-9, the CDRs of the V_κ and V_H DNAs have been recombinantly linked to the framework (FR) sequences of the human V_κ and V_H regions, respectively, which are subsequently linked, respectively, to the human kappa and γ1 constant regions, so as to express in mammalian cells as described above hMu-9.

[0082] In another embodiment of the present invention, hMu-9, the CDRs of the light chain variable region comprise CDR1 comprising amino acids 24 to 34 of SEQ ID NO: (figure 9B), CDR2 comprising amino acids 50 to 56 of SEQ ID NO: (figure 9B), and CDR3 comprising amino acids 89 to 97 of SEQ ID NO: (figure 9B); and the CDRs of the heavy chain variable region comprise CDR1 comprising amino acids 31 to 35 of SEQ ID NO: (figure 9A), CDR2 comprising amino acids 50 to 64 of SEQ ID NO: (figure, 9A), and CDR3. comprising amino acids 95 to 97 of SEQ ID NO: (figure 9A).

[0083] Other preferred embodiments of the invention include anti-CSAp antibody fragments comprising the light chain variable region of SEQ ID NO: (figure 4B) and/or the heavy chain variable region of SEQ ID NO: (figure 4A).

[0084] In this specification, the expressions "cMu-9" or "cMu-9 mAb" are intended to refer to the chimeric monoclonal antibody constructed by joining or subcloning the non-human Vk and VH regions to the human constant light and heavy chains, respectively. The expressions "hMu-9" or "hMu-9 mAb" are intended to refer to the humanization of the chimeric monoclonal antibody by replacing the non-human FR sequences in cMu-9 with that of human framework regions. Preferably, the anti-CSAp humanized antibodies and fragments thereof of the present disclosure comprise framework region sequences where at least one amino acid of the corresponding non-human light or heavy chain framework regions is retained. Preferably, an amino acid from the murine antibody FR is retained in the same position of the corresponding humanized antibody.

[0085] 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 is 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.

[0086] A humanized antibody is a recombinant protein in which the CDRs from an antibody from one species; e.g., a rodent antibody, is 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 is derived from those of a human antibody.

[0087] The present disclosure also contemplates anti-CSAp antibody fragments. The antibody fragments are antigen binding portions of an antibody, such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, and the like. The antibody fragments contain one or more CDRs of the intact antibody and bind to the same antigen that is recognized by the intact antibody. For example, an anti-colon-specific antigen-p (CSAp) monoclonal antibody fragment binds to an epitope of colon-specific antigen-p.

[0088] Also, the present disclosure provides a bi-specific antibody or antibody fragment having at least one arm that is reactive against a targeted tissue and at least one other arm that is reactive against a targetable construct. The targetable construct is comprised of a carrier portion and at least 2 units of a recognizable hapten. Examples of recognizable haptens include, but are not limited to, histamine succinyl glycine(HSG) and fluorescein isothiocyanate. The targetable construct may be conjugated to a variety of agents useful for treating or identifying diseased tissue. Examples of conjugated agents include, but are not limited to, chelators, metal chelate complexes, drugs, toxins (e.g., ricin, abrin, ribonuclease, DNase I, *Staphylococcal* enterotoxin-A, pokeweed antiviral protein, gelonin, diphtherin toxin, *Pseudomonas* exotoxin, *Pseudomonas* endotoxin) and other effector molecules. Additionally, enzymes useful for activating a prodrug or increasing the target-specific toxicity of a drug can be conjugated to the targetable construct. Thus, the use of bsAb which are reactive to a targetable construct allows a variety of therapeutic and diagnostic/detection applications to be performed without raising new bsAb for each application.

[0089] Additionally, the present disclosure encompasses a method for detecting or treating target cells, tissues or pathogens in a mammal, comprising administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate. As used herein, the term "pathogen" includes, but is not limited to fungi, viruses (e.g., human immunodeficiency virus (HIV), herpes virus, cytomegalovirus, rabies virus, influenza virus, hepatitis B virus, Sendai virus, feline leukemia virus, Reo virus, polio virus, human serum parvo-like virus, simian virus 40, respiratory syncytial virus, mouse mammary tumor virus, Varicella-Zoster virus, Dengue virus, rubella virus, measles virus, adenovirus, human T-cell leukemia viruses, Epstein-Barr virus, murine leukemia virus, mumps virus, vesicular stomatitis virus, Sindbis virus, lymphocytic choriomeningitis virus, wart virus and blue tongue virus), parasites and bacteria (e.g., Streptococcus agalactiae, Legionella pneumophilia, Streptococcus pyogenes, Escherichia coli, Neisseria gonorrhoeae, Neisseria meningitidis, Pneumococcus, Hemophilis influenzae B, Treponema pallidum, Lyme disease spirochetes, Pseudomonas aeruginosa, Mycobacterium leprae, Brucella abortus, Mycobacterium tuberculosis and Tetanus toxin). *See* U.S. Patent No. 5,332,567.

[0090] Antibodies that do not target the CSAp antigen can be used in this disclosure. For example, antibodies against other antigens associated with carcinomas, particularly carcinomas of the gastrointestinal system (colon, rectum, pancreas tumors) and ovarian cancer, can be combined with CSAp antibodies and also used as fusion partners with CSAp

antibodies. Antibodies against intracellular and other antigens associated with necrosis, angiogenesis factors, immune response factors (e.g., CD40), as well as products of oncogenes, may also be used in combination with CSAp antibodies and as fusion partners for CSAp antibodies. Anti-necrosis antibodies are described in Epstein et al., U.S. Pat. Nos. 6,071,491, 6.017,514, 5,019,368 and 5,882,626,

[0091] Immunoconjugates between chimeric, humanized and human anti-CSAp antibodies or fragments thereof and a diagnostic or therapeutic reagent, formulated in pharmaceutically acceptable vehicles (see, e.g., Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, Pa., 1990) can be prepared. An immunoconjugate is a conjugate of an antibody component with a therapeutic or diagnostic agent. As random (non-specific) conjugation often results in products with reduced binding activity, it is preferred to use conjugates in which the reagent is site-specifically bound to the antibody through, for example, carbohydrate moieties, such as through oxidized carbohydrate derivatives. Carbohydrade moieties can be introduced into an antibody by site-specific mutagenesis without altering the immunoreactivity. Methods for the production of such conjugates and their use in diagnostics and therapeutics are provided, for example in Shih et al., U.S. Pat. No. 5,057,313; Shih et al., Int. J. Cancer 41: 832 (1988); and Hansen et al., U.S. Pat. No. 5,443,953. Direct linkage of the reagent to oxidized carbohydrate without the use of a polymeric carrier is described in McKearn et al., U.S. Pat. No. 5,156, 840,

[0092] A wide variety of diagnostic/detection and therapeutic reagents can be advantageously conjugated to the antibodies of the disclosure. These include, but are not limited to, different classes of chemotherapeutic agents, such as anthracyclines, antibiotics, alkylating agents, anti-mitotic agents, anti-angiogenesis agents, plant alkaloids, COX-inhibitors, antimetabolites e.g., doxorubicin, CPT-11, oxaliplatin, methotrexate, taxol and other taxanes, and the like; chelators, such as DTPA, to which detectable labels such as fluorescent molecules or cytotoxic agents, such as heavy metals or radionuclides can be complexed; and toxins such as Pseudomonas exotoxin, RNAse, gelonin, and the like.

[0093] 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, enzymes, enzyme-inhibitors, nucleases, hormones, hormone antagonists, immunomodulators, chelators, boron compounds, uranium atoms, photoactive agents or dyes and radionuclides. Radionuclides in therapeutic agents, which substantially decay by beta-particle emission include, but are not limited to: P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, and Bi-213. 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 Auger-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.

[0094] Enzymes are also useful therapeutic agents. For example, alkaline phosphatase for use in combination with phosphate-containing prodrugs (U.S. Pat. No. 4,975,278); arylsulfatase for use in combination with sulfate-containing prodrugs (U.S. Pat. No. 5,270,196); peptidases and proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidase (U.S. Pat. Nos. 5,660,829; 5,587,161; 5,405,990) and cathepsins (including cathepsin B and L), for use in combination with peptide-based prodrugs; D-alanylcarboxypeptidases for use in combination with D-amino acid-modified prodrugs; carbohydrate-cleaving enzymes such as beta-galactosidase and neuraminidase for use in combination with glycosylated prodrugs (U.S. Pat. Nos. 5,561,119; 5,646,298); beta-lactamase for use in combination with .beta.-lactam-containing prodrugs; penicillin amidases, such as penicillin V amidase (U.S. Pat. No. 4,975,278) or penicillin G amidase, for use in combination with drugs derivatized at their amino nitrogens with phenoxyacetamide or phenylacetamide groups; and cytosine deaminase (U.S. Pat. Nos. 5,338,678; 5,545,548) for use in combination with 5-fluorocytosine-based prodrugs (U.S. Pat. No. 4,975,278) are suitable therapeutic agents for the present disclosure.

[0095] Anti-angiogenic agents (or angiogenesis inhibitors) suitable for use in combination therapy or for conjugating to antibodies include angiostatin, endostatin, vasculostatin, canstatin and maspin.

[0096] Other useful therapeutic agents include metals, such as those as part of a photodynamic therapy, and nuclides, such as those valuable in therapies based on neutron capture procedures. Specifically, zinc, aluminum, gallium, lutetium and palladium are useful for photodynamic therapy and B-10, Gd-157 and U-235 are useful for neutron capture therapy.

[0097] 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/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 agents are selected from the group consisting of radioisotopes for nuclear imaging, intraoperative and endoscopic 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. 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 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.

[0098] Paramagnetic ions suitable for the present disclosure 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.

[0099] 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.

[0100] Metals are also useful in diagnostic 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 purpose. Chelates are coupled to the peptide antigens 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 disclosure. 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 disclosure.

[0101] 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.

[0102] As used herein, the term "subject" refers to any animal (i.e., vertebrates and invertebrates) including, but not limited to humans and other primates, rodents (e.g., mice, rats, and guinea pigs), lagamorphs (e.g., rabbits), bovines (e.g, cattle), ovines (e.g., sheep), caprines (e.g., goats), porcines (e.g., swine), equines (e.g., horses), canines (e.g., dogs), felines (e.g., cats), domestic fowl (e.g., chickens, turkeys, ducks, geese, other gallinaceous birds, etc.), as well as feral or wild animals, including, but not limited to, such animals as ungulates (e.g., deer), bear, fish, lagamorphs, rodents, birds, etc. It is not intended that the term be limited to a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are encompassed by the term.

II. Preparation of Antibodies

[0103] Monoclonal antibodies (MAbs) are a homogeneous population of antibodies to a particular antigen and the

antibody comprises only one type of antigen binding site and binds to only one epitope on an antigenic determinant. Rodent monoclonal antibodies to 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, monoclonal antibodies can be obtained by injecting mice with a composition comprising an 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 the antigen, culturing the clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures.

[0104] 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).

[0105] Abs to peptide backbones are generated by well-known methods for Ab production. For example, injection of an immunogen, such as (peptide)n-KLH, wherein KLH is keyhole limpet hemocyanin, and n=1-30, in complete Freund's adjuvant, followed by two subsequent injections of the same immunogen suspended in incomplete Freund's adjuvant into immunocompetent animals, is followed three days after an i.v. boost of antigen, by spleen cell harvesting. Harvested spleen cells are then fused with Sp2/0-Ag14 myeloma cells and culture supernatants of the resulting clones analyzed for anti-peptide reactivity using a direct-binding ELISA. Fine specificity of generated Abs can be analyzed for by using peptide fragments of the original immunogen. These fragments can be prepared readily using an automated peptide synthesizer. For Ab production, enzyme-deficient hybridomas are isolated to enable selection of fused cell lines. This technique also can be used to raise antibodies to one or more of the chelates comprising the linker, e.g., In(III)-DTPA chelates. Monoclonal mouse antibodies to an In(III)-di-DTPA are known (Barbet '395 *supra*).

[0106] The antibodies used in the present disclosure are specific to a variety of cell surface or intracellular tumor-associated antigens as marker substances. These markers may be substances produced by the tumor or may be substances which accumulate at a tumor site, on tumor cell surfaces or within tumor cells, whether in the cytoplasm, the nucleus or in various organelles or sub-cellular structures, or even as part of the endothelium of vessels nourishing tumors or elaborated by the tumor vasculature. Among such tumor-associated markers are those disclosed by Herberman, "Immunodiagnosis of Cancer," in Fleisher ed., "The Clinical Biochemistry of Cancer," page 347 (American Association of Clinical Chemists, 1979) and in U.S. Patent Nos. 4,150,149; 4,361,544; and 4,444,744.

[0107] Tumor-associated markers have been categorized by Herberman, *supra,* in a number of categories including oncofetal antigens, placental antigens, oncogenic or tumor virus associated antigens, tissue associated antigens, organ associated antigens, ectopic hormones and normal antigens or variants thereof. Occasionally, a sub-unit of a tumor-associated marker is advantageously used to raise antibodies having higher tumor-specificity, e.g., the beta-subunit of human chorionic gonadotropin (HCG) or the gamma region of carcino embryonic antigen (CEA), which stimulate the production of antibodies having a greatly reduced cross-reactivity to non-tumor substances as disclosed in U.S. Patent Nos. 4,361,644 and 4,444,744.

[0108] Another marker of interest is transmembrane activator and CAML-interactor (TACI). *See* Yu et al. Nat. Immunol. 1:252-256 (2000). Briefly, TACI is a marker for B-cell malignancies (e.g., lymphoma). Further it is known that TACI and B cell maturation antigen (BCMA) are bound by the tumor necrosis factor homolog a proliferation-inducing ligand (APRIL). APRIL stimulates *in vitro* proliferation of primary B and T cells and increases spleen weight due to accumulation of B cells *in vivo.* APRIL also competes with TALL-I (also called BLyS or BAFF) for receptor binding. Soluble BCMA and TACI specifically prevent binding of APRIL and block APRIL-stimulated proliferation of primary B cells. BCMA-Fc also inhibits production of antibodies against keyhole limpet hemocyanin and Pneumovax in mice, indicating that APRIL and/or TALL-I signaling via BCMA and/or TACI are required for generation of humoral immunity. Thus, APRIL-TALL-I and BCMA-TACI form a two ligand-two receptor pathway involved in stimulation of B and T cell function.

[0109] After the initial raising of antibodies to the immunogen, the antibodies can be sequenced and subsequently prepared by recombinant techniques. Humanization and chimerization of murine antibodies and antibody fragments are well known to those skilled in the art. For example, humanized monoclonal antibodies are produced by transferring mouse complementary determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then, substituting human residues in the framework regions of the murine counterparts. In a preferred embodiment, some human residues in the framework regions of the humanized anti-CSAp antibody or fragments thereof are replaced by their murine counterparts. It is also preferred that a combination of framework sequences from 2 different human antibodies are used for V_H. Still preferred, the two human antibodies are EU and NEWM. The constant domains of the antibody molecule is derived from those of a human antibody. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions.

[0110] A human antibody can be recovered from a transgenic mouse possessing human immunoglobulin loci. The

mouse humoral immune system is humanized by inactivating the endogenous immunoglobulin genes and introducing human immunoglobulin loci. The human immunoglobulin loci are exceedingly complex and comprise a large number of discrete segments which together occupy almost 0.2 % of the human genome. To ensure that transgenic mice are capable of producing adequate repertoires of antibodies, large portions of human heavy- and light-chain loci must be introduced into the mouse genome. This is accomplished in a stepwise process beginning with the formation of yeast artificial chromosomes (YACs) containing either human heavy- or light-chain immunoglobulin loci in germline configuration. Since each insert is approximately 1 Mb in size, YAC construction requires homologous recombination of overlapping fragments of the immunoglobulin loci. The two YACs, one containing the heavy-chain loci and one containing the light-chain loci, are introduced separately into mice via fusion of YAC-containing yeast spheroblasts with mouse embryonic stem cells. Embryonic stem cell clones are then microinjected into mouse blastocysts. Resulting chimeric males are screened for their ability to transmit the YAC through their germline and are bred with mice deficient in murine antibody production. Breeding the two transgenic strains, one containing the human heavy-chain loci and the other containing the human light-chain loci, creates progeny which produce human antibodies in response to immunization.

[0111] Unrearranged human immunoglobulin genes also can be introduced into mouse embryonic stem cells via microcell-mediated chromosome transfer (MMCT). See, e.g., Tomizuka et al., Nature Genetics, 16: 133 (1997). In this methodology microcells containing human chromosomes are fused with mouse embryonic stem cells. Transferred chromosomes are stably retained, and adult chimeras exhibit proper tissue-specific expression.

[0112] As an alternative, an antibody or antibody fragment of the present invention may be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, e.g., Barbas et al., METHODS: A Companion to Methods in Enzymology 2: 119 (1991), and Winter et al., Ann. Rev. Immunol. 12: 433 (1994). Many of the difficulties associated with generating monoclonal antibodies by B-cell immortalization can be overcome by engineering and expressing antibody fragments in *E. coli,* using phage display. To ensure the recovery of high affinity, monoclonal antibodies a combinatorial immunoglobulin library must contain a large repertoire size. A typical strategy utilizes mRNA obtained from lymphocytes or spleen cells of immunized mice to synthesize cDNA using reverse transcriptase. The heavy- and light-chain genes are amplified separately by PCR and ligated into phage cloning vectors. Two different libraries are produced, one containing the heavy-chain genes and one containing the light-chain genes. Phage DNA is islolated from each library, and the heavy- and light-chain sequences are ligated together and packaged to form a combinatorial library. Each phage contains a random pair of heavy- and light-chain cDNAs and upon infection of *E. coli* directs the expression of the antibody chains in infected cells. To identify an antibody that recognizes the antigen of interest, the phage library is plated, and the antibody molecules present in the plaques are transferred to filters. The filters are incubated with radioactively labeled antigen and then washed to remove excess unbound ligand. A radioactive spot on the autoradiogram identifies a plaque that contains an antibody that binds the antigen. Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA).

[0113] 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). for constructing chimeric antibodies are well known to those of skill in the art. As an example, Leung et al., Hybridoma 13:469 (1994), describe how they produced an LL2 chimera by combining DNA sequences encoding the V_κ and V_H domains of LL2 monoclonal antibody, an anti-CD22 antibody, with respective human κ and IgG_1 constant region domains. This publication also provides the nucleotide sequences of the LL2 light and heavy chain variable regions, V_κ and V_H, respectively. Techniques for producing humanized MAbs are described, for example, by Jones et al., Nature 321: 522 (1986), Riechmann et al., Nature 332: 323 (1988), Verhoeyen et al., Science 239: 1534 (1988), Carter et al., Proc. Nat'l Acad. Sci. USA 89: 4285 (1992), Sandhu, Crit. Rev. Biotech. 12: 437 (1992), and Singer et al., J. Immun. 150: 2844 (1993),

[0114] A chimeric antibody is a recombinant protein that contains the variable domains including the CDRs derived from one species of animal, such as a rodent antibody, while the remainder of the antibody molecule; i.e., the constant domains, is derived from a human antibody. Accordingly, a chimeric monoclonal antibody can also be humanized by replacing the sequences of the murine FR in the variable domains of the chimeric MAb with one or more different human FR. Specifically, mouse CDRs are transferred from heavy and light variable chains of the mouse immunoglobulin into the corresponding variable domains of a human antibody. As simply transferring mouse CDRs into human FRs often results in a reduction or even loss of antibody affinity, additional modification might be required in order to restore the original affinity of the murine antibody. This can be accomplished by the replacement of one or more some human residues in the FR regions with their murine counterparts to obtain an antibody that possesses good binding affinity to its epitope. See, for example, Tempest et al., Biotechnology 9:266 (1991) and Verhoeyen et al., Science 239: 1534 (1988). Further, the affinity of humanized, chimeric and human MAbs to a specific epitope can be increased by mutagenesis of the CDRs, so that a lower dose of antibody may be as effective as a higher dose of a lower affinity MAb prior to mutagenesis. See for example, WO0029584A1

[0115] Another method for producing the antibodies of the present disclosure is by production in the milk of transgenic livestock. See, e.g., Colman, A., Biochem. Soc. Symp., 63: 141-147, 1998; U.S. Patent 5,827,690, Two DNA constructs

are prepared which contain, respectively, DNA segments encoding paired immunoglobulin heavy and light chains. The DNA segments are cloned into expression vectors which contain a promoter sequence that is preferentially expressed in mammary epithelial cells. Examples include, but are not limited to, promoters from rabbit, cow and sheep casein genes, the cow α-lactoglobulin gene, the sheep β-lactoglobulin gene and the mouse whey acid protein gene. Preferably, the inserted fragment is flanked on its 3' side by cognate genomic sequences from a mammary-specific gene. This provides a polyadenylation site and transcript-stabilizing sequences. The expression cassettes are coinjected into the pronuclei of fertilized, mammalian eggs, which are then implanted into the uterus of a recipient female and allowed to gestate. After birth, the progeny are screened for the presence of both transgenes by Southern analysis. In order for the antibody to be present, both heavy and light chain genes must be expressed concurrently in the same cell. Milk from transgenic females is analyzed for the presence and functionality of the antibody or antibody fragment using standard immunological methods known in the art. The antibody can be purified from the milk using standard methods known in the art.

Preparation of chimeric, humanized and human anti-CSAp antibodies

[0116] Also contemplated in the present disclosure are human, humanized and chimeric anti-CSAp monoclonal antibodies used in combination with other human or reengineered (e.g., chimerized, humanized) antibodies, such as carcinoma associated antibodies including those expressed by colorectal and ovarian carcinomas. In a preferred embodiment, antibodies against CEA, MUC1, MUC2, MUC3, MUC 4, PAM-4, KC4, BrE3, Le-Y (e.g., B3 antibody), EGFR, EGP-1, RS5 (GA733 antigen target, such as for antibodies EGP-2, 17-1A, KS1-4, Ep-CAM), TAG-72, the A33 antibody-determinant, KS-1, A3 and HER2/neu are used for combination therapy with humanized, chimeric or human anti-CSAp antibodies. See, e.g., Mendez et al., Nature Genetics, 15: 146-156 (1997); U.S. Patent No. 5,633,425, in . The BrE3 antibody is described in Couto, J, Christian, R, Peterson, J, and Ceriani, R., Cancer Res. 1995;55 (Suppl.23):5973s-5977s. TheEGP-1 antibody is described in U.S. WO03074566 , some of the EGP-2 antibodies are cited in Birbenet et al., in Staib et al.; and Schwartzberg in the references cited at the end of this application. The KS -1 antibody is cited in Koda et al.; the A33 antibody is cited in Ritter et al. at end; Le(y) antibody B3 described in Di Carlo et al; A3 antibody is described in Tordsson et al., all listed in the references cited at the end of this application. Preferably, antibodies against marker antigens or receptors of gastrointestinal and ovarian carcinomas are well suited for use in combination with CSAp antibodies, and in particular with the Mu-9 antibodies. In a preferred embodiment, a gastrointestinal cancer is a colorectal cancer.

[0117] 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, by . Antibodies against certain immune response modulators, such as antibodies to CD40, are described in Todryk et al. and Turner et al., listed in the references cited at the end of this application. Other antibodies suitable for combination therapy include anti-necrosis antibodies as described in Epstein et al. (*infra*).

[0118] Cell lines and culture media used in the present disclosure include Mu-9 hybridoma cells and Sp2/0-Ag14 myeloma cells (ATCC, Rockville, MD). The monoclonal hybridoma producing Mu-9 was obtained by fusing the spleen from a mouse that had been immunized with colon-specific antigen-p (CSAp) with SP2/0Ag14. These cells may be cultured in Hybridoma serum-free media (HSFM) (life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Hyclone Laboratories, Logan, UT) and antibiotics (complete media). Alternatively, they may be cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% FCS (Gibco/BRL, Gaithersburg, Mass.) containing 10% of FCS and 75 g/ml gantamicin (complete HSFM) or, where indicated, in HSFM containing only antibiotics. Selection of the transfectomas may be carried out in complete HSFM containing 500 units/ml of hygromycin (Calbiochem, San Diego, CA). All cell lines are preferably maintained at 37°C in 5% CO_2.

Obtaining V_κ and V_H Gene Segments

[0119] Isolation of the V_κ and V_H gene segments can be accomplished by several means that are well-known in the art. Two such means include, but are not limited to, PCR cloning and cDNA library screening.

[0120] PCR cloning techniques are well-known in the art. In brief, however, PCR cloning of V_κ and V_H gene fragments may be accomplished as follows. Poly A mRNA may be isolated from a Mu-9 hybridoma cell line using commercially available kits such as the Fast Track mRNA Isolation kit (Invitrogen, San Diego, CA). The first strand cDNA may then be reverse transcribed from poly A mRNA using a cDNA cycle kit (Invitrogen). In this process, poly A mRNA is annealed to a murine IgG CH1-specific primer or a murine Ck-specific primer. Examples of such primers include CH1B (5' - ACA GTC ACT GAG CTG G - 3') and Ck3-BH1 (5' - GCC GGA TCC TGA CTG GAT GGT GGG AAG ATG GAT ACA - 3'), respectively. The first strand cDNA may be used as templates to amplify the V_H and V_κ sequences by PCR, as described by *Orlandi et al.* For the V_κ region, a primer pair such as VK1Back (5' - GAC ATT CAG CTG ACC CAG TCT CCA - 3') and IgGKC3' (5' - CTC ACT GGA TGG TGG GAA GAT GGA TAC AGT TGG - 3') may be used. For the V_H region, a

primer pair such as VH1Back (5' - AGG T(C/G)(A/C) A(A/G)C TGC AG(C/G) AGT C(A/T)G G - 3') and CH1B may be used. After amplification, the V_κ and V_H fragments may then be gel-purified and cloned into a cloning vector such as the TA cloning vector (Invitrogen) for sequence analyses by the dideoxytermination method. Sequences confirmed to be of immunoglobulin origin may then be used to construct chimeric expression vectors using methods described by Leung et al.

[0121] As a preferred alternative to isolating the V_κ and V_H gene segments by PCR cloning, cDNA library screening may be utilized. cDNA screening methods also are well known in the art. In brief, however, a cDNA library may be constructed from the mRNA extracted from the murine Mu-9 hybridoma cells in pSPORT vector (Life Technologies). The first strand cDNA may be synthesized by priming poly A RNA from Mu-9 hybridoma with an oligo dT primer-NotI adaptor (Life Technologies). After the second strand synthesis and attachment of SalI adaptors, the cDNA pool may be size fractionated through a cDNA size fractionation column. Fractionated cDNA may then be ligated to pSPORT vector and subsequently transformed into *Escherichia coli* DH5. A library may then be plated, transferred to filters, and amplified.

[0122] Screening of the cDNA library may be accomplished by hybridization with labeled probes specific for the heavy and light chains. For example [32-P]-labeled probes such as MUCH-1 (5' - AGA CTG CAG GAG AGC TGG GAA GGT GTG CAC - 3') for heavy chain and MUCK-1 (5' - GAA GCA CAC GAC TGA GGC ACC TCC AGA TGT - 3') for light chain. Clones that are positive on a first screening may be transferred to duplicate plates and screened a second time with the same probes.

[0123] RNA isolation, cDNA synthesis, and amplification can be carried out as follows. Total cell RNA can be prepared from a Mu-9 hybridoma cell line, using a total of about 10^7 cells, according to Sambrook et al., (Molecular Cloning: A Laboratory Manual, Second ed., Cold Spring Harbor Press, 1989), First strand cDNA can be reverse transcribed from total RNA conventionally, such as by using the SuperScript preamplification system (Gibco/BRL., Gaithersburg, Md.). Briefly, in a reaction volume of 20 μl, 50 ng of random hexamer primers can be annealed to 5 μg of RNAs in the presence of 2 μl of 10X synthesis buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl, 25 mM $MgCl_2$, 1 mg/ml BSA], 1 μl of 10 mM dNTP mix, 2 μl of 0.1 M DTT, and 200 units of SuperScript reverse transcriptase. The elongation step is initially allowed to proceed at room temperature for 10 min followed by incubation at 42° C. for 50 min. The reaction can be terminated by heating the reaction mixture at 90° C. for 5 min.

[0124] Synthesizing and labeling the screening probes can be accomplished by well-known means. Depending on the detection systems utilized, probe labeling will vary. Many kits for this purpose are commercially available. One method for 32-P labeling of oligonucleotides includes the use of with [-32P]ATP (Amersham Arlington Heights, IL) and T4 polynucleotide kinase (New England Biolabs, Beverly, MA), followed by column purification.

Reparation of a chimeric anti-CSAp antibody

[0125] In general, to prepare chimeric anti-CSAp MAb, V_H and $V_\kappa$chains of a CSAp antibody may be obtained by methods such as those described above and amplified by PCR. In a preferred embodiment, the chimeric anti-CSAp antibody is a Mu-9 antibody. The V_κ PCR products may be subcloned into a pBR327 based staging vector (VKpBR), as described by Leung et al. (Hybridoma, 13:469-476 (1994)). The V_H PCR products may be subcloned into a similar pBluescript-based staging vector (VHpBS). The fragments containing the V_κ and V_H sequences, along with the promoter and signal peptide sequences, can be excised from the staging vectors using HindIII and BamHI restriction endonucleases. The V_κ fragments (about 600 bp) can be subcloned into a mammalian expression vector (for example, pKh) conventionally. pKh is a pSVhyg-based expression vector containing the genomic sequence of the human kappa constant region, an Ig enhancer, a kappa enhancer and the hygromycin-resistant gene. Similarly, the about 800 bp V_H fragments can be subcloned into pG1g, a pSVgpt-based expression vector carrying the genomic sequence of the human IgG1 constant region, an Ig enhancer and the xanthine-guanine phosphoribosyl transferase (gpt) gene. The two plasmids may be co-transfected into mammalian cells, such as Sp2/0-Ag14 cells, by electroporation and selected for hygromycin resistance. Colonies surviving selection are expanded, and supernatant fluids monitored for production of cMu-9 mAb by an ELISA method. A transfection efficiency of about 1-10 x 10^6 cells is desirable. An antibody expression level of between 0.10 and 2.5 μg/ml can be expected with this system.

[0126] Alternately, the V and VH expression cassettes can be assembled in the modified staging vectors, VKpBR2 and VHpBS2, excised as XbaI/BamHI and XhoI/BamHI fragments, respectively, and subcloned into a single expression vector, such as pdHL2, as described by Gilles et al. J. Immunol. Methods 125:191 (1989), Losman et al., Clin. Cancer Res. 5:3101 (1999) and in Losman et al., Cancer, 80:2660 (1997) for the expression in Sp2/0-Ag14 cells. Another vector that is useful in the present invention is the GS vector, as described in Barnes et al., Cytotechnology 32:109-123 (2000), which is preferably expressed in the NS0 cell line and CHO cells. Other appropriate mammalian expression systems are described in Werner et al., Arzneim.-Forsch./Drug Res. 48(II), Nr. 8, 870-880 (1998).

[0127] The V_κ and V_H sequences can amplified by PCR as described by Orlandi et al., (Proc. Natl. Acad. Sci., U.S.A., 86: 3833 (1989)) Vk sequences may be amplified using the primers CK3BH and V_κ5-3 (Leung et al., BioTechniques, 15: 286 (1993),), while V_H sequences can be amplified using the primer CH1B which anneals to the CH1 region of

murine 1gG, and VHIBACK (Orlandi et al., 1989 above). The PCR reaction mixtures containing 10 μl of the first strand cDNA product, 9 μl of 10X PCR buffer [500 mM KCl, 100 mM Tris-HCl (pH 8.3), 15 mM $MgCl_2$, and 0.01% (w/v) gelatin] (Perkin Elmer Cetus, Norwalk, Conn.), can be subjected to 30 cycles of PCR. Each PCR cycle preferably consists of denaturation at 94° C. for 1 min, annealing at 50° C. for 1.5 min, and polymerization at 72° C. for 1.5 min. Amplified Vk and VH fragments can be purified on 2% agarose (BioRad, Richmond, Calif.).

Preparation of a humanized anti-CSAp antibody

[0128] In a preferred embodiment, the humanized anti-CSAp antibody is a humanized Mu-9 antibody. Once the sequences for the hMu-9V_κ and V_H domains are designed, CDR engrafting can be accomplished by gene synthesis using long synthetic DNA oligonucleotides as templates and short oligonucleotides as primers in a PCR reaction. In most cases, the DNA encoding the V_κ or VH domain will be approximately 350 bp long. By taking advantage of codon degeneracy, a unique restriction site may easily be introduced, without changing the encoded amino acids, at regions close to the middle of the V gene DNA sequence. For example, at DNA nucleotide positions 132-137 (amino acid positions 44-46) for the hMu-9 VH domain, a unique XbaI site can be introduced while maintaining the originally designed amino acid sequence (see the sequence in Figure 11A). Two long non-overlapping single-stranded DNA oligonucleotides (~ 150 bp) upstream and downstream of the XbaI site can be generated by automated DNA oligonucleotide synthesizer (Cyclone Plus DNA Synthesizer, Milligen-Biosearch). As the yields of full length DNA oligonucleotides may be expected to be low, they can be amplified by two pairs of flanking oligonucleotides in a PCR reaction. The primers can be designed with the necessary restriction sites to facilitate subsequent sequence assembly and subcloning. Primers for the oligonucleotides should contain overlapping sequence at the XbaI site so that the resultant PCR products can be joined in-frame at the XbaI site to form a full length DNA sequence encoding the hMu-9 VH domain. The ligation of the PCR products for the oligos at the XbaI site and their subcloning into the PstII/BstEII sites of the staging vector, VHpBS, can be completed in a single three-fragment ligation step. The subcloning of the correct sequence into VHpBS can be first analyzed by restriction digestion analysis and subsequently conformed by sequencing reaction according to Sanger et al., Proc. Natl. Acad. Sci. USA 74 5463 (1977).

[0129] The HindIII/BamHI fragment containing the Ig promoter, leader sequence and the hMu-9 V_H sequence can be excised from the staging vector and subcloned to the corresponding sites in a pSVgpt-based vector, pG1g, which contains the genomic sequence of the human IgG constant region, an Ig enhancer and a gpt selection marker, forming the final expression vector, hMu-9pG1g. Similar strategies can be employed for the construction of the hMu-9 V_κ sequence. The restriction site chosen for the ligation of the PCR products for the long oligonucleotides can be NruI in this case.

[0130] The DNA sequence containing the Ig promoter, leader sequence and the hMu-9 V_κ sequence can be excised from the staging vector VKpBR by treatment with BamHI/HindIII, and can be subcloned into the corresponding sites of a pSVhyg-based vector, pKh, which contains the genomic sequence of human kappa chain constant regions, a hygromycin selection marker, an Ig and a kappa enhancer, forming the final expression vector, hMu-9pKh.

[0131] The two plasmids can be co-transfected into an appropriate cell, e.g., myeloma Sp2/0-Ag14, colonies selected for hygromycin resistance, and supernatant fluids monitored for production of hMu-9 antibodies by, for example, an ELISA assay, as described below. Alternately, the V and VH expression cassettes can be assembled in the modified staging vectors, VKpBR2 and VHpBS2, excised as XbaI/BamHI and XhoI/BamHI fragments, respectively, and subcloned into a single expression vector, such as pdHL2, as described by Gilles et al. J. Immunol. Methods 125:191 (1989), Losman et al., Clin. Cancer Res. 5:3101 (1999) and in Losman et al., Cancer, 80:2660 (1997) for the expression in Sp2/0-Ag14 cells. Another vector that is useful in the present disclosure is the GS vector, as described in Barnes et al., Cytotechnology 32:109-123 (2000), which is preferably expressed in the NS0 cell line and CHO cells. Other appropriate mammalian expression systems are described in Werner et al., Arzneim.-Forsch./Drug Res. 48(II), Nr. 8, 870-880 (1998).

[0132] Transfection, and assay for antibody secreting clones by ELISA, can be carried out as follows. About 10 μg of hMu-9pKh (light chain expression vector) and 20 μg of hMu-9pG1g (heavy chain expression vector) can be used for the transfection of 5 x 10^6 SP2/0 myeloma cells by electroporation (BioRad, Richmond, Calif.) according to Co et al., J. Immunol., 148: 1149 (1992) . Following transfection, cells may be grown in 96-well microtiter plates in complete HSFM medium (GIBCO, Gaithersburg, Md.) at 37° C., 5% CO_2. The selection process can be initiated after two days by the addition of hygromycin selection medium (Calbiochem, San Diego, Calif.) at a final concentration of 500 μg/ml of hygromycin. Colonies typically emerge 2-3 weeks post-electroporation. The cultures can then be expanded for further analysis.

Screening the Clones and Isolating Antibodies

[0133] Transfectoma clones that are positive for the secretion of chimeric or humanized heavy chain can be identified by ELISA assay. Briefly, supernatant samples (100 μl) from transfectoma cultures are added in triplicate to ELISA microtiter plates precoated with goat anti-human (GAH)-IgG, $F(ab')_2$ fragment-specific antibody (Jackson ImmunoRe-

search, West Grove, Pa.). Plates are incubated for 1 h at room temperature. Unbound proteins are removed by washing three times with wash buffer (PBS containing 0.05% polysorbate 20). Horseradish peroxidase (HRP) conjugated GAH-IgG, Fc fragment-specific antibodies (Jackson ImmunoResearch, West Grove, Pa.) are added to the wells, (100 μl of antibody stock diluted x 10^4, supplemented with the unconjugated antibody to a final concentration of 1.0 μg/ml). Following an incubation of 1 h, the plates are washed, typically three times. A reaction solution, [100 μl, containing 167 μg of orthophenylene-diamine (OPD) (Sigma, St. Louis, Mo.), 0.025% hydrogen peroxide in PBS], is added to the wells. Color is allowed to develop in the dark for 30 minutes. The reaction is stopped by the addition of 50 μl of 4 N HCl solution into each well before measuring absorbance at 490 nm in an automated ELISA reader (Bio-Tek instruments, Winooski, Vt.). Bound chimeric antibodies are than determined relative to an irrelevant chimeric antibody standard (obtainable from Scotgen, Ltd., Edinburg, Scotland).

[0134] Antibodies can be isolated from cell culture media as follows. Transfectoma cultures are adapted to serum-free medium. For production of chimeric 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 micron 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 absorbancies at 280/260 nm. Peak fractions are pooled, dialyzed against PBS, and the antibody concentrated, for example, with the Centricon 30 (Amicon, Beverly, Mass.). 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.

[0135] The affinity of a chimeric, humanized or human anti-CSAp antibody may be evaluated using a direct binding assay or a competitive binding assay, as exemplified below.

Modifying/Optimizing the Recombinant Antibodies

[0136] As humanization sometimes results in a reduction or even loss of antibody affinity, additional modification might be required in order to restore the original affinity (See, for example, Tempest et al., Bio/Technology 9: 266 (1991); Verhoeyen et al., Science 239: 1534 (1988)), which. Knowing that cMu-9 exhibits a binding affinity comparable to that of its murine counterpart, defective designs, if any, in the original version of hMu-9 can be identified by mixing and matching the light and heavy chains of cMu-9 to those of the humanized version. Preferably, some human residues in the framework regions are replaced by their murine counterparts. Also preferred, a combination of framework sequences from 2 different human antibodies, such as EU and NEWM are used for V_H. For example, FR1-3 can come from EU and FR 4 from NEWM.

[0137] Other modifications, such as Asn-linked glycosylation sites, can be introduced into a chimerical, human, or humanized Mu-9 antibody by conventional oligonucleotide directed site-specific mutagenesis. Detailed protocols for oligonucleotide-directed mutagenesis and related techniques for mutagenesis of cloned DNA are well-known. For example, see Sambrook *et al.* and Ausubel *et al.* above.

[0138] For example, to introduce an Asn in position 18 of hMu-9 V_κ (figure 4B), one may alter codon 18 from CGA for Arg to AAC. To accomplish this, a single stranded DNA template containing the antibody light chain sequence is prepared from a suitable strain of E. coli (e.g., dut^-.ung-) in order to obtain a single strand DNA molecule containing a small number of uracils in place of thymidine. Such a DNA template can be obtained by M13 cloning or by in vitro transcription using a SP6 promoter. See, for example, Ausubel et al., eds., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, 1987. An oligonucleotide containing the mutated sequence is synthesized conventionally, annealed to the single-stranded template and the product treated with T4 DNA polymerase and T4 DNA ligase to produce a double-stranded DNA molecule. Transformation of wild type E. coli (dut^+, ung^+) cells with the double-stranded DNA provides an efficient recovery of mutated DNA.

[0139] Alternatively, an Asn-linked glycosylation site can be introduced into an antibody light chain using an oligonucleotide containing the desired mutation as the primer and DNA clones of the variable regions for the Vk chain, or by using RNA from cells that produce the antibody of interest as a template. Also see, Huse, in ANTIBODY ENGINEERING: A PRACTICAL GUIDE, Boerrebaeck, ed., W. H. Freeman & Co., pp. 103-120, 1992. Site-directed mutagenesis can be performed, for example, using the TRANSFORMER™ kit (Clonetech, Palo Alto, Calif.) according to the manufacturer's instructions.

[0140] Alternatively, a glycosylation site can be introduced by synthesizing an antibody chain with mutually priming oligonucleotides, one such containing the desired mutation. See, for example, Uhlmann, Gene 71: 29 (1988); Wosnick et al., Gene 60: 115 (1988); Ausubel et al., above,

[0141] Although the general description above referred to the introduction of an Asn glycosylation site in position 18 of the light chain of an antibody, it will occur to the skilled artisan that it is possible to introduce Asn-linked glycosylation sites elsewhere in the light chain, or even in the heavy chain variable region.

Determining Antibody Binding Affinity

[0142] Comparative binding affinities of the isolated murine, human, humanized and chimeric Mu-9 antibodies thus isolated may be determined by direct radioimmunoassay. Mu-9 can be labeled with ^{131}I or ^{125}I using the chloramine T method (see, for example, Greenwood et al., Biochem. J., 89: 123 (1963) The specific activity of the iodinated antibody is typically adjusted to about 10 μCi/μg. Unlabeled and labeled antibodies are diluted to the appropriate concentrations using reaction medium (HSFM supplemented with 1 % horse serum and 100 μg/ml gentamicin). The appropriate concentrations of both labeled and unlabeled antibodies are added together to the reaction tubes in a total volume of 100 μl. A culture of GW-39 tumor cells is sampled and the cell concentration determined. The culture is centrifuged and the collected cells washed once in reaction medium followed by resuspension in reaction medium to a final concentration of about 10^7 cells/ml. All procedures are carried out in the cold at 4° C. The cell suspension, 100 μl, is added to the reaction tubes. The reaction is carried out at 4° C. for 2 h with periodic gentle shaking of the reaction tubes to resuspend the cells. Following the reaction period, 5 ml of wash buffer (PBS containing 1 % BSA) is added to each tube. The suspension is centrifuged and the cell pellet washed a second time with another 5 ml of wash buffer. Following centrifugation, the amount of remaining radioactivity remaining in the cell pellet is determined in a gamma counter (Minaxi, Packard Instruments, Sterling, Va.).

III. Production of Antibody Fragments

[0143] 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. Other antibody fragments include, but are not limited to: the $F(ab)'_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab' fragments, which can be generated by reducing disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab' expression 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.

[0144] A single chain Fv molecule (scFv) comprises a VL domain and a VH domain. The VL and VH 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 VL-L-VH if the VL domain is the N-terminal part of the scFv molecule, or as VH-L-VL if the VH 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).

[0145] To obtain high-affinity scFv, an scFv library with a large repertoire can be constructed by isolating V-genes from non-immunized human donors using PCR primers corresponding to all known V_H, V_κ and V_λ gene families. See, e.g., Vaughn et al., Nat. Biotechnol., 14: 309-314 (1996). Following amplification, the V_κ and V_λ pools are combined to form one pool. These fragments are ligated into a phagemid vector. The scFv linker, $(Gly_4, -Ser)_3$, is then ligated into the phagemid upstream of the V_L fragment. The V_H and linker-V_L fragments are amplified and assembled on the J_H region. The resulting V_H-linker-V_L fragments are ligated into a phagemid vector. The phagemid library can be panned using filters, as described above, or using immunotubes (Nunc; Maxisorp). Similar results can be achieved by constructing a combinatorial immunoglobulin library from lymphocytes or spleen cells of immunized rabbits and by expressing the scFv constructs in *P. pastoris.* See, e.g., Ridder et al., Biotechnology, 13: 255-260 (1995). Additionally, following isolation of an appropriate scFv, antibody fragments with higher binding affinities and slower dissociation rates can be obtained through affinity maturation processes such as CDR3 mutagenesis and chain shuffling. See, e.g., Jackson et al., Br. J. Cancer, 78: 181-188 (1998); Osbourn et al., Immunotechnology, 2: 181-196 (1996).

[0146] 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 a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic 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.

[0147] 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 to synthesize the variable region from RNA of antibody-producing cells. See, for example, 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).

[0148] 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.

Preparation of a Bispecific Antibody

[0149] The bsAbs can be prepared by techniques known in the art, for example, an anti-CEA or anti-CSAp Ab and an anti-peptide Ab are both separately digested with pepsin to their respective $F(ab')_2$s. The anti-CEA-Ab-$F(ab')_2$ or anti-CSAp-Ab-$F(ab')_2$ is reduced with cysteine to generate Fab' monomeric units which are further reacted with the cross-linker bis(maleimido) hexane to produce Fab'-maleimide moieties. The anti-peptide Ab-$F(ab')_2$ is reduced with cysteine and the purified, recovered anti-peptide Fab'-SH reacted with the anti-CEA-Fab'-maleimide or anti-CSAp-Fab'-maleimide, respectively, to generate the Fab' x *Fab'* bi-specific Ab. Alternatively, the anti-peptide Fab'-SH fragment may be coupled with the anti-CEA $F(ab')_2$ or anti-CSAp $F(ab')_2$, respectively, to generate a $F(ab')_2$ x *Fab'* construct, or with anti-CEA or Mu-9 IgG to generate an IgG x *Fab'* bi-specific construct. In one embodiment, the IgG x *Fab'* construct can be prepared in a site-specific manner by attaching the antipeptide *Fab'* thiol group to anti-CEA or Mu-9 IgG heavy-chain carbohydrate which has been periodate-oxidized, and subsequently activated by reaction with a commercially available hydrazide-maleimide cross-linker. The component Abs used can be chimerized or humanized by known techniques. A chimeric antibody is a recombinant protein that contains the variable domains and complementary determining regions derived from a rodent antibody, while the remainder of the antibody molecule is derived from a human antibody. Humanized antibodies are recombinant proteins in which murine complementarity determining regions of a monoclonal antibody have been transferred from heavy and light variable chains of the murine immunoglobulin into a human variable domain.

[0150] A variety of recombinant methods can be used to produce bi-specific antibodies and antibody fragments. For example, bi-specific antibodies and antibody fragments can be produced in the milk of transgenic livestock. See, e.g., Colman, A., Biochem. Soc. Symp., 63: 141-147, 1998; U.S. Patent No. 5,827,690. Two DNA constructs are prepared which contain, respectively, DNA segments encoding paired immunoglobulin heavy and light chains. The fragments are cloned into expression vectors which contain a promoter sequence that is preferentially expressed in mammary epithelial cells. Examples include, but are not limited to, promoters from rabbit, cow and sheep casein genes, the cow α-lactoglobulin gene, the sheep β-lactoglobulin gene and the mouse whey acid protein gene. Preferably, the inserted fragment is flanked on its 3' side by cognate genomic sequences from a mammary-specific gene. This provides a polyadenylation site and transcript-stabilizing sequences. The expression cassettes are coinjected into the pronuclei of fertilized, mammalian eggs, which are then implanted into the uterus of a recipient female and allowed to gestate. After birth, the progeny are screened for the presence of both transgenes by Southern analysis. In order for the antibody to be present, both heavy and light chain genes must be expressed concurrently in the same cell. Milk from transgenic females is analyzed for the presence and functionality of the antibody or antibody fragment using standard immunological methods known in the art. The antibody can be purified from the milk using standard methods known in the art.

[0151] A chimeric Ab is constructed by ligating the cDNA fragment encoding the mouse light variable and heavy variable domains to fragment encoding the C domains from a human antibody. Because the C domains do not contribute to antigen binding, the chimeric antibody will retain the same antigen specificity as the original mouse Ab but will be closer to human antibodies in sequence. Chimeric Abs still contain some mouse sequences, however, and may still be immunogenic. A humanized Ab contains only those mouse amino acids necessary to recognize the antigen. This product is constructed by building into a human antibody framework the amino acids from mouse complementarity determining regions.

[0152] Other recent methods for producing bsAbs include engineered recombinant Abs which have additional cysteine residues so that they crosslink more strongly than the more common immunoglobulin isotypes. See, e.g., FitzGerald et al., Protein Eng. 10(10): 1221-1225, 1997. Another approach is to engineer recombinant fusion proteins linking two or more different single-chain antibody or antibody fragment segments with the needed dual specificities. See, e.g., Coloma et al., Nature Biotech. 15:159-163, 1997. A variety of bi-specific fusion proteins can be produced using molecular engineering. In one form, the bi-specific 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 bi-specific

fusion protein is divalent, consisting of, for example, an IgG with two binding sites for one antigen and two scFv with two binding sites for a second antigen.

[0153] Functional bi-specific single-chain antibodies (bscAb), also called diabodies, can be produced in mammalian cells using recombinant methods. See, e.g., Mack et al., Proc. Natl. Acad. Sci. , 92: 7021-7025, 1995. For example, bscAb are produced by joining two single-chain Fv fragments via a glycine-serine linker using recombinant methods. The V light-chain (V_L) and V heavy-chain (V_H) domains of two antibodies of interest are isolated using standard PCR methods. The V_L and V_H cDNA's obtained from each hybridoma are then joined to form a single-chain fragment in a two-step fusion PCR. The first PCR step introduces the $(Gly_4\text{-}Ser_1)_3$ linker, and the second step joins the V_L and V_H amplicons. Each single chain molecule is then cloned into a bacterial expression vector. Following amplification, one of the single-chain molecules is excised and sub-cloned into the other vector, containing the second single-chain molecule of interest. The resulting bscAb fragment is subcloned into an eukaryotic expression vector. Functional protein expression can be obtained by transfecting the vector into chinese hamster ovary cells. Bi-specific fusion proteins are prepared in a similar manner. Bi-specific single-chain antibodies and bi-specific fusion proteins are included within the scope of the present invention. Diabody, triabody and tetrabody bispecific fusion proteins produced in E.coli and yeast are described in U.S. Publications and 60/342,103, US 2003148409, US20030342103

[0154] Bi-specific fusion proteins linking two or more different single-chain antibodies or antibody fragments are produced in similar manner.

[0155] Large quantities of bscAb and fusion proteins can be produced using *Escherichia coli* expression systems. See, e.g., Zhenping et al., Biotechnology, 14: 192-196, 1996. A functional bscAb can be produced by the coexpression in *E. coli* of two "cross-over" scFv fragments in which the V_L and V_H domains for the two fragments are present on different polypeptide chains. The V light-chain (V_L) and V heavy-chain (V_H) domains of two antibodies of interest are isolated using standard PCR methods. The cDNA's are then ligated into a bacterial expression vector such that C-terminus of the V_L domain of the first antibody of interest is ligated via a linker to the N-terminus of the V_H domain of the second antibody. Similarly, the C-terminus of the V_L domain of the second antibody of interest is ligated via a linker to the N-terminus of the V_H domain of the first antibody. The resulting dicistronic operon is placed under transcriptional control of a strong promoter, e.g., the *E. coli* alkaline phosphatase promoter which is inducible by phosphate starvation. Alternatively, single-chain fusion constructs have successfully been expressed in *E. coli* using the *lac* promoter and a medium consisting of 2% glycine and 1% Triton X-100. See, e.g., Yang et al., Appl. Environ. Microbiol. , 64: 2869-2874, 1998. An *E. coli,* heat-stable, enterotoxin II signal sequence is used to direct the peptides to the periplasmic space. After secretion, the two peptide chains associate to form a non-covalent heterodimer which possesses both antigen binding specificities. The bscAb is purified using standard procedures known in the art, e.g., Staphylococcal protein A chromatography.

[0156] Functional bscAb and fusion proteins also can be produced in the milk of transgenic livestock. See, e.g., Colman, A., Biochem. Soc. Symp., 63: 141-147, 1998; U.S. Patent No. 5,827,690. The bscAb fragment, obtained as described above, is cloned into an expression vector containing a promoter sequence that is preferentially expressed in mammary epithelial cells. Examples include, but are not limited to, promoters from rabbit, cow and sheep casein genes, the cow α-lactoglobulin gene, the sheep β-lactoglobulin gene and the mouse whey acid protein gene. Preferably, the inserted bscAb is flanked on its 3' side by cognate genomic sequences from a mammary-specific gene. This provides a polyadenylation site and transcript-stabilizing sequences. The expression cassette is then injected into the pronuclei of fertilized, mammalian eggs, which are then implanted into the uterus of a recipient female and allowed to gestate. After birth, the progeny are screened for the presence of the introduced DNA by Southern analysis. Milk from transgenic females is analyzed for the presence and functionality of the bscAb using standard immunological methods known in the art. The bscAb can be purified from the milk using standard methods known in the art. Transgenic production of bscAb in milk provides an efficient method for obtaining large quantities of bscAb.

[0157] Functional bscAb and fusion proteins also can be produced in transgenic plants. See, e.g., Fiedler et al., Biotech., 13: 1090-1093, 1995; Fiedler et al., Immunotechnology, 3: 205-216, 1997. Such production offers several advantages including low cost, large scale output and stable, long term storage. The bscAb fragment, obtained as described above, is cloned into an expression vector containing a promoter sequence and encoding a signal peptide sequence, to direct the protein to the endoplasmic recticulum. A variety of promoters can be utilized, allowing the practitioner to direct the expression product to particular locations within the plant. For example, ubiquitous expression in tobacco plants can be achieved by using the strong cauliflower mosaic virus 35S promoter, while organ specific expression is achieved via the seed specific legumin B4 promoter. The expression cassette is transformed according to standard methods known in the art. Transformation is verified by Southern analysis. Transgenic plants are analyzed for the presence and functionality of the bscAb using standard immunological methods known in the art. The bscAb can be purified from the plant tissues using standard methods known in the art.

[0158] Additionally, transgenic plants facilitate long term storage of bscAb and fusion proteins. Functionally active scFv proteins have been extracted from tobacco leaves after a week of storage at room temperature. Similarly, transgenic tobacco seeds stored for 1 year at room temperature show no loss of scFv protein or its antigen binding activity.

[0159] Functional bscAb and fusion proteins also can be produced in insect cells. See, e.g., Mahiouz et al., J. Immunol. Methods, 212: 149-160 (1998). Insect-based expression systems provide a means of producing large quantities of homogenous and properly folded bscAb. The baculovirus is a widely used expression vector for insect cells and has been successfully applied to recombinant antibody molecules. See, e.g., Miller, L.K., Ann. Rev. Microbiol., 42: 177 (1988); Bei et al., J. Immunol. Methods, 186: 245 (1995). Alternatively, an inducible expression system can be utilized by generating a stable insect cell line containing the bscAb construct under the transcriptional control of an inducible promoter. See, e.g., Mahiouz et al., J. Immunol. Methods, 212: 149-160 (1998). The bscAb fragment, obtained as described above, is cloned into an expression vector containing the *Drosphila* metallothionein promoter and the human HLA-A2 leader sequence. The construct is then transfected into *D. melanogaster* SC-2 cells. Expression is induced by exposing the cells to elevated amounts of copper, zinc or cadmium. The presence and functionality of the bscAb is determined using standard immunological methods known in the art. Purified bscAb is obtained using standard methods known in the art.

[0160] Preferred bispecific antibodies of the instant disclosure are those which incorporate the Fv of MAb Mu9 and the Fv of MAb 679 or the Fv of MAb MN14 and the Fv of MAb 679, and their human, chimerized or humanized counterparts. Accordingly, an anti-CSAp antibody fragments are also contemplated in the present invention. Preferably, the anti-CSAp antibody fragment is a Mu-9 antibody fragment. The MN14, as well as its chimerized and humanized counterparts, are disclosed in U.S. Patent No. 5,874,540. Also preferred are bispecific antibodies which incorporate one or more of the CDRs of Mu9 or 679. The antibody can also be a fusion protein or a bispecific antibody that incorporates a Class III anti-CEA antibody and the Fv of 679. Class III antibodies, including Class III anti-CEA are discussed in detail in U.S. Patent No. 4,818,709.

IV. Antibodies for Treatment and Diagnosis/Detection

Humanized, chimeric and human anti-CSAp antibodies for treatment and diagnosis/detection

[0161] Contemplated in the present disclosure is the use of humanized, chimeric and human anti-CSAp antibodies and fragments thereof in therapeutic and diagnostic methods. Preferably, the chimeric, humanized and human anti-CSAp antibodies and fragments thereof are chimeric, humanized or human Mu-9 antibodies. Still preferred, the chimeric, humanized and human Mu-9 antibodies and fragments thereof of the present invention are used in methods for treating malignancies. For example, a malignancy of particular interest in this patent is a cancer of the gastrointestingal system, more preferably of the colon and rectum, pancreas, as well as ovarian cancer.

[0162] The compositions for treatment contain at least one naked humanized, chimeric or human anti-CSAp antibody or fragment thereof alone or in combination with other anti-CSAp antibodies or antibody fragments thereof, such as other anti-CSAp humanized, chimeric or human antibodies. The present disclosure also contemplates treatment with at least one naked humanized, chimeric or human anti-CSAp antibody or fragment thereof in combination with other antibodies or antibody fragments thereof that are not anti-CSAp antibodies, whereby these other antibodies can be administered unconjugated (naked) or conjugated with a therapeutic compound. For example, other antibodies suitable for combination therapy include, but are not limited to, carcinoma-associated antibodies and fragments thereof such as antibodies against CEA, EGP-1, Ga 733 antigen target, such as for antibodies EGP-2, 17-1A, KS1-4 and Ep-CAM, MUC1 to MUC-4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, BrE3, Le-Y, K-1, A3, anti-necrosis antibodies, and the A33 antibody determinant. Suitable antibodies could also include those targeted against oncogene markers or products, or antibodies against tumor-vasculature markers, such as the angiogenesis factor, VEGF, and antibodies against certain immune response modulators such as antibodies to CD40. Additionally, treatment can be effected with at least one humanized, chimeric or human anti-CSAp immunoconjugate or fragment thereof alone or in combination with other anti-CSAp antibodies or antibody fragments thereof, such as other anti-CSAp humanized, chimeric or human antibodies. Still preferred, compositions for treatment can contain at least one humanized, chimeric or human anti-CSAp immunoconjugate or fragment thereof in combination with other antibodies or antibody fragments thereof that are not anti-CSAp antibodies, these being either naked or conjugated to a therapeutic agent.

[0163] Similarly, conjugated and naked anti-CSAp humanized, chimeric or human antibodies may be used alone or may be administered with, but unconjugated to, the various diagnostic or therapeutic agents described herein. Also, naked or conjugated antibodies to the same or different epitope or antigen may be also combined with one or more of the antibodies of the present disclosure.

[0164] Accordingly, the present disclosure contemplates the administration of humanized, chimeric and human Mu-9 antibodies and fragments thereof alone, as a naked antibody, or administered as a multimodal therapy. Multimodal therapies of the present disclosure further include immunotherapy with naked or conjugated CSAp antibodies supplemented with administration of other antibodies in the form of naked antibodies, fusion proteins, or as immunoconjugates. For example, a humanized, chimeric or human Mu-9 antibody may be combined with another naked humanized, naked chimeric or naked human Mu-9 antibody, or a humanized, chimeric or human Mu-9 antibody immunoconjugate, such

as a humanized, chimeric or human Mu-9 antibody conjugated to an isotope, one or more chemotherapeutic agents, cytokines, enzymes, enzyme-inhibitors, hormones or hormone antagonists, metals, toxins or a combination thereof. A fusion protein of a humanized, chimeric or human Mu-9 antibody and a toxin or may also be used in this disclosure. Many different antibody combinations may be constructed, either as naked antibodies or as partly naked and partly conjugated with a therapeutic agent or immunomodulator, or merely in combination with another therapeutic agents, such as a cytotoxic drug or with radiation.

[0165] The compositions for treatment contain at least one humanized, chimeric or human monoclonal CSAp antibody or fragment thereof alone or in combination with other antibodies and fragments thereof, such as other naked or conjugated humanized, chimeric, or human antibodies, fusion proteins, or therapeutic agents. In particular, combination therapy with a fully human antibody is also contemplated and is produced by the methods as set forth above.

[0166] Naked or conjugated antibodies and fragments thereof to the same or different epitope or antigen may be also combined with one or more of the antibodies or fragments thereof of the present disclosure. For example, a humanized, chimeric or human naked Mu-9 antibody may be combined with another naked humanized, naked chimeric or naked human Mu-9 antibody, a humanized, chimeric or human naked Mu-9 antibody may be combined with a Mu-9 immunoconjugate, a naked Mu-9 antibody may be combined with a different antibody radioconjugate or an different naked antibody may be combined with a humanized, chimeric or human Mu-9 antibody conjugated to an isotope, or to one or more chemotherapeutic agents, cytokines, toxins, enzymes, enzyme inhibitors, hormones, hormone antagonists, or a combination thereof. A fusion protein of a humanized, chimeric or human Mu-9 antibody and a toxin or immunomodulator may also be used in this invention. Many different antibody combinations, targeting at least two different antigens may be constructed, either as naked antibodies or as partly naked and partly conjugated with a therapeutic agent or immunomodulator, or merely in combination with another therapeutic agents, such as a cytotoxic drug or with radiation.

[0167] Multimodal therapies of the present disclosure further include immunotherapy with naked Mu-9 antibodies or fragments thereof supplemented with administration of antibodies against antigens expressed by colorectal or ovarian carcinomas in the form of naked antibodies, fusion proteins, immunoconjugates, and fragments thereof. In a preferred embodiment, antibodies or fragments thereof for multimodal therapy include, but are not limited to, antibodies against CEA, EGP-1, EGP-2, TAG-72, MUC1, MUC2, MUC3, MUC4, KC4, PAM-4, EGFR, BrE3, Le-Y, KS-1, A3, the A33 antibody and HER2/neu antibodies and fragments thereof, as well as antibodies against angiogenesis factors (e.g., VEGF) or antibodies against oncogene markers or products, as well as antibodies against immunomodulators, (e.g., CD40). These antibodies include polyclonal, monoclonal, chimeric, human or humanized antibodies and fragments thereof that recognize at least one epitope on these antigenic determinants. In another form of multimodal therapy, subjects receive naked antibodies or fragments thereof, and/or immunoconjugates or fragments thereof, in conjunction with standard cancer chemotherapy. 5-fluorouracil in combination with folinic acid, alone or in combination with irinotecan (CPT-11), is a regimen used to treat colorectal cancer. Other suitable combination chemotherapeutic regimens are well known, such as with oxaliplatin alone, or in combination with these other drugs, to those of skill in the art. In ovarian cancer, still other chemotherapeutic agents may be preferred, such as any one of the taxanes and platinum agents, Thio-TEPA and other alkylating agents (e.g., chlorambucil), as well as gemcitabine and other more recent classes of cytotoxic drugs. In a preferred multimodal therapy, both chemotherapeutic drugs and cytokines are co-administered with an antibody, immunoconjugate or fusion protein according to the present invention. The cytokines, chemotherapeutic drugs and antibody or immunoconjugate can be administered in any order, or together.

[0168] A variety of other chemotherapeutic agents may be used in for combination treatment or for making immunoconjugates. Such chemotherapeutic agents include, but are not limited to, adriamycin, dactinomycin, mitomycin, carminomycin, daunomycin, doxorubicin, tamoxifen, taxol, taxotere, vincristine, vinblastine, vinorelbine, etoposide (VP-16), 5-fluorouracil (5FU), cytosine arabinoside, cyclophohphamide, thiotepa, methotrexate, camptothecin, actinomycin-D, mitomycin C, cisplatin (CDDP), aminopterin, combretastatin(s), neomycin, podophyllotoxin(s), TNF-α, $\alpha 2\beta 3$ antagonists, calcium ionophores, calcium-flux inducing agents and derivatives and prodrugs thereof. Anti-metabolites such as cytosine arabinoside, aminopterin; anthracyclines; vinca alkaloids and other alkaloids; antibiotics, demecolcine; etopside; mithramycin; and other anti-tumor alkylating agents are also contemplated for use with the antibodies of the present disclosure.

[0169] In addition, a therapeutic composition of the present disclosure can contain a mixture or hybrid molecules of monoclonal naked Mu-9 antibodies or their fragments directed to different, non-blocking epitopes on the CSAp molecule. Accordingly, the present disclosure contemplates therapeutic compositions comprising a mixture of monoclonal Mu-9 antibodies or their fragments that bind at least two CSAp epitopes. Additionally, the therapeutic composition described herein may contain a mixture of Mu-9 antibodies and fragments thereof with varying CDR sequences.

Naked antibody therapy

[0170] A therapeutically effective amount of the naked chimeric, humanized or human anti-CSAp antibodies, or their fragments can be formulated in a pharmaceutically acceptable excipient. The efficacy of the naked Mu-9 antibodies can also be enhanced by supplementing naked antibodies with one or more other naked antibodies (such as against tumor-

associated antigens, or also agonist or antagonist antibodies to immunomodulators, such as CD40 antigen or ligand), with one or more immunoconjugates of Mu-9, with one or more immunoconjugates of the antibodies against tumor-associated antigens other than CSAp, and conjugated with therapeutic agents, including drugs, toxins, cytokines, immunomodulators, hormones, hormone antagonists, enzymes, enzyme inhibitors, therapeutic radionuclides, etc., with one or more therapeutic agents, including drugs, toxins, cytokines, immunomodulators, hormones, enzymes, enzyme inhibitors, therapeutic radionuclides, etc., administered concurrently or sequentially or according to a prescribed dosing regimen, with the Mu-9 antibodies.

Humanized, chimeric and human anti-CSAp immunoconjugates

[0171] Alternatively, conjugates of the Mu-9 antibodies or fragments thereof of the present disclosure may be administered. For therapy, these conjugates preferably contain a cytotoxic agent. More preferably, the cytotoxic agent is a toxin. An immunoconjugate, as described herein, is a molecule comprising an antibody component and a therapeutic or diagnostic agent, including a peptide which may bear the diagnostic or therapeutic agent. An immunoconjugate retains the immunoreactivity of the antibody component, i.e., the antibody moiety has about the same or slightly reduced ability to bind the cognate antigen after conjugation as before conjugation.

[0172] A wide variety of diagnostic/detection and therapeutic reagents can be advantageously conjugated to the antibodies and fragments thereof, of the disclosure. 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 and other alkaloids, anthracyclines, epidophyllotoxins, taxanes, antimetabolites, alkylating agents, antibiotics, Cox-2 inhibitors, 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 the preparation of immunoconjugates and antibody fusion proteins include nitrogen mustards, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, COX-2 inhibitors, pyrimidine analogs, purine analogs, platinum coordination complexes, hormones, toxins (e.g., RNAse, Psudomonas exotoxin), 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.

[0173] 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 composite. 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.,* Cancer Res. 45:4380 (1985); Oseroff et al., Proc. Natl. Acad. Sci. USA 83:8744 (1986); *idem.,* Photochem. 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 disclosure contemplates the therapeutic use of immunoconjugates comprising photoactive agents or dyes.

[0174] A toxin, such as *Pseudomonas* exotoxin, may also be complexed to or form the therapeutic agent portion of an immunoconjugate. For example, the toxin may be complexed with a Mu-9 antibody of the present disclosure, or when used in combination with the naked Mu-9 antibody of conjugates of the Mu-9 antibody, also complexed to the other, non-CSAp antibodies used in the present disclosure. 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, disclosure. These can be derived, for example, from animal, plant and microbial sources.

[0175] An immunomodulator, such as a cytokine may also be conjugated to, or form the therapeutic agent portion of the anti-CSAp immunoconjugate, or be administered unconjugated to the chimeric, humanized or human anti-CSAp antibodies of the disclosure. 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 and IL-18), colony stimulating factors (*e.g.*, granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF)), interferons (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, interferon-γ, TNF-α, and the like. Alternatively, subjects can receive naked Mu-9 antibodies and a separately administered cytokine, which can be administered before, concurrently or after administration of the naked Mu-9 antibodies. The Mu-9 antibody may also be conjugated to the immunomodulator. The immunomodulator may also be conjugated to a hybrid antibody consisting of one or more antibodies binding to different antigens. Such an antigen may also be an immunomodulator. For example, CD40 or other immunomodulators may be administered in combination with the anti-CSAp or anti-CSAp/non-CSAp antibody combination either together, before or after the antibody combinations are administered. The Mu-9 antibody may also be used in combination with, or conjugated to, as a fusion protein, an immunomodulating antibody, such as against CD40.

[0176] Furthermore, the present disclosure includes methods of diagnosing or detecting a malignancy in a subject. Diagnosis/detection may be accomplished by administering a diagnostically effective amount of a diagnostic conjugate, formulated in a pharmaceutically acceptable excipient, and detecting said label. For example, radioactive and non-radioactive agents can be used as diagnostic agents. A suitable non-radioactive diagnostic agent is a contrast agent suitable for magnetic resonance imaging, a radiopaque compound for X-rays or computed tomography, or a contrast agent suitable for 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, by In a preferred embodiment, the contrast agent is an ultrasound enhancing agent. Still preferred, the ultrasound enhancing agent is a liposome. 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 incolude 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.

[0177] Furthermore, a radiolabeled antibody or immunoconjugate may comprise a γ-emitting radioisotope or a positron-emitter useful for diagnostic imaging. Examples of diagnostic/detection agents include diverse labels, radionuclides, chelators, dyes, contrast agents, fluorescent compounds, chromagens, and other marker moieties. 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 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.

[0178] Additionally, radionuclides suitable for treating a diseased tissue include, **but are not limited to**, P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, and Bi-213, Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, I-125, Ho-161, Os-189m, Ir-192, 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.

[0179] Suitable diagnostic imaging isotopes are usually in the range of 20 to 2,000 keV, while suitable therapeutic radionuclides are usually in the range of 20 to 10,000 keV. See for example, U.S. Patent Application entitled "Labeling Targeting Agents with Gallium-68"- Inventors G.L.Griffiths and W.J. McBride, (U.S. Provisional Application No. 60/342,104), which discloses positron emitters, such as ^{18}F, ^{68}Ga, ^{94m}Tc. and the like, for imaging purposes and which is.

Bispecific antibody therapy

[0180] The present disclosure also encompasses the use of the bsAb and a therapeutic agent associated with the linker moieties discussed above in intraoperative, intravascular, and endoscopic tumor and lesion detection, biopsy and therapy as described in U.S. Patent No. 6,096,289.

[0181] The antibodies and antibody fragments of the present disclosure can be employed not only for therapeutic or imaging purposes, but also as aids in performing research *in vitro.* For example, the bsAbs of the present disclosure can be used *in vitro* to ascertain if a targetable construct can form a stable complex with one or more bsAbs. Such an assay would aid the skilled artisan in identifying targetable constructs which form stable complexes with bsAbs. This would, in turn, allow the skilled artisan to identify targetable constructs which are likely to be superior as therapeutic and/or imaging agents.

[0182] The assay is advantageously performed by combining the targetable construct in question with at least two molar equivalents of a bsAb. Following incubation, the mixture is analyzed by size-exclusion HPLC to determine whether or not the construct has bound to the bsAb. Alternatively, the assay is performed using standard combinatorial methods wherein solutions of various bsAbs are deposited in a standard 96 well plate. To each well, is added solutions of targetable

construct(s). Following incubation and analysis, one can readily determine which construct(s) bind(s) best to which bsAb (s).

[0183] It should be understood that the order of addition of the bsAb to the targetable construct is not crucial; that is, the bsAb may be added to the construct and vice versa. Likewise, neither the bsAb nor the construct needs to be in solution; that is, they may be added either in solution or neat, whichever is most convenient. Lastly, the method of analysis for binding is not crucial as long as binding is established. Thus, one may analyze for binding using standard analytical methods including, but not limited to, FABMS, high-field NMR or other appropriate method in conjunction with, or in place of, size exclusion HPLC.

[0184] The present disclosure provides a bispecific antibody or antibody fragment having at least a binding region that specifically binds a targeted cell marker and at least one other binding region that specifically binds a targetable conjugate. The targetable conjugate comprises a carrier portion which comprises or bears at least one epitope recognized by at least one binding region of the bispecific antibody or antibody fragment.

[0185] For example, the anti-CSAp antibodies and fragments thereof, as well as other antibodies with different specificities and fragments thereof, for use in combination therapy, described herein, can also be made as multispecific antibodies (comprising at least one binding site to a CSAp epitope or antigen and at least one binding site to another epitope on CSAp or another antigen) and multivalent antibodies (comprising mutliple binding sites to the same epitope or antigen).

[0186] A variety of recombinant methods can be used to produce bispecific antibodies and antibody fragments as described above.

[0187] In a preferred embodiment, the multivalent antibody is a Mu-9 antibody. A Mu-9 multivalent antibody is also contemplated in the present disclosure. This multivalent antibody is constructed by association of a first and a second polypeptide. The first polypeptide comprises a first single chain Fv molecule covalently linked to a first immunoglobulin-like domain which preferably is an immunoglobulin light chain variable region domain. The second polypeptide comprises a second single chain Fv molecule covalently linked to a second immunoglobulin-like domain which preferably is an immunoglobulin heavy chain variable region domain. Each of the first and second single chain Fv molecules forms a target binding site, and the first and second immunoglobulin-like domains associate to form a third target binding site.

[0188] A single chain Fv molecule with the VL-L-VH configuration, wherein L is a linker, may associate with another single chain Fv molecule with the VH-L-VL configuration to form a bivalent dimer. In this case, the VL domain of the first scFv and the VH domain of the second scFv molecule associate to form one target binding site, while the VH domain of the first scFv and the VL domain of the second scFv associate to form the other target binding site.

[0189] Another embodiment disclosure is a Mu-9 bispecific, trivalent antibody comprising two heterologous polypeptide chains associated non-covalently to form three binding sites, two of which have affinity for one target and a third which has affinity for a hapten that can be made and attached to a carrier for a diagnostic and/or therapeutic agent. Preferably, the binding protein has two CSAp binding sites and one other antigen binding site. The bispecific, trivalent targeting agents have two different scFvs, one scFv contains two V_H domains from one antibody connected by a short linker to the V_L domain of another antibody and the second scFv contains two V_L domains from the first antibody connected by a short linker to the V_H domain of the other antibody. The methods for generating multivalent, multispecific agents from V_H and V_L domains provide that individual chains synthesized from a DNA plasmid in a host organism are composed entirely of V_H domains (the V_H-chain) or entirely of V_L domains (the V_L-chain) in such a way that any agent of multivalency and multispecificity can be produced by non-covalent association of one V_H-chain with one V_L-chain. For example, forming a trivalent, trispecific agent, the V_H-chain will consist of the amino acid sequences of three V_H domains, each from an antibody of different specificity, joined by peptide linkers of variable lengths, and the V_L-chain will consist of complementary V_L domains, joined by peptide linkers similar to those used for the V_H-chain. Since the V_H and V_L domains of antibodies associate in an anti-parallel fashion, the preferred method in this disclosure has the V_L domains in the V_L-chain arranged in the reverse order of the V_H domains in the V_H-chain.

[0190] Bispecific antibodies and fragments thereof of the present disclosure useful in pretargeting methods and provide a preferred way to deliver two therapeutic agents or two diagnostic/detection agents to a subject. U.S. Serial No. 09/382,186 discloses a method of pretargeting using a bispecific antibody, in which the bispecific antibody is labeled with ^{125}I and delivered to a subject, followed by a divalent peptide labeled with ^{99m}Tc. The delivery results in excellent tumor/normal tissue ratios for ^{131}I and ^{99m}Tc, thus showing the utility of two diagnostic radioisotopes. Any combination of known therapeutic agents or diagnostic agents can be used to label the antibodies and antibody fusion proteins. The binding specificity of the antibody component of the mAb conjugate, the efficacy of the therapeutic agent or diagnostic agent and the effector activity of the Fc portion of the antibody can be determined by standard testing of the conjugates.

Preparation of humanized, chimeric and human Mu-9 immunoconjugates

[0191] Any of the anti-CSAp antibodies or fragments thereof or antibody fusion proteins or fragments thereof of the present disclosure can be conjugated with one or more therapeutic or diagnostic agents. Generally, one therapeutic or

diagnostic agent is attached to each antibody or antibody fragment but more than one therapeutic agent or diagnostic agent can be attached to the same antibody or antibody fragment. The antibody fusion proteins of the present disclosure comprise two or more antibodies or fragments thereof and each of the antibodies that compose this fusion protein can contain a therapeutic agent or diagnostic agent. In other words, the antibody fusion protein or fragment thereof can comprise at least one first anti-CSAp MAb or fragment thereof and at least one second MAb or fragment thereof that is not an anti-CSAp MAb. Preferably, the second MAb is a carcinoma-associated antibody, such as an antibody against CEA, EGP-1, EGP-2, MUC-1, MUC2, MUC3, MUC4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, BrE3, Le-Y, A3, KS-1, VEGF and other angiogenesis antibodies, oncogene antibodies, anti-necrosis antibodies, or the antibody A33. Additionally, one or more of the antibodies of the antibody fusion protein can have more than one therapeutic 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. Particulary, 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 agents also can be attached to reduced SH groups and to the carbohydrate side chains.

[0192] Also preferred, the antibody fusion protein of the present disclosure comprises at least two anti-CSAp monoclonal antibodies or fragments thereof, and these may be to different epitopes of the CSAp antigen or of different human immunoglobulin backbone sequences (or IgGs).

[0193] A therapeutic or diagnostic agent can be attached at the hinge region of a reduced antibody component via disulfide bond formation. As an alternative, such peptides 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 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 peptide that is bound to a thiol group, or the carbohydrate moiety can be used to bind a different peptide.

[0194] 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.

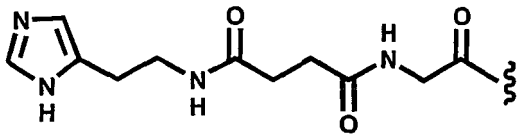
[0195] The Fc region is absent if the antibody used as the antibody component of the immunoconjugate is an antibody fragment. However, it is possible to introduce a carbohydrate moiety into the light chain variable region of a full length antibody or antibody fragment. See, for example, Leung et al., 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 agent.

V. Constructs Targetable to Antibodies

[0196] The targetable construct can be of diverse structure, but is selected not only to avoid eliciting an immune elicit sufficient immune responses, but also for rapid *in vivo* clearance when used within the bsAb targeting method. Hydrophobic agents are best at eliciting strong immune responses, whereas hydrophilic agents are preferred for rapid *in vivo* clearance, thus, a balance between hydrophobic and hydrophilic needs to be established. This is accomplished, in part, by relying on the use of hydrophilic chelating agents to offset the inherent hydrophobicity of many organic moieties. Also, subunits of the targetable construct may be chosen which have opposite solution properties, for example, peptides, which contain amino acids, some of which are hydrophobic and some of which are hydrophilic. Aside from peptides, carbohydrates may be used.

[0197] Peptides having as few as two amino-acid residues may be used, preferably two to ten residues, if also coupled to other moieties such as chelating agents. The linker should be a low molecular weight conjugate, preferably having a molecular weight of less than 50,000 daltons, and advantageously less than about 20,000 daltons, 10,000 daltons or 5,000 daltons, including the metal ions in the chelates. For instance, the known peptide DTPA-Tyr-Lys(DTPA)-OH (wherein DTPA is diethylenetriaminepentaacetic acid) has been used to generate antibodies against the indium-DTPA portion of the molecule. However, by use of the non-indium-containing molecule, and appropriate screening steps, new Abs against the tyrosyl-lysine dipeptide can be made. More usually, the antigenic peptide will have four or more residues, such as the peptide DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2, wherein DOTA is 1,4,7,10-tetraazacyclododecane-

tetraacetic acid and HSG is the histamine succinyl glycyl group of the formula:



[0198] The non-metal-containing peptide may be used as an immunogen, with resultant Abs screened for reactivity against the Phe-Lys-Tyr-Lys backbone.

[0199] The disclosure also contemplates the incorporation of unnatural amino acids, e.g., D-amino acids, into the backbone structure to ensure that, when used with the final bsAb/linker system, the arm of the bsAb which recognizes the linker moiety is completely specific. The disclosure further contemplates other backbone structures such as those constructed from non-natural amino acids and peptoids.

[0200] The peptides to be used as immunogens are synthesized conveniently on an automated peptide synthesizer using a solid-phase support and standard techniques of repetitive orthogonal deprotection and coupling. Free amino groups in the peptide, that are to be used later for chelate conjugation, are advantageously blocked with standard protecting groups such as an acetyl group. Such protecting groups will be known to the skilled artisan. *See* Greene and Wuts Protective Groups in Organic Synthesis, 1999 (John Wiley and Sons, N.Y.). When the peptides are prepared for later use within the bsAb system, they are advantageously cleaved from the resins to generate the corresponding C-terminal amides, in order to inhibit *in vivo* carboxypeptidase activity.

[0201] The haptens of the immunogen comprise an immunogenic recognition moiety, for example, a chemical hapten. Using a chemical hapten, preferably the HSG hapten, high specificity of the linker for the antibody is exhibited. This occurs because antibodies raised to the HSG hapten are known and can be easily incorporated into the appropriate bispecific antibody. Thus, binding of the linker with the attached hapten would be highly specific for the antibody or antibody fragment.

Chelate Moietie

[0202] The presence of hydrophilic chelate moieties on the linker moieties helps to ensure rapid *in vivo* clearance. In addition to hydrophilicity, chelators are chosen for their metal-binding properties, and are changed at will since, at least for those linkers whose bsAb epitope is part of the peptide or is a non-chelate chemical hapten, recognition of the metal-chelate complex is no longer an issue.

[0203] Particularly useful metal-chelate combinations include 2-benzyl-DTPA and its monomethyl and cyclohexyl analogs, used with ^{47}Sc, ^{52}Fe, ^{55}Co, ^{67}Ga, ^{68}Ga, ^{111}In, ^{89}Zr, ^{90}Y, ^{161}Tb, ^{177}Lu, ^{212}Bi, ^{213}Bi, and ^{225}Ac for radio-imaging and RAIT. The same chelators, when complexed with non-radioactive metals, such as Mn, Fe and Gd can be used for MRI, when used along with the bsAbs of the invention. Macrocyclic chelators such as NOTA (1,4,7-triaza-cyclononane-N,N', N"-triacetic acid), DOTA, and TETA (p-bromoacetamido-benzyl-tetraethylaminetetraacetic acid) are of use with a variety of metals and radiometals, most particularly with radionuclides of Ga, Y and Cu, respectively.

[0204] DTPA and DOTA-type chelators, where the ligand includes hard base chelating functions such as carboxylate or amine groups, are most effective for chelating hard acid cations, especially Group IIa and Group IIIa metal cations. Such metal-chelate complexes can be made very stable by tailoring the ring size to the metal of interest. Other ring-type chelators such as macrocyclic polyethers are of interest for stably binding nuclides such as ^{223}Ra for RAIT. Porphyrin chelators may be used with numerous radiometals, and are also useful as certain cold metal complexes for bsAb-directed immuno-phototherapy. More than one type of chelator may be conjugated to a carrier to bind multiple metal ions, *e.g.*, cold ions, diagnostic radionuclides and/or therapeutic radionuclides. Particularly useful therapeutic radionuclides include, but are not limited to ^{32}P, ^{33}P, ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{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. Particularly useful diagnostic radionuclides include, but are not limited to, ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{57}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, ^{94m}Tc, ^{14}Tc, ^{99m}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, 154-^{158}Gd and ^{175}Lu.

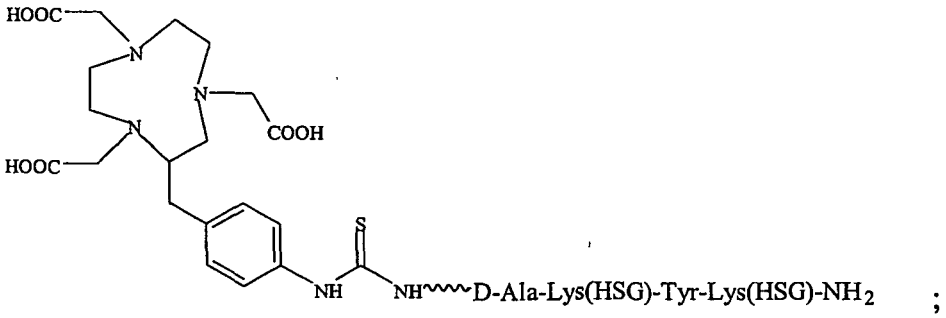
[0205] Chelators such as those disclosed in U.S. Patent 5,753,206, especially thiosemicarbazonylglyoxylcysteine (Tscg-Cys) and thiosemicarbazinyl-acetylcysteine (Tsca-Cys) chelators are advantageously used to bind soft acid cations of Tc, Re, Bi and other transition metals, lanthanides and actinides that are tightly bound to soft base ligands, especially sulfur- or phosphorus-containing ligands. It can be useful to link more than one type of chelator to a peptide, e.g., a DTPA or similar chelator for, say In(III) cations, and a thiol-containing chelator, e.g., Tscg-Cys, for Tc cations. Because antibodies to a di-DTPA hapten are known (Barbet '395, *supra*) and are readily coupled to a targeting antibody to form a bsAb, it is possible to use a peptide hapten with cold diDTPA chelator and another chelator for binding a radioisotope, in a pretargeting protocol, for targeting the radioisotope. One example of such a peptide is Ac-Lys(DTPA)-Tyr-Lys

(DTPA)-Lys(Tscg-Cys-)-NH_2. This peptide can be preloaded with In(III) and then labeled with 99-m-Tc cations, the In (III) ions being preferentially chelated by the DTPA and the Tc cations binding preferentially to the thiol-containing Tscg-Cys. Other hard acid chelators such as NOTA, DOTA, TETA and the like can be substituted for the DTPA groups, and Mabs specific to them can be produced using analogous techniques to those used to generate the anti-di-DTPA Mab.

[0206] It will be appreciated that two different hard acid or soft acid chelators can be incorporated into the linker, e.g., with different chelate ring sizes, to bind preferentially to two different hard acid or soft acid cations, due to the differing sizes of the cations, the geometries of the chelate rings and the preferred complex ion structures of the cations. This will permit two different metals, one or both of which may be radioactive or useful for MRI enhancement, to be incorporated into a linker for eventual capture by a pretargeted bsAb.

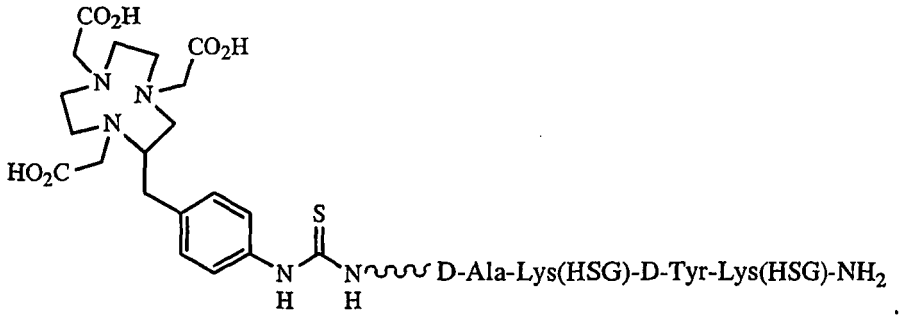
[0207] Preferred chelators include NOTA, DOTA and Tscg and combinations thereof. These chelators have been incorporated into a chelator-peptide conjugate motif as exemplified in the following constructs:

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



;

and
(e)



[0208] The chelator-peptide conjugates (d) and (e), above, has been shown to bind ^{68}Ga and is thus useful in positron emission tomography (PET) applications.

[0209] Chelators are coupled to the linker moieties using standard chemistries which are discussed more fully in the working Examples below. Briefly, the synthesis of the peptide Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys-)-NH_2 was accomplished by first attaching Aloc-Lys(Fmoc)-OH to a Rink amide resin on the peptide synthesizer. The protecting group abbreviations "Aloc" and "Fmoc" used herein refer to the groups allyloxycarbonyl and fluorenylmethyloxy carbonyl. The Fmoc-Cys(Trt)-OH and TscG were then added to the side chain of the lysine using standard Fmoc automated synthesis protocols to form the following peptide: Aloc-Lys(Tscg-Cys(Trt)-rink resin. The Aloc group was then removed. The peptide synthesis was then continued on the synthesizer to make the following peptide: (Lys(Aloc)-D-Tyr-Lys (Aloc)-Lys(Tscg-Cys(Trt)-)-rink resin. Following N-terminus acylation, and removal of the side chain Aloc protecting groups. The resulting peptide was then treated with activated N-trityl-HSG-OH until the resin gave a negative test for amines using the Kaiser test. *See* Karacay et al. Bioconjugate Chem. 11:842-854 (2000). The synthesis of Ac-Lys(HSG) D-Tyr-Lys(HSG)-Lys(Tscg-Cys-)-NH_2, as well as the syntheses of DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2; and DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 are described in greater detail below.

Preparation of Metal Chelates

[0210] Chelator-peptide conjugates may be stored for long periods as solids. They may be metered into unit doses for metal-binding reactions, and stored as unit doses either as solids, aqueous or semi-aqueous solutions, frozen solutions or lyophilized preparations. They may be labeled by well-known procedures. Typically, a hard acid cation is introduced as a solution of a convenient salt, and is taken up by the hard acid chelator and possibly by the soft acid chelator. However, later addition of soft acid cations leads to binding thereof by the soft acid chelator, displacing any hard acid cations which may be chelated therein. For example, even in the presence of an excess of cold $^{111}InCl_3$, labeling with 99m-Tc(V) glucoheptonate or with Tc cations generated *in situ* with stannous chloride and Na99m-TcO4 proceeds quantitatively on the soft acid chelator. Other soft acid cations such as ^{186}Re, ^{188}Re, ^{213}Bi and divalent or trivalent cations of Mn, Co, Ni, Pb, Cu, Cd, Au, Fe, Ag (monovalent), Zn and Hg, especially ^{64}Cu and ^{67}Cu, and the like, some of which are useful for radioimmunodiagnosis or radioimmunotherapy, can be loaded onto the linker peptide by analogous methods. Re cations also can be generated *in situ* from perrhenate and stannous ions or a prereduced rhenium glucoheptonate or other transchelator can be used. Because reduction of perrhenate requires more stannous ion (typically above 200 μg/mL final concentration) than is needed for the reduction of Tc, extra care needs to be taken to ensure that the higher levels of stannous ion do not reduce sensitive disulfide bonds such as those present in disulfide-cyclized peptides. During radiolabeling with rhenium, similar procedures are used as are used with the Tc-99m. A preferred method for the preparation of ReO metal complexes of the Tscg-Cys- ligands is by reacting the peptide with $ReOCl_3(P(Ph_3)_2$ but it is also possible to use other reduced species such as $ReO(ethylenediamine)_2$.

VI. Methods of Administration

[0211] It should be noted that much of the discussion presented herein below focuses on the use of the bispecific antibodies and targetable constructs in the context of treating diseased tissue. The invention contemplates, however, the use of the bispecific antibodies and targetable constructs in treating and/or imaging normal tissue and organs using the methods described in U.S. Patent Nos. 6,126,916; 6,077,499; 6,010,680; 5,776,095; 5,776,094; 5,776,093; 5,772,981; 5,753,206; 5,746,996; 5,697,902; 5,328,679; 5,128,119; 5,101,827; and 4,735,210. As used herein, the term "tissue" refers to tissues, including but not limited to, tissues from the ovary, thymus, parathyroid or spleen.

[0212] The administration of a bsAb and a therapeutic agent associated with the linker moieties discussed above may be conducted by administering the bsAb at some time prior to administration of the therapeutic agent which is associated with the linker moiety. The doses and timing of the reagents can be readily devised by a skilled artisan, and are dependent on the specific nature of the reagents employed. If a bsAb-$F(ab')_2$ derivative is given first, then a waiting time of 24-72 hr before administration of the linker moiety would be appropriate. If an IgG-Fab' bsAb conjugate is the primary targeting vector, then a longer waiting period before administration of the linker moiety would be indicated, in the range of 3-10 days.

[0213] As used herein, the term "therapeutic agent" includes, but is not limited to a drug, prodrug and/or toxin. The terms "drug," "prodrug," and "toxin" are defined throughout the specification. A diagnostic agent is more often used to determine the kind of disease present, while a detection agent is more often used for localization and diagnosis. However, as described herein, the term diagnostic agent can also be used to refer to a detection agent.

[0214] After sufficient time has passed for the bsAb to target to the diseased tissue, the diagnostic/detection agent is administered. Subsequent to administration of the diagnostic/detection agent, imaging can be performed. Tumors can be detected in body cavities by means of directly or indirectly viewing various structures to which light of the appropriate wavelength is delivered and then collected. Lesions at any body site can be viewed so long as nonionizing radiation can be delivered and recaptured from these structures. For example, PET which is a high resolution, non-invasive, imaging technique can be used with the inventive antibodies for the visualization of human disease. In PET, 511 keV gamma photons produced during positron annihilation decay are detected.

[0215] The disclosure generally contemplates the use of diagnostic agents which emit 25-600 keV gamma particles and/or positrons. Examples of such agents include, but are not limited to ^{18}F, ^{52}Fe,^{62}Cu, ^{14}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, ^{94m}Tc, ^{94}Tc, ^{99m}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154\text{-}158}Gd$ and ^{175}Lu.

[0216] The present antibodies or antibody fragments can be used in a method of photodynamic therapy (PDT) as discussed in U.S. Patent Nos. 6,096,289; 4,331,647; 4,818,709; 4,348,376; 4,361,544; 4,444,744; 5,851,527.

[0217] In PDT, a photosensitizer, e.g., a hematoporphyrin derivative such as dihematoporphyrin ether, is administered to a subject. Anti-tumor activity is initiated by the use of light, e.g., 630 nm. Alternate photosensitizers can be utilized, including those useful at longer wavelengths, where skin is less photosensitized by the sun. Examples of such photosensitizers include, but are not limited to, benzoporphyrin monoacid ring A (BPD-MA), tin etiopurpurin (SnET2), sulfonated aluminum phthalocyanine (AlSPc) and lutetium texaphyrin (Lutex).

[0218] Additionally, in PDT, a diagnostic agent is injected, for example, systemically, and laser-induced fluorescence can be used by endoscopes to detect sites of cancer which have accreted the light-activated agent. For example, this has been applied to fluorescence bronchoscopic disclosure of early lung tumors. Doiron *et al. Chest 76*:32 (1979). In

another example, the antibodies and antibody fragments can be used in single photon emission. For example, a Tc-99m-labeled diagnostic agent can be administered to a subject following administration of the inventive antibodies or antibody fragments. The subject is then scanned with a gamma camera which produces single-photon emission computed tomographic images and defines the lesion or tumor site.

[0219] Therapeutically useful immunoconjugates can be obtained by conjugating photoactive agents or dyes to an antibody composite. Fluorescent 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*., Cancer Res. 45:4380 (1985); Oseroff et al., Proc. Natl. Acad. Sci. USA 83:8744 (1986); *idem*., Photochem. 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.

[0220] The linker moiety may also be conjugated to an enzyme capable of activating a prodrug at the target site or improving the efficacy of a normal therapeutic by controlling the body's detoxification pathways. Following administration of the bsAb, an enzyme conjugated to the linker moiety, a low MW hapten recognized by the second arm of the bsAb, is administered. After the enzyme is pretargeted to the target site, a cytotoxic drug is injected, which is known to act at the target site. The drug may be one which is detoxified by the mammal's ordinary detoxification processes. For example, the drug may be converted into the potentially less toxic glucuronide in the liver. The detoxified intermediate can then be reconverted to its more toxic form by the pretargeted enzyme at the target site. Alternatively, an administered prodrug can be converted to an active drug by the pretargeted enzyme. The pretargeted enzyme improves the efficacy of the treatment by recycling the detoxified drug. This approach can be adopted for use with any enzyme-drug pair.

[0221] Certain cytotoxic drugs that are useful for anticancer therapy are relatively insoluble in serum. Some are also quite toxic in an unconjugated form, and their toxicity is considerably reduced by conversion to prodrugs. Conversion of a poorly soluble drug to a more soluble conjugate, *e.g.* , a glucuronide, an ester of a hydrophilic acid or an amide of a hydrophilic amine, will improve its solubility in the aqueous phase of serum and its ability to pass through venous, arterial or capillary cell walls and to reach the interstitial fluid bathing the tumor. Cleavage of the prodrug deposits the less soluble drug at the target site. Many examples of such prodrug-to-drug conversions are disclosed in Hansen U.S. Patent No. 5,851,527.

[0222] Conversion of certain toxic substances such as aromatic or alicyclic alcohols, thiols, phenols and amines to glucuronides in the liver is the body's method of detoxifying them and making them more easily excreted in the urine. One type of antitumor drug that can be converted to such a substrate is epirubicin, a 4-epimer of doxorubicin (Adriamycin), which is an anthracycline glycoside and has been shown to be a substrate for human beta-D-glucuronidase See, e.g., Arcamone Cancer Res. 45:5995 (1985). Other analogues with fewer polar groups are expected to be more lipophilic and show greater promise for such an approach. Other drugs or toxins with aromatic or alicyclic alcohol, thiol or amine groups are candidates for such conjugate formation. These drugs, or other prodrug forms thereof, are suitable candidates for the site-specific enhancement methods of the present invention.

[0223] The prodrug CPT-11 (irinotecan) is converted *in vivo* by carboxylesterase to the active metabolite SN-38. One application of the invention, therefore, is to use a bsAb targeted against a tumor and a hapten (*e.g.* di-DTPA) followed by injection of a di-DTPA-carboxylesterase conjugate. Once a suitable tumor-to-background localization ratio has been achieved, the CPT-11 is given and the tumor-localized carboxylesterase serves to convert CPT-11 to SN-38 at the tumor. Due to its poor solubility, the active SN-38 will remain in the vicinity of the tumor and, consequently, will exert an effect on adjacent tumor cells that are negative for the antigen being targeted. This is a further advantage of the method. Modified forms of carboxylesterases have been described and are within the scope of the invention. See, e.g., Potter et al., Cancer Res. 58:2646-2651 (1998) and Potter et al., Cancer Res. 58:3627-3632 (1998).

[0224] Etoposide is a widely used cancer drug that is detoxified to a major extent by formation of its glucuronide and is within the scope of the invention. See, e.g., Hande et al. Cancer Res. 48:1829-1834 (1988). Glucuronide conjugates can be prepared from cytotoxic drugs and can be injected as therapeutics for tumors pre-targeted with mAb-glucuronidase conjugates. See, e.g., Wang et al. Cancer Res. 52:4484-4491 (1992). Accordingly, such conjugates also can be used with the pre-targeting approach described here. Similarly, designed prodrugs based on derivatives of daunomycin and doxorubicin have been described for use with carboxylesterases and glucuronidases. See, e.g., Bakina et al. J. Med Chem. 40:4013-4018 (1997). Other examples of prodrug/enzyme pairs that can be used within the present invention include, but are not limited to, glucuronide prodrugs of hydroxy derivatives of phenol mustards and beta-glucu.ronidase; phenol mustards or CPT-11 and carboxypeptidase; methotrexate-substituted alpha-amino acids and carboxypeptidase A; penicillin or cephalosporin conjugates of drugs such as 6-mercaptopurine and doxorubicin and beta-lactamase; etoposide phosphate and alkaline phosphatase.

[0225] The enzyme capable of activating a prodrug at the target site or improving the efficacy of a normal therapeutic by controlling the body's detoxification pathways may aternatively be conjugated to the hapten. The enzyme-hapten conjugate is administered to the subject following administration of the pre-targeting bsAb and is directed to the target site. After the enzyme is localized at the target site, a cytotoxic drug is injected, which is known to act at the target site, or a prodrug form thereof which is converted to the drug *in situ* by the pretargeted enzyme. As discussed above, the drug is one which is detoxified to form an intermediate of lower toxicity, most commonly a glucuronide, using the mammal's ordinary detoxification processes. The detoxified intermediate, *e.g.,* the glucuronide, is reconverted to its more toxic form by the pretargeted enzyme and thus has enhanced cytotoxicity at the target site. This results in a recycling of the drug. Similarly, an administered prodrug can be converted to an active drug through normal biological processess. The pretargeted enzyme improves the efficacy of the treatment by recycling the detoxified drug. This approach can be adopted for use with any enzyme-drug pair.

[0226] The invention further contemplates the use of the inventive bsAb and the diagnostic agent(s) in the context of Boron Neutron Capture Therapy (BNCT) protocols. BNCT is a binary system designed to deliver ionizing radiation to tumor cells by neutron irradiation of tumor-localized ^{10}B atoms. BNCT is based on the nuclear reaction which occurs when a stable isotope, isotopically enriched ^{10}B (present in 19.8% natural abundance), is irradiated with thermal neutrons to produce an alpha particle and a ^{7}Li nucleus. These particles have a path length of about one cell diameter, resulting in high linear energy transfer. Just a few of the short-range 1.7 MeV alpha particles produced in this nuclear reaction are sufficient to target the cell nucleus and destroy it. Success with BNCT of cancer requires methods for localizing a high concentration of ^{10}B at tumor sites, while leaving non-target organs essentially boron-free. Compositions and methods for treating tumors in subjects using pre-targeting bsAb for BNCT are described in co-pending Patent Appl. Serial No. 09/205,243 and can easily be modified for the purposes of the present invention.

[0227] It should also be noted that a bispecific antibody or antibody fragment can be used in the present method, with at least one binding site specific to an antigen at a target site and at least one other binding site specific to the enzyme component of the antibody-enzyme conjugate. Such an antibody can bind the enzyme prior to injection, thereby obviating the need to covalently conjugate the enzyme to the antibody, or it can be injected and localized at the target site and, after non-targeted antibody has substantially cleared from the circulatory system of the mammal, enzyme can be injected in an amount and by a route which enables a sufficient amount of the enzyme to reach a localized antibody or antibody fragment and bind to it to form the antibody-enzyme conjugate *in situ.*

[0228] It should also be noted that the invention also contemplates the use of multivalent target binding proteins which have at least three different target binding sites as described in Patent Appl. Serial No. 09/911,610. Multivalent antibodies have been made by cross-linking several Fab-like fragments via chemical linkers. *See* U.S. Patent Nos. 5,262,524; 5,091,542 and Landsdorp et al. Euro. J. Immunol. 16: 679-83 (1986). Multivalent antibodies also have been made by covalently linking several single chain Fv molecules (scFv) to form a single polypeptide. *See* U.S. Patent No. 5,892,020. A multivalent antibody which is basically an aggregate of scFv molecules has been disclosed in U.S. Patent Nos. 6,025,165 and 5,837,242. A trivalent target binding protein comprising three scFv molecules has been described in Krott et al. Protein Engineering 10(4): 423-433 (1997).

[0229] A clearing agent may be used which is given between doses of the bsAb and the linker moiety. The present inventors have discovered that a clearing agent of novel mechanistic action may be used with the invention, namely a glycosylated anti-idiotypic Fab' fragment targeted against the disease targeting arm(s) of the bsAb. Anti-CEA (MN 14 Ab) x anti-peptide bsAb is given and allowed to accrete in disease targets to its maximum extent. To clear residual bsAb, an anti-idiotypic Ab to MN-14, termed WI2, is given ,preferably as a glycosylated Fab' fragment. The clearing agent binds to the bsAb in a monovalent manner, while its appended glycosyl residues direct the entire complex to the liver, where rapid metabolism takes place. Then the therapeutic which is associated with the linker moiety is given to the subject. The WI2 Ab to the MN-14 arm of the bsAb has a high affinity and the clearance mechanism differs from other disclosed mechanisms (see Goodwin *et al*., *ibid*), as it does not involve cross-linking, because the WI2-Fab' is a monovalent moiety. Alternatively, an anti-CSAp (Mu-9 antibody) x anti-peptide bsAb is given and allowed to accrete in disease targets to its maximum extent.

VII. Pharmaceutically suitable excipient

[0230] The humanized, chimeric or human anti-CSAp antibodies and fragments thereof to be delivered to a subject can consist of the antibody alone, immunoconjugate, fusion protein, or can comprise one or more pharmaceutically suitable excipients, one or more additional ingredients, or some combination of these. Preferably, the anti-CSAp antibody is a Mu-9 antibody.

[0231] The Mu-9 immunoconjugate, naked antibody, fusion protein, and fragments thereof of the present disclosure 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.

[0232] The immunoconjugate, naked antibody, fusion protein, and fragments thereof of the present disclosure 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 multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0233] Additional pharmaceutical methods may be employed to control the duration of action of the therapeutic or diagnostic conjugate or naked antibody. Control release preparations can be prepared through the use of polymers to complex or adsorb the immunoconjugate or naked antibody. 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 an immunoconjugate or antibody from such a matrix depends upon the molecular weight of the immunoconjugate or antibody, the amount of immunoconjugate, 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.

[0234] The immunoconjugate, antibody fusion proteins, or naked antibody may also be administered to a mammal subcutaneously or even by other parenteral routes. 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 4-10 weeks, preferably once per week for 8 weeks, and more preferably, once per week for 4 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.

[0235] For purposes of therapy, the immunoconjugate, fusion protein, or naked antibody, and fragments thereof are administered to a subject in a therapeutically effective amount. A suitable subject for the present invention is a mammal, preferably a human but a non-human mammal such as a dog, cat or horse 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.

[0236] For diagnostic purposes, the immunoconjugate, fusion protein, or naked antibody, and fragments thereof are administered to a subject in a diagnostically effective amount. An antibody preparation is said to be administered in a "diagnostically effective amount" if the amount administered is generally sufficient to diagnose or detect a condition, malignancy, disease or disorder in a subject, usually without any pharmacological effects on the host.

VIII. Expression vectors

[0237] The present disclosure also embraces nucleic acids that encode chimeric, humanized or human anti-CSAp antibodies, fusion proteins and fragments thereof. Expression vectors that comprise such nucleic acids also are included in the invention. The DNA sequence encoding a humanized, chimeric or human Mu-9 antibody can be recombinantly engineered into a variety of known host vectors that provide for replication of the nucleic acid. These vectors can be designed, using known methods, to contain the elements necessary for directing transcription, translation, or both, of the nucleic acid in a cell to which it is delivered. Known methodology can be used to generate expression constructs that have a protein-coding sequence operably linked with appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques and synthetic techniques. For example, see Sambrook et al., 1989, MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory (New York); Ausubel et al., 1997, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons (New York). Also provided for in this invention is the delivery of a polynucleotide not associated with a vector.

[0238] Vectors suitable for use in the instant disclosure can be viral or non-viral. Particular examples of viral vectors include adenovirus, AAV, herpes simplex virus, lentivirus, and retrovirus vectors. An example of a non-viral vector is a plasmid. In a preferred embodiment, the vector is a plasmid.

[0239] An expression vector, as described herein, is a polynucleotide 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.

[0240] Preferably, the expression vector of the instant disclosure comprises the DNA sequence encoding a humanized, chimeric or human Mu-9 antibody, which includes both the heavy and the light chain variable and constant regions. However, two expression vectors may be used, with one comprising the heavy chain variable and constant regions and the other comprising the light chain variable and constant regions. Still preferred, the expression vector further comprises a promoter, a DNA sequence encoding a secretion signal peptide, a genomic sequence encoding a human IgG1 heavy chain constant region, an Ig enhancer element and at least one DNA sequence encoding a selection marker.

[0241] The representative embodiments described below are simply used to illustrate the invention.

IX. Examples

Example 1. Synthesis of Ac-Lys(HSG)-D-Tyr-Lys(HSG)-Lys(Tscg-Cys-)-NH_2 (IMP 243)

[0242] The peptide was synthesized as described by Karacay et. al. Bioconjugate Chem. 11:842-854 (2000) except D-tyrosine was used in place of the L-tyrosine and the N-trityl-HSG-OH was used in place of the DTPA. The final coupling of the N-trityl-HSG-OH was carried out using a ten fold excess of N-trityl-HSG-OH relative to the peptide on the resin. The N-trityl-HSG-OH (0.28 M in NMP) was activated using one equivalent (relative to HSG) of N-hydroxybenzotriazole, one equivalent of benzotrazole-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP) and two equivalents of diisopropylethylamine. The activated substrate was mixed with the resin for 15 hr at room temperature.

Example 2. Tc-99m Kit Formulation Comprising IMP 243

[0243] A formulation buffer was prepared which contained 22.093 g hydroxypropyl-β-cyclodextrin, 0.45 g 2,4-dihydroxybenzoic acid, 0.257 g acetic acid sodium salt, and 10.889 g α-D-glucoheptonic acid sodium salt dissolved in 170 mL nitrogen degassed water. The solution was adjusted to pH 5.3 with a few drops of 1 M NaOH then further diluted to a total volume of 220 mL. A stannous buffer solution was prepared by diluting 0.2 mL of $SnCl_2$ (200 mg/mL) with 3.8 mL of the formulation buffer. The peptide Ac-Lys(HSG)-D-Tyr-Lys(HSG)-Lys(Tscg-Cys-)-NH_2 (0.0026g), was dissolved in 78 mL of the buffer solution and mixed with 0.52 mL of the stannous buffer. The peptide solution was then filtered through a 0.22 m Millex GV filter in 1.5 mL aliquots into 3 mL lyophilization vials. The filled vials were frozen immediately, lyophilized and crimp sealed under vacuum.

[0244] Pertechnetate solution (27 mCi) in 1.5 mL of saline was added to the kit. The kit was incubated at room temperature for 10 min and heated in a boiling water bath for 25 min. The kit was cooled to room temperature before use.

Example 3. Peptides for Carrying Therapeutic/Imaging Radioisotopes to Tumors via Bispecific Antibody Tumor Pretargeting

[0245] DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 (IMP 237) was synthesized to deliver therapeutic radioisotopes such as ^{90}Y or ^{177}Lu to tumors via bispecific antibody tumor pretargeting. The bispecific antibody is composed of one portion which binds to an antigen on the tumor and another portion which binds to the HSG peptide. The antibody which binds the HSG peptide is 679. This system can also be used to deliver imaging isotopes such as ^{111}In-111.

Synthesis of IMP 237

[0246] IMP 237 was synthesized on Sieber Amide resin (Nova-Biochem) using standard Fmoc based solid phase peptide synthesis to assemble the peptide backbone with the following protected amino acids, in order: Fmoc-Lys (Aloc)-OH, Fmoc-Tyr(But)-OH, Fmoc-Lys(Aloc)-OH, Fmoc-Phe-OH, (Reagents from Advanced Chemtech) tri-t-butyl DOTA (Macrocyclics). The side lysine side chains were then deprotected with $Pd[P(Ph)_3]_4$ by the method of Dangles et.al. J. Org. Chem. 52:4984-4993 (1987). The HSG ligands were then added as Trityl HSG (synthesis described below) using the BOP/HBTU double coupling procedure used to attach the amino acids. The peptide was cleaved from the resin and the protecting groups were removed by treatment with TFA. The peptide was purified by HPLC to afford 0.6079 g of peptide from 1.823 g of Fmoc-Lys(Aloc)-Tyr(But)-Lys(Aloc)-NH-Sieber amide resin.

Synthesis of N-Trityl-HSG-OH

[0247] Glycine t-butyl ester hdyrochloride (15.263 g, 9.1 x1 0^{-2} mol) and 19.760 g Na_2CO_3 were mixed, then suspended in 50 mL H_2O and cooled in an ice bath. Succinic anhydride (9.142 g, 9.14 x 10^{-2} mol) was then added to the reaction solution which was allowed to warm slowly to room temperature and stir for 18 hr. Citric acid (39.911 g) was dissolved in 50 mL H_2O and slowly added to the reaction solution and then extracted with 2 x 150 mL EtOAc. The organic extracts

were dried over Na_2SO_4, filtered and concentrated to afford 25.709 g of a white solid.

[0248] The crude product (25.709 g) was dissolved in 125 mL dioxane, cooled in a room temperature water bath and mixed with 11.244 g of N-hydroxysuccinimide. Diisopropylcarbodiimide 15.0 mL was added to the reaction solution which was allowed to stir for one hour. Histamine dihydrochloride (18.402 g, 1.00 x 10^{-1} mol) was then dissolved in 100 mL DMF and 35 mL diisopropylethylamine. The histamine mixture was added to the reaction solution which was stirred at room temperature for 21 hr. The reaction was quenched with 100 mL water and filtered to remove a precipitate. The solvents were removed under hi-vacuum on the rotary evaporator. The crude product was dissolved in 300 mL dichloromethane and extracted with 100 mL saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated to afford 34.19 g of crude product as a yellow oil.

[0249] The crude product (34.19 g) was dissolved in 50 mL chloroform and mixed with 31 mL diisopropylethylamine. Triphenylmethyl chloride (25.415g) was dissolved in 50 ml chloroform and added dropwise to the stirred reaction solution which was cooled in an ice bath. The reaction was stirred for 45 min and then quenched with 100 mL H_2O. The layers were separated and the organic solution was dried over Na_2SO_4 and concentrated to form a green gum. The gum was triturated with 100 mL Et_2O to form a yellow precipitate which was washed with 3 x 50 mL portions of Et_2O. The solid was vacuum dried to afford 30.641 g (59.5 % overall yield) of N-trityl-HSG-t-butyl ester.

[0250] N-trityl-HSG-t-butyl ester (20.620 g, 3.64 x 10^{-2} mol) was dissolved in a solution of 30 mL chloroform and 35 mL glacial acetic acid. The reaction was cooled in an ice bath and 15 mL of BF_3•Et_2O was slowly added to the reaction solution. The reaction was allowed to warm slowly to room temperature and mix for 5 hr. The reaction was quenched by pouring into 200 mL 1M NaOH and the product was extracted with 200 mL chloroform. The organic layer was dried over Na_2SO_4 and concentrated to afford a crude gum which was triturated with 100 mL Et_2O to form a precipitate. The crude precipitate was poured into 400 mL 0.5 M pH 7.5 phosphate buffer and extracted with 2 x 200 mL EtOAc. The aqueous layer was acidified to pH 3.5 with 1 M HCl and extracted with 2 x 200 mL chloroform. A precipitate formed and was collected by filtration (8.58 g). The precipitate was the desired product by HPLC comparison to a previous sample (ESMS MH + 511).

Radiolabeling

^{90}Y Kit Preparation

[0251] DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 was dissolved in 0.25 M NH_4OAc/ 10 % HPCD buffer at concentrations of 9, 18, 35, 70 and 140 μg/mL. The solutions were sterile filtered through a 0.22 μm Millex GV filter in one mL aliquots into acid washed lyophilization vials. The filled vials were frozen immediately on filling and lyophilized. When the lyophilization cycle was complete the vials were sealed under vacuum and crimp sealed upon removal from the lyophilizer.

[0252] The ^{90}Y (~400 μCi/kit) was diluted to 1mL in deionized water and added to the lyophilized kits. The kits were heated in a boiling water bath for 15 min, the vials were cooled to room temperature and the labeled peptides were evaluated by reverse phase HPLC (HPLC conditions: Waters Nova-Pak C-18, 8x100 mm RCM column eluted at 3 mL/min with a linear gradient from 100 % (0.1 % TFA in H_2O) to 100 % (90 % CH_3CN, 0.1 % TFA, 10 % H_2O)). The HPLC analysis revealed that the minimum concentration of peptide needed for complete labeling, with this formulation, was 35 μg/mL. The reverse phase HPLC trace showed a sharp ^{90}Y labeled peptide peak. The labeled peptide was completely bound when mixed with excess 679 IgG by size exclusion HPLC.

Labeling with ^{111}In

[0253] The ^{111}In (~300 μCi/kit) was diluted to 0.5 mL in deionized water and added to the lyophilized kits. The kits were heated in a boiling water bath for 15 min, the vials were cooled and 0.5 mL of 2.56 x 10^{-5} M In in 0.5 M acetate buffer was added and the kits were again heated in the boiling water bath for 15 min. The labeled peptide vials were cooled to room temperature and evaluated by reverse phase HPLC (HPLC conditions: Waters Nova-Pak C-18, 8x100 mm RCM column eluted at 3 mL/min with a linear gradient from 100 % (0.1 % TFA in H_2O) to 100 % (90 % CH_3CN, 0.1 % TFA, 10 % H_2O)). The HPLC analysis revealed that the minimum concentration of peptide needed for labeling (4.7 % loose ^{111}In), with this formulation, was 35 μg/mL. The reverse phase HPLC trace showed a sharp ^{111}In labeled peptide peak. The labeled peptide was completely bound when mixed with excess 679 IgG by size exclusion HPLC.

In-Vivo Studies

[0254] Nude mice bearing GW-39 human colonic xenograft tumors (100-500 mg) were injected with the bispecific antibody hMN-14 x m679 (1.5 x 10^{-10} mol). The antibody was allowed to clear for 24 hr before the ^{111}In labeled peptide (8.8 μCi, 1.5 x 10^{-11} mol) was injected. The animals were sacrificed at 3, 24, 48 hr post injection.

[0255] The results of the biodistribution studies of the peptide in the mice pretargeted with hMN-14 x m679 are shown in Table 1. The tumor to non-tumor ratios of the peptides in the pretargeting study are show in Table 2.

Table 1

Pretargeting With ^{111}In Labeled Peptide 24 hr After Injection of hMN-14 x m679 % Injected/g Tissue			
Tissue	3 hr After ^{111}In IMP 237	24 hr After ^{111}In IMP 237	48 hr After ^{111}In IMP 237
GW-39	7.25 ± 2.79	8.38 ± 1.70	5.39 ± 1.46
Liver	0.58 ± 0.13	0.62 ± 0.09	0.61 ± 0.16
Spleen	0.50 ± 0.14	0.71 ± 0.16	0.57 ± 0.15
Kidney	3.59 ± 0.75	2.24 ± 0.40	1.27 ± 0.33
Lungs	1.19 ± 0.26	0.44 ± 0.10	0.22 ± 0.06
Blood	2.42 ± 0.61	0.73 ±0.17	0.17 ± 0.06
Stomach	0.18 ± 0.03	0.09 ± 0.02	0.07 ± 0.02
Sm. Int.	0.65 ± 0.74	0.18 ± 0.03	0.11 ± 0.02
Lg. Int.	0.30 ± 0.07	0.17 ± 0.03	0.13 ± 0.03

Table 2

Pretargeting With ^{111}In Labeled Peptides 24 hr After Injection of hMN-14 x m679 Tumor/Non-Tumor Tissue Ratios			
Tissue	3 hr After ^{111}In IMP 237	24 hr After ^{111}In IMP 237	48 hr After ^{111}In IMP 237
Liver	12.6 ± 4.44	13.6 ± 2.83	8.88 ± 1.78
Spleen	15.1 ± 6.32	12.1 ± 2.86	9.50 ± 1.62
Kidney	2.04 ± 0.74	3.84 ± 1.04	4.25 ± 0.19
Lungs	6.11 ± 1.96	19.6 ± 5.91	25.4 ± 6.00
Blood	3.04 ± 1.13	11.9 ± 3.20	31.9 ± 4.79
Stomach	40.5 ± 16.5	104. ± 39.6	83.3 ± 16.5
Sm. Int.	18.9 ± 12.6	47.5 ± 10.3	49.5 ± 7.83
Lg. Int.	25.2 ± 10.6	50.1 ± 16.7	43.7 ± 9.35

Serum Stability of DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH2 (IMP 237) and DOTA-Phe-Lys(HSG)-D-Tyr-Lys (HSG)-NH2 (IMP 241)

Peptide Labeling and HPLC Analysis

[0256] The peptides, IMP 237 and IMP 241, were labeled according to the procedure described by Karacay et. al. Bioconjugate Chem. 11:842-854 (2000). The peptide, IMP 241 (0.0019 g), was dissolved in 587 μl 0.5 M NH_4Cl, pH 5.5. A 1.7 μL aliquot of the peptide solution was diluted with 165 μl 0.5 M NH_4Cl, pH 5.5. The ^{111}In (1.8 mCi) in 10 μL was added to the peptide solution and the mixture was heated in a boiling water bath for 30 min.

[0257] The labeled peptide was analyzed by HPLC using a Waters 8x100 mm radial-pak, nova-pak C-18 RCM cartridge column. The column was eluted at 3 mL/min with a linear gradient which started with 100 % of 0.1 % TFA in water and went to 100 % of 0.1 %TFA in 90% acetonitrile and 10 % water over 10 min. There was about 6% loose ^{111}In in this labeling which came out at the void volume of the column (1.6 min). There were also some ^{111}In labeled peaks at 5 min and 6.6 to 8 min. The ^{111}In labeled peptide was eluted at 8.8 min as a single peak. The HPLC profile of ^{111}In IMP 237 was nearly identical to ^{111}In IMP 241.

Serum Stability

[0258] An aliquot (30 μL) of ^{111}In IMP 241 was placed in 300 μL of fresh mouse serum and placed in a 37° C incubator. The peptide was monitored as described above by HPLC. An aliquot (24 μL) of ^{111}In IMP 237 was placed in 230 μL of fresh mouse serum and placed in a 37° C incubator. The peptide was monitored as described above by HPLC. The analysis showed that the ^{111}In IMP 241 may have decomposed slightly (~5%) after heating 22 hr in mouse serum at 37° C. The ^{111}In IMP 237 was about 70 % converted to the shorter retention time peak after incubation for 22 hr at 37° C.

Conclusion

[0259] The D-tyrosine in the IMP 241 peptide slows the decomposition of the peptide in mouse serum compared to IMP 237.

III Vivo Stability of IMP 237 and IMP 241 Compared

[0260] The *in vivo* stabilities of ^{111}In IMP 237 and ^{111}In IMP 241 were compared by examining (by HPLC) urine samples from mice at 30 and 60 min. The peptides, IMP 241 and IMP 237, were ^{111}In-111 labeled as described above.

[0261] The labeled peptides were injected into Balb/c mice which were sacrificed at 30 min and 60 min post injection of the peptides using one mouse per time point. The attached HPLC traces indicate that ^{111}In IMP 241 was excreted intact while ^{111}In IMP 237 was almost completely metabolized to a new ^{111}In labeled peptide.

Conclusion

[0262] The replacement of Tyr with D-Tyr in the peptide backbone minimized metabolism of the peptide in-vivo.

Additional In Vivo Studies

[0263] Nude mice bearing GW-39 human colonic xenograft tumors (100-500 mg) were injected with the bispecific antibody mMu9 x m679 (1.5×10^{-10} mol). The antibody was allowed to clear for 48 hr before the ^{111}In labeled peptides (8.8 μCi, 1.5×10^{-11} mol) were injected. The animals were sacrificed at 3, 24, 48 hr post injection.

[0264] The results of the biodistribution studies of the peptides in the mice pretargeted with mMU9 x m679 are shown in Table 3. The tumor to non-tumor ratios of the peptides in the pretargeting study are show in Table 4. The data in Table 5 shows the biodistribution of the peptides in mice that were not pretreated with the bispecific antibody.

Table 3

Pretargeting With ^{111}In Labeled Peptides 48 hr After Injection of mMU9 x m679 % Injected/g Tissue						
Tissue	3 hr After ^{111}In Peptide		24 hr After ^{111}In Peptide		48 hr After ^{111}In Peptide	
	IMP 237	IMP 241	IMP 237	IMP 241	IMP 237	IMP 241
GW-39	18.3 ± 7.17	26.7 ± 14.1	16.7 ± 8.22	14.8 ± 4.56	12.9 ± 1.10	12.3 ± 2.11
Liver	0.41 ± 0.10	0.66 ± 0.34	0.32 ± 0.08	0.32 ± 0.09	0.28 ± 0.09	0.32 ± 0.21
Spleen	0.34 ± 0.12	0.63 ± 0.38	0.34 ± 0.12	0.25 ± 0.07	0.28 ± 0.07	0.31 ± 0.22
Kidney	3.62 ± 0.71	4.28 ± 0.77	2.51 ± 0.54	2.34 ± 0.70	1.78 ± 0.38	1.17 ± 0.43
Lungs	0.61 ± 0.15	1.03 ± 0.65	0.22 ± 0.07	0.21 ± 0.07	0.12 ± 0.04	0.14 ± 0.08
Blood	1.16 ± 0.48	1.78 ± 1.49	0.21 ± 0.13	0.15 ± 0.05	0.08 ± 0.03	0.10 ± 0.09
Stomach	0.12 ± 0.04	0.21 ± 0.09	0.05 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.03 ± 0.02
Sm. Int.	0.23 ± 0.04	0.50 ± 0.27	0.12 ± 0.02	0.09 ± 0.06	0.11 ± 0.08	0.07 ± 0.06
Lg. Int.	0.34 ± 0.16	0.38 ± 0.15	0.15± 0.07	0.10 ± 0.02	0.12 ± 0.07	0.09 ± 0.05

Table 4

Pretargeting With ^{111}In Labeled Peptides 48 hr After Injection of mMU9 x m679 Tumor/Non-Tumor Tissue Ratios						
Tissue	3 hr After ^{111}In Peptide		24 hr After ^{111}In Peptide		48 hr After ^{111}In Peptide	
	IMP 237	IMP 241	IMP 237	IMP 241	IMP 237	IMP 241
Liver	45.6 ± 17.8	41.8 ± 19.6	49.8 ± 16.6	47.1 ± 8.68	49.1 ± 13.6	45.1 ± 13.9
Spleen	56.8 ± 23.8	43.5 ± 9.77	47.4 ± 14.7	59.6 ± 13.0	47.5 ± 10.6	50.2 ± 19.0
Kidney	5.13 ± 2.18	6.05 ± 2.41	6.43 ± 2.24	6.58 ± 2.42	7.43 ± 1.02	11.2 ± 2.61
Lungs	30.5 ± 10.6	28.4 ± 12.8	76.4 ± 34.1	72.7 ± 21.9	115. ± 36.6	102. ± 37.1
Blood	18.6 ± 12.0	19.0 ± 11.8	86.9 ± 36.2	108. ± 41.0	187. ± 76.3	181. ± 86.6
Stomach	156. ± 86.1	126. ± 49.6	303. ± 95.9	328. ± 96.7	344. ± 101.	456. ± 193.
Tissue	3 hr After ^{111}In Peptide		24 hr After ^{111}In Peptide		48 hr After ^{111}In Peptide	
Sm. Int.	80.7 ± 29.0	59.0 ± 31.0	143. ± 60.7	193. ± 83.7	153. ± 67.7	217. ± 73.5
Lg. Int.	56.3 ± 19.7	78.6 ± 54.4	116. ± 36.9	155. ± 42.4	133. ± 47.6	153. ± 43.1

Table 5

Biodistribution of ^{111}In Labeled Peptides Alone						
Tissue	30 min After In-111 Peptide		3 hr After In-111 Peptide		24 hr After In-111 Peptide	
	IMP 237	IMP 241	IMP 237	IMP 241	IMP 237	IMP 241
GW-39	2.99 ± 1.11	2.73 ± 0.37	0.17 ± 0.05	0.31 ± 0.12	0.11 ± 0.02	0.11 ± 0.08
Liver	0.48 ± 0.06	0.50 ± 0.09	0.15 ± 0.02	1.07 ± 1.61	0.15 ± 0.01	0.09 ± 0.04
Spleen	0.42 ± 0.08	0.43 ± 0.22	0.09 ± 0.04	0.13 ± 0.05	0.13 ± 0.02	0.08 ± 0.03
Kidney	5.85 ± 0.37	7.31 ± 0.53	3.55 ± 0.44	3.21 ± 0.45	2.18 ± 0.24	2.61 ± 0.51
Lungs	1.26 ± 0.24	1.12 ± 0.26	0.13 ± 0.02	0.15 ± 0.06	0.06 ± 0.00	0.07 ± 0.06
Blood	1.62 ± 0.34	1.59 ± 0.29	0.12 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.00 ± 0.00
Stomach	0.59 ± 0.32	0.52 ± 0.16	0.04 ± 0.01	0.07 ± 0.03	0.03 ± 0.01	0.04 ± 0.04
Sm. Int.	0.55 ± 0.13	2.52 ± 3.73	0.09 ± 0.01	0.17 ± 0.08	0.08 ± 0.01	0.04 ± 0.01
Lg. Int.	0.33 ± 0.05	0.30 ± 0.07	0.33 ± 0.15	0.32 ± 0.14	0.05 ± 0.01	0.07 ± 0.03

Example 4. PCR Cloning of the Mu-9 Variable Regions

[0265] Poly A mRNA was isolated from Mu-9 hybridoma cell line (3 x 10^7 cells) using the Fast Track mRNA Isolation kit (Invitrogen, San Diego, CA). The first strand cDNA was reverse transcribed from poly A mRNA using the cDNA cycle kit (Invitrogen). Briefly, 1 g of poly A mRNA was annealed to murine IgG CH1-specific primer, CH1B (5' ACA GTC ACT GAG CTG G 3'), or murine Ck-specific primer, Ck3-BH1 (5' GCC GGA TCC TCA CTG GAT GGT GGG AAG ATG GAT ACA 3'), at a final concentration of 1 M at 42 for 60 minutes in the presence of 1 1 of RNAse inhibitor (10 U/ 1), 4.0 1 of 5x reverse transcriptase buffer (500 mM Tris-HCL, pH 8.2, 200 mM KCl, 50 mM $MgCl_2$, and 2.5 mM spermidine), 1 l of 100 mM dNTPs; 1 1 of 80 mM sodium pyrophosphate, and 5 U of AMV reverse transcriptase. The RNA-cDNA hybrids were then denatured at 95 C for 2 minutes. The first strand cDNAs were then used as templates to amplify the VH and Vκ sequences by PCR as described by Orlandi et al. , Proc. Natl. Acad. Sci. USA 1989, 86: 3833. The Vκ region was amplified using primers VK1BACK (5'-GAC ATT CAG CTG ACC CAG TCT CCA 3') and IgKC3' (5'-CTC ACT GGA TGG TGG GAA GAT GGA TAC AGT TGG 3'). The VH region was amplified using primers VH1BACK (5' AGG T(C/G)(A/C) A(A/G)C TGC AG(C/G) AGT C(A/T)G G 3') and CH1B. In a preferred embodiment, the VH region is amplified using the

primer indicated by the arrow underline in figure 8. The PCR reaction mixtures containing 10 1 of the first strand cDNA product, 10 1 of 10x PCR buffer (15 mM $MgCl_2$, 500 mM KCl, 100 mM Tris-HCl, pH 8.3, and 0.01 % (w/v) gelatin), 1 M of each primer, 16 1 of dNTPs, and 5 U of AmpliTaq DNA polymerase (Perkin-Elmer, Applied Biosystems Division, Foster City, CA) were subjected to 30 cycles of PCR (denaturation at 94 C for 1 minute, annealing at 45 C for 1 minute and polymerization at 72 C for 1 minute for 5 cycles, combined with denaturation at 94 C for 1 minute, annealing at 55 C for 1 minute, and polymerization at 72 C for 1 minute for 25 cycles). The amplified Vk and VH fragments were gel-purified and cloned into the TA cloning vector (Invitrogen) for sequence analyses by the dideoxytermination method. Sequences confirmed to be of immunoglobulin origin were then used to construct chimeric expression vectors using methods described by Leung et al., Hybridoma 1994, 13: 469.

[0266] Nucleotide sequencing of multiple clones confirmed the isolation of one Vκ (MU9Vκ1) and one V_H (Mu9 V_H) sequence (Figure 8). The chimeric Mu-9 (cMu-9-1) constructed from the V_H and Vκ1 cloned by this RT-PCR method did not demonstrate any binding to CSAp antigen, suggesting the possible existence of other "functional" V-region sequence(s) that might be overlooked by RT-PCR cloning procedures.

Example 5. Cloning the Mu-9 Variable Regions by cDNA Library Screening

[0267] The cDNA library was constructed from the murine Mu-9 hybridoma in pSPORT vector (Life Technologies). The first strand cDNA was synthesized by pairing poly A mRNA from murine Mu-9 hybridoma with an oligo dT primer-NotI adaptor (Life Technologies). After the second strand synthesis and attachment of SalI adaptors, the cDNA pool was size fractionated through a cDNA size fractionation column. The fractionated cDNA was ligated to pSPORT vector and then transformed into *Escherichia coli* DH5. The library was plated onto LB-amp (100 g/ml) plates, colonies transferred to Nytran filters (Schleicher and Schuell, Keene, NH), and then amplified on LB-chloramphenicol plates. The amplified colonies were treated successively with 0.5 N NaOH/1.5 M NaCl for 5 minutes; 1 M Tris-HCl, pH 8.0, for 5 minutes; 0.1 M Tris-HCl, pH 7.5/2x SSC for 5 minutes; and finally with 2x SSC for 5-10 minutes. The DNA was immobilized on the filters by baking at 80 C for 30 minutes. The filters were incubated in prehybridization buffer containing 6x SSC, 5X Denhardt's (0.1 % Ficoll, 0.1 % polyvinylpyrrolidone, and 0.1 % bovine serum albumin), 0.5% SDS, 0.05% sodium pyrophosphate, and 100 g/ml herring sperm DNA (Life Technologies) for 2 hours at 50 C. Hybridization with the ^{32}P-labeled probes (MUCH-1 (5'-AGA CTG CAG GAG AGC TGG GAA GGT GTG CAC 3') specific for murine heavy chain and MUCk-1 (5'-GAA GCA CAC GAC TGA GGC ACC TCC AGA TGT 3') specific for murine light chain) at 10^6 cpm/ml was done overnight at their respective Tms in the prehybridization solution supplemented with 10% (w/v) dextran sulfate (Pharmacia Biotech, Piscataway, NJ). The filters were washed four times in 0.2x SSC, 0.1 % SDS at 37 C for 10 minutes, twice at 42 C for 15 minutes, and once at 50 C for 15 minutes until the radioactivities on the filters were constant as determined with a Geiger counter. After a final rinse in 2x SSC, the wet filters were exposed to Kodak XAR-5 film (Rochester, NY) at 70 C. The clones that were positive on the first screening were transferred to duplicate LB-amp plates. Duplicate Nytran filters were hybridized to the same probes as described above. Only clones that positively hybridized on both the filters were picked for further screening. For the tertiary screening, MUCH-1- positive colonies were screened in duplicate with VHCDR3Mu9 (specific for the V_H sequenced cloned by RT-PCR in Example 4) and MUCκ-1-positive colonies were secreened with VKCDR1Mu9 and VKCDR3Mu9 (specific for the CDR1 and CDR3 coding sequences of Vκ-1 cloned by RT-PCR in Example 4).

[0268] All clones (25) that were confirmed to be positive with MUCH-1 were also positive with VHCDR3Mu9, indicating that there was only one type of heavy chain sequence expressed in the hybridoma. Ten clones that positively hybridized with the VHCDR3Mu9 were sequenced and found to be identical to the RT-PCR cloned Mu9VH, the sequence of which is disclosed in Figure 5.

[0269] Of the 34 clones that were positive for MUCk-1 in both primary and secondary screenings only 14 hybridized to the Mu9Vk1-specific probes, VKCDR1Mu9 and VKCDR3Mu9. Sequence analyses revealed that these clones were identical to the Mu9Vk1. Of the remaining 20 clones that were negative for the Mu9Vk1-specific probes, 8 were subjected to DNA sequencing. Seven of these clones encoded a kappa light chain sequence with a Vk domain that was different from Mu-9 Vκ1 and designated as Mu-9 Vκ2, the sequence of which is disclosed in Figure 4. The chimeric Mu-9 (cMu-9-2) constructed from the VH and Vκ2 and expressed in Sp2/0 cells showed comparable binding affinity to CSAp antigen as that of murine Mu-9 (see Example 8-9 for details).

Example 6. Probe Labeling for cDNA Library Screening

[0270] Oligonucleotides were synthesized on an automated 392 DNA/RNA synthesized (Applied Biosystems) and then purified on a PD 10 column (Pharmacia Biotech). The purified oligonucleotides were labeled with [-^{32}P]ATP (Amersham, Arlington Heights, IL) using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). A typical reaction mixture contained in a final volume of 20 1 was as follows: 50 pmol oligonucleotide, 60 Ci of [-^{32}P]ATP (6000 Ci/mmol), and 2 1 of 10x kinase buffer (New England Biolabs). The reaction mixture was incubated at 37 C for 1 hour, and the

reaction was terminated with 20 1 of 0.1 M EDTA. The unincorporated [- ^{32}P]ATP was separated from the labeled oligonucleotide on a TE-10 Chromaspin column (Clonetech, Palo Alto, CA). The labeled probe was used at 10^6 cpm/m. for hybridization.

Example 7. Transfection of SP2/0 Cells

[0271] The putative V_K and VH sequences for Mu-9 were subcloned into the light (pKh or pKh*) and heavy chain (pG1g) expression vectors, respectively, as describe by Leung et al., supra. pKh* is essentially identical to the pKh, except that is has a XhoI/PacI linker introduced into the BstXI site of the pKh. Mu-9 V_K2 obtained by cDNA screening contained an internal BstXI site, it was subcloned into pKh*, which could then be linearized with either XhoI or PacI for transfection.

[0272] Approximately 10 and 30 g of linearized light (Mu-9-1pKh or Mu-9-2pKh*) and heavy (Mu-9pG1g) chain expression vectors were cotransfected into SP2/0 cells by electroporation. Transfected cells were grown in 96-well cell culture plates in complete medium for 2 days and then selected by the addition of hygromycin at a final concentration of 500 U/ml. Typically, the colonies began to emerge 2-3 weeks after electroporation and were assayed for antibody secretion by enzyme-linked immunosorbent assay (ELISA). The chimeric antibodies were purified from the culture supernatant by affinity chromatography on Protein A-Sepharose 4B column. The purified antibodies (5-μg) were analyzed by SDS-PAGE on a 4-20% gradient gel under reducing conditions.

Example 8. Mu-9 Direct Binding Assay

[0273] ELISA microtiter plates were coated with a void volume fraction of GW-39 tumor extracts (which contains the CSAp antigen) eluted from a Sepharose 4B-CL column and left overnight at 4 °C. The next day, the nonspecific binding was blocked with phosphate-buffered saline (PBS) containing 1 % BSA and 0.05 % Tween 20. Chimeric antibody supernatant (100 μl) or purified antibody (0-1 μg/ml) was added and incubated at room temperature for 1 hour. Unbound proteins were removed by washing six times with wash buffer (PBS containing 0.05 % Tween 20). Purified murine Mu-9 protein was used as the standard. The bound antibodies were allowed to react with peroxidase-conjugated goat anti-human IgG, Fc fragment-specific (Jackson ImmunoResearch, West Grove PA) and peroxidase conjugated goat anti-mouse IgG, Fc fragment-specific antibodies (Jackson ImmunoResearch). After washing the plate six times with wash buffer, 100 μl of OPD substrate solution (10 mg of orthophenylenediamine dihydrochloride (Sigma, St. Louis, MO) in 25 ml of 0.32x PBS and 0.12% H_2O_2) was added to each well. The color was developed in the dark for 1 hour, the reaction was stopped with 50 μl of 4N H_2SO_4, and the absorbance at 490 nm was measured in a Dynatech plate reader (Dynatech Labs, Sussex, UK).

[0274] Direct binding of a cMu-9 (cMu-9-2) to CSAp antigen occurred, as shown in Figure 12. The binding profile of cMu-9-2 was virtually superimposable on that of the murine Mu-9. These data demonstrated that the immunoreactivity of cMu-9-2 is comparable to that of murine Mu-9. The DNA and amino acid sequences of the functional cMu-9 V_K and VH are shown in Figure 2A and 2B, respectively.

Example 9. Competitive Binding Assay

[0275] Murine Mu-9 IgG was conjugated with horseradish peroxidase (HRP) (Sigma). The peroxidase-conjugated Mu-9 was first tested for binding on microwells coated with CSAp antigen, and the optimum concentration was determined to be 0.2 μg/ml. Peroxidase-conjugated Mu-9 was mixed with various concentrations of either murine or chimeric Mu-9 (0-50 μg/ml) before addition to the antigen-coated wells. Binding of the peroxidase-conjugated Mu-9 to the antigen in the presence of the competing antibodies was measured at 490 nm after the addition of the substrate as described earlier.

[0276] Figure 5(b) displays the results of the competitive binding assay. Murine Mu-9 and cMu-9-2 competed equally well with the binding of HRP-conjugated Mu-9 to the CSAp antigen. These data demonstrated that the immunoreactivity of cMu-9-2 is comparable to that of murine Mu-9.

Example 10. Choice of human frameworks and sequence design for the humanization of Mu9 monoclonal antibody.

[0277] By comparing the variable (V) region framework (FR) sequences of Mu9 to that of human antibodies in the Kabat data base, the FRs of Mu-9 VH and V_K were found to exhibit the highest degree of sequence homology to that of the human antibodies, EU VH and WOL V , respectively. In Figures 10A and 10B, the amino acid sequences of the Mu9VH and V_K are aligned and compared with the corresponding human sequences. Therefore, the FRs of EU VH and WOL V were selected as the human frameworks onto which the CDRs for Mu-9VH and V were grafted, respectively. The FR4 sequence of NEWM, however, rather than that of EU, was used for the humanization of Mu9 heavy chain

(Figure 10A). A few amino acid residues in Mu9 FRs that are close to the putative CDRs were maintained in hMu9 based on the guideline described previously (Qu et al., Clin. Cancer Rec. 5:3095s-3100s (1999)). These residues are.L37, V58 and Q100 of V (Figure 10B) and Y27, T30, K38, R40, I48, K66, A67, K74, T93, R94 and G103 of VH (Figure 10A). The gene sequences of hMu-9VH and V were then designed and shown with the amino acid sequences in Figure 4A and 4B, respectively.

Example 11. PCR/gene synthesis of the humanized V genes.

[0278] The strategy as described by Leung et al. (Leung et al., 1994)) was used to construct the designed hMu-9 V_K and VH genes using a combination of long oligonucleotide syntheses and PCR. Each variable chain was constructed in two parts, a 5'- and 3'-half, designated as "A" and "B," respectively. Each half was produced by PCR amplification of a single strand synthetic oligonucleotide template with two short flanking primers, using Taq polymerase. The amplified fragments were first cloned into the pCR4 TA cloning vector from Invitrogen (Carlsbad, CA) and subjected to DNA sequencing. The templates and primer pairs are listed as follows:

Template	Primers	PCR product
Oligo G	Oligo14/Oligo 15	hMu9VHA
Oligo H	Oligo 16/Oligo 17	hMu9VHB
Oligo J	Oligo 18/Oligo 19	hMu9VkA
Oligo K	Oligo 20/Oligo 21	hMu9VkB

Heavy chain

[0279] For constructing the full-length DNA of the hMu9VH sequence, Oligo G (102 mer) and H (179 mer) were synthesized on an automated RNA/DNA synthesizer (Applied Biosystems). Oligo G sequence represents the minus strand of hMu9VH domain complementary to nt 19 to 120:

5'- AGGTCTCTGT TTTACCCAGG TAATAACATA CTCAGTGAAG GTGTATCCAG AAGCCTTGCA GGAGACCTTC ACTGAGCTCC CAGGCTTTTT CACCTCAGCT CC -3'

[0280] Oligo H sequence represents the nt 147 to 325 of hMu9VH domain:

5'- GATTTATCCT GGAAGTGGTA GTACTTCCTA CAATGAAAAG TTCAAGGGCA AGGCCACAAT CACTGCTGAC AAATCCACTA ACACAGCCTA CATGGAGCTC AGCAGCCTGA GATCTGAGGA CACTGCGTTC TATTTCTGTA CAAGAGAGGA TCTTGGGGGC CAAGGGTCTC TGGTCACCG-3'

[0281] Oligo G and H were cleaved from the support and deprotected by treatment with concentrated ammonium hydroxide. After samples were vacuum-tried and resuspended in 100 1 of water, incomplete oligomers (less than 100-mer) were removed by centrifugation through a ChormaSpin-100 column (Clontech, Palo Alto, CA). All flanking primers were prepared similarly, except ChromaSpin-30 columns were used to remove synthesis by-products. 1 1 of ChromaSpin column purified Oligo G was PCR amplified in a reaction volume of 100 1 containing 10 1 of 10X PCR buffer [500 mM KCl, 100 mM Tris-HCl (pH 8.3), 15 mM $MgCl_2$, and 0.01 % (w/v) gelatin] (Perkin Elmer Cetus, Norwalk, CT), 250 M of each dNTP, 200 nM of Oligo14 (5'-GTGCAGCTGC AGCAGTCAGG AGCTGAGGTG-3') and Oligo 15 (5'-ACTCTAGACC CTGTCCAGGT CTCTGTTTTA CCCAGGTAAT AACATA-3'), and 5 units of Taq DNA polymerase (Perkin Elmer Cetus). This reaction mixture was subjected to 30 cycles of PCR reaction consisting of denaturation at 94°C for 1 min, annealing at 50 °C for 1.5 min, and polymerization at 72°C for 1.5 min. Oligo H was PCR-amplified by the primer pair Oligo 16 (5'-GGTCTAGAGT GGATTGGAGA GATTTATCCT GGAAGTGGTA GTACTT-3') and Oligo 17 (5'-TGAAGAGACG GT-GACCAGAG ACCCTTGGCC CCCAAGATCC TCTCTTGTAC AGAAATAGAA CGC-3') under similar condition. Resulting PCR fragments, VHA and VHB were purified on 2% agarose (BioRad, Richmond, CA). Unique restriction sites were

designed at the ends of each fragment to facilitate joining through DNA ligation. The amplified VHA fragment contained a PstI restriction site, CTGCAG, at its 5'-end and a XbaI restriction site, TCTAGA, at the 3'-end. The amplified VHB fragment contained a XbaI restriction site at its 5'-end and a BstEII restriction site, GGTCACC, at the 3'-end. Assembly of the full-length VH chain was accomplished by restriction enzyme digestion of each fragment with the appropriate 5'- and 3'-enzymes and ligation into the VHpBS vector (Leung et al., Hybridoma, 13:469 (1994)) previously digested with PstI and BstEII. The resulting ligated product contains the A fragment ligated to the PstI site, the B fragment to the BstEII site, and the A and B fragments joined together at the XbaI site (Figure 4A). Upon confirmation of a correct open reading frame by DNA sequencing, the intact VH gene sequence along with the promoter and the secretion signal peptide coding sequence was removed from VHpBS as a HindIII to BamHI fragment and ligated into the VHpG1g expression vector (Leung et al., Hybridoma, 13:469 (1994)), resulting in hMu9VHpG1g.

Light chain

[0282] For the construction of V , the long oligonucleotide templates synthesized were Oligo J (130 mer) representing the minus strand of hMu9V domain complementary to nt 21 to 150:

5'-CCTTGGAGCC TGGCCTGGTT TCTGCAGGTA CCATTCTAAA TAGGTGTTGC CATTACTATG CACAATGCTC TGACTAGACC TGCAAGACAG AGTGGCTCGC TCTCCAGGAC TGAGGGACAG GGTGCCTGGG-3'

and Oligo K (150 mer) representing the nt 151 to 300 of hMu9V domain:

5'-CTCCTGATCT ACAAAGTTTC CAACCGATTT TCCGGAGTCC CAGACAGGTT CAGTGGCTCT GGATCAGGGA CAGATTTCAC ACTTACTATC AGCAGACTGG AGCCTGAGGA TTTTGCTGTG TATTACTGCT TTCAAGGTTC ACGTGTTCCG-3'

[0283] These oligos were PCR-amplified by their respective primer pairs as listed:

Oligo 18 5'-GATATCCAGC TGACCCAATC CCCAGGCACC CTGTCCCTCA GTCCTGGAG-3'

Oligo 19 5'-AGATCAGGAG CCTTGGAGCC TGGCCTGGTT TCTGCA-3'

Oligo 20 5'-TACCTGCAGA AACCAGGCCA GGCTCCAAGG CTCCTGATCT ACAAAGTTTC CAACCG-3'

Oligo 21 5'-TTAATCTCCA CCTTGGTCCC CCCTCCGAAC GTGTACGGAA CACGTGAACC TTGAAAGCAG TAATACA-3'

[0284] The same construction method as done for VH was carried out for V , with the following modifications: the 5'-end restriction site of the A fragments was PvuII (CAGCTG) and the 3'-end restriction site of B fragments was BglII (AGATCT). These fragments were joined together upon ligation into the VKpBR vector at a common PstI site (CTGCAG), resulting in full-length V sequence (Figure 4B) and confirmed by DNA sequencing. The assembled V gene was subcloned

as HindIII-BamHI restriction fragment into the light expression vector, resulting in hMu9VKpKh.

Example 12. Transfection, expression and binding activity assays for hMu9.

[0285] The methods for expression and binding activity assays for hMu9 were same as described for cMu9. Approximately 10 and 30 g of linearized hMu9VkpKh and hMu9VHpG1g were co-transfected into SP2/0 cells by electroporation. Transfected cells were grown in 96-well cell culture plates in complete medium for 2 days and then selected by the addition of hygromycin at a final concentration of 500 U/ml. Typically, the colonies began to emerge 2-3 weeks after electroporation and were assayed for antibody secretion by enzyme-linked immunosorbent assay (ELISA). The chimeric antibodies were purified from the culture supernatant by affinity chromatography on Protein A-Sepharose 4B column. The purified antibodies (5 μg) were analyzed by SDS-PAGE on a 4-20% gradient gel under reducing conditions.

[0286] Direct binding assay showed that the purified hMu-9 bound to CSAp antigen. The binding affinity of hMu-9 was compared in a competitive binding assay as described in Example 6. Figure 13 displays the results of the competitive binding assay. hMu-9 or murine Mu-9 competed equally well with the binding of HRP-conjugated Mu-9 to the CSAp antigen. These data demonstrated that the immunoreactivity of hMu-9 is comparable to that of murine Mu-9.

Example 13. Therapy of a Patient with ^{90}Y-labeled Humanized Mu-9 Antibody

[0287] A 62-year-old man, with a history of Dukes' C rectal carcinoma that was resected 3 years earlier, at which time radiation therapy followed by 5-fluorouracil/folinic acid chemotherapy were given, began showing a rise in his plasma CEA titer over the last 6 months, reaching a level of 30 ng/mL. The patient, who was seeing his oncologist twice annually, learned of this result and underwent various diagnostic procedures because of a suspected recurrence. It was found, by computed tomorgraphy, that there were two metastases present in his liver, one being 3 cm in diameter in his right lobe, and the other being somewhat smaller in the left lobe, close to the interlobe ligament. The patient opted not to undergo chemotherapy, and was then given a dose of 25 mCi ^{90}Y conjugated to the humanized Mu-9 antibody, given at a protein dose of 50 mg by intravenous infusion over a period of 2 hours. This therapy was then repeated one month later. The patients had a drop of his white blood cells and platelets, measured 2-4 weeks after the last therapy infusion, but recuperated at the 8-week post-therapy evaluation. The computed tomography findings at 3 months post-therapy revealed a 40% shrinkage of the major tumor metastasis of the right liver lobe, and a lesser reduction in the left-lobe tumor. At this time, the patient's blood CEA dropped to 15 ng/mL. At the 6-month follow-up, his tumor lesions had been reduced, in two-diameter CT-measurements, by about 70 percent, his plasma CEA was at 8 ng/mL, and his general condition was fine, with no apparent toxicity or adverse events related to the therapy. The patient is now 9 months post-therapy with no change in the size of his liver metastases and a stable serum CEA titer at about 5-8 ng/mL. He is being followed every 3 months, so that if the disease begins to grow, he is scheduled to receive another course of this radioimmunotherapy, followed by a course of naked Mu-9 antibody, at a weekly dose of 300 mg/m^2, once weekly for 6 weeks, concomitantly with a therapy course of irinotecan (CPT-11).

X. References

[0288] Additional references of interest, as well as references cited therein, include the following,

Bamias, A., and Epenetos,A.A. Two-step strategies for the diagnosis and treatment of cancer with bioconjugates. Antibody, Immunoconjugates, Radiopharm. 1992; 5: 385-395.

Barbet, J., Peltier, P., Bardet, S., Vuillez, JP., Bachelot, I., Denet, S., Olivier, P., Lecia, F., Corcuff, B., Huglo, D., Proye,C., Rouvier, E., Meyer,P., Chatal,J.F. Radioimmunodetection of medullary thyroid carcinoma using indium-111 bivalent hapten and anti-CEA x anti-DTPA-indium bispecifc antibody. J.Nucl.Med. 1998; 39:1172-1178.

Bos, ES., Kuijpers, WHA., Meesters-Winters, M., Pham, DT., deHaan, AS., van Doormalen,Am., Kasperson,F.M., vanBoeckel, CAA and Gouegeon-Bertrand, F. In vitro evaluation of DNA-DNA hybridization as a two-step approach in radioimmunotherapy of cancer. Cancer Res. 1994; 54:3479-3486.

Carr et al., WO00/34317.

Di Carlo, A., Mariano, A., D'Alessandro, V., Belli, G., Romano, G., Macchia, V. Evaluation of epidermal growth factor receptor, carcinoembryonic antigen and Lewis carbohydrate antigens in human colorectal and liver neoplasias. Oncol. Rep. 2001; 8:387-392.

Epstein et al. U.S. Patents No. 5,019,368; 5,882,626;6,017,514; and the patents and references cited therein.

Gautherot, E., Bouhou, J., LeDoussal, J-M., Manetti, C., Martin, M., Rouvier, E., Barbet, J. Therapy for colon carcinoma xenografts with bi-specific antibody-targeted, iodine-131-labeled bivalent hapten. Cancer suppl. 1997; 80: 2618-2623.

Gautherot, E., Bouhou, J., Loucif, E., Manetti, C., Martin, M., LeDoussal, J.M., Rouvier, E., Barbet, J. Radioimmunotherapy of LS174T colon carcinoma in nude mice using an iodine-131-labeled bivalent hapten combined with an anti-CEA x anti-indium-DTPA bi-specific antibody. J.Nucl.Med. Suppl. 1997; 38: 7p.

Goodwin, D.A., Meares, CF., McCall, MJ., McTigue,M., Chaovapong, W. Pre-targeted immunoscintigraphy of murine tumors with indium-111-labeled bifunctional haptens. J.Nucl.Med. 1988; 29:226-234.

Greenwood, F.C. and Hunter, W.M. The preparation of I-131 labeled human growth hormone of high specific radioactivity. Biochem. 1963; 89:114-123.

Hawkins, G.A., McCabe, R.P., Kim, C.-H., Subramanian, R., Bredehorst, R., McCullers, G.A., Vogel,C.-W., Hanna, M.G.Jr., and Pomata, N. Delivery of radionuclides to pretargeted monoclonal antibodies using dihydrofolate reductase and methotrexate in an affinity system. Cancer Res. 1993; 53: 2368-2373. Infusa, H., Adachi, T., Kiyokawa, T., Nakatani, Y., et al., Ley glycolipid-recognizing monoclonal antibody inhibits procoagulant activity and metastasis of uman adenocarcinoma. Int. J. Oncol., 2001; 19:941-946.

Koda, K., Glassy, M.C., McKnight, M.E., Yasutomi, J., Saito, N., Dan, M., Nakajima, N. Immunotherapy for recurrent colorectal cancers with human monoclonal antibody SK-1. Anticancer Res. 2001; 21:621-627.

Kranenborg, M.h., Boerman, O.C., Oosterwijk-Wakka, j., weijert, M., Corstens, F., Oosterwijk, E. Development and characterization of anti-renal cell carcinoma x antichelate bi-specific monoclonal antibodies for two-phase targeting of renal cell carcinoma. Cancer Res.(suppl) 1995; 55: 5864s-5867s.

Losman M.J., Qu Z., Krishnan I.S., Wang J., Hansen H.J., Goldenberg D.M., Leung S.O. Clin. Cancer Res. 1999; 5(10 Suppl.):3101s-3105s.

Maeta, M. Saito, H. Oka, S., Tsujitani, S., Ikeguchi, M., Kaibara, N. Mutated p53 in tumors, mutant p53 and p53-specific antibodies in the circulation in patients with gastric cancer. J. Exp. Clin. Cancer Res. 2000; 19:489-95.

Penefsky, H.S. A centrifuged column procedure for the measurement of ligand binding by beef heart F1. Part G. Methods Enzymol. 1979; 56:527-530.

Ritter, G., Cohen, L.S., Williams, C., Jr., Richards, E.C., Old, L.J., Welt S. Serological analysis of human anti-human antibody responses in colon cancer patients treated with repeated doses of humanized monoclonal antibody A33. Cancer Res. 2001; 61:6851-6859.

Schuhmacher, J., Klivenyi, G., Matys,R., Stadler, M., Regiert, T., Hauser,H. , Doll, J., Maier-Borst, W., Zoller, M. Multistep tumor targeting in nude mice using bi-specific antibodies and a gallium chelate suitable for immunocintigraphy with positron emission tomography. Cancer Res. 1995; 55, 115-123.

Schwartzberg, L.S. Clinical experience with edrecolomab: a monoclonal antibody therapy for colorectal carcinoma. Crit. Rev. Oncol. Hematol. 2001; 40:17-24.

Sharkey, RM., Karacay, Griffiths, GL., Behr, TM., Blumenthal,RD., Mattes,MJ.,Hansen, HJ., Goldenberg. Development of a streptavidin-anti-carcinoembryonic ntigen antibody, radiolabeled biotin pretargeting method for radioimmunotherapy of colorectal cancer. Studies in a human colon cancer xenograft model. Bioconjugate Chem 1997; 8: 595-604.

Staib, L., Birebent, B., Somasundaram, R., Purev, E., Braumuller, H., et al. Immunogenicity of recombinant GA733-2E antigen (CO17-1A, EGP, Dsi-4, KSA, Ep-CAM) in gastro-intestinal carcinoma patients. Int. J. Cancer 2001; 92:79-87.

Stickney, DR., Anderson, LD., Slater, JB., Ahlem, CN.,Kirk, GA., Schweighardt, SA and Frincke, JM. Bifunctional

antibody: a binary radiopharmaceutical delivery system for imaging colorectal carcinoma. Cancer Res. 1991;51: 6650-6655.

Thorpe et al., U.S. Patents No. 6,342,221; 6,004,554; and patents and references cited therein.

Todryk, S.M., Turr, A.L., Green, M.H., Smallwood, J.A., Halanek, N., Dalgleish, A.G., Glennie, M.J. CD40 ligation for immunotherapy of solid tumours. J. Immunol Methods 2001; 248:139-147.

Tordsson, J., Lavasani, S., Ohlsson, L., Karlstrom, P., Svedberg, H., Abrahmsen, L., Brodin, T. A3—a novel colon and pancreatic cancer reactive antibody from a primate phage library seleted using intact tumour cells. Int. J. Cancer 2000; 87:559-568.

Turner, J.G., Rakhmilevich, A.L. Burdelya, L., Neal., Z., Imboden, M., Sondel, P.M., Yu, H. Anti-CD40 antibody induces antitumor and antimetastatic effects: the role of NK cells. J. Immunol. 2001; 166:89-94.Res. 1991;51: 6650-6655.

Claims

1. A humanized monoclonal (MAb) antibody or fragment thereof that binds to colon-specific antigen-p mucin (CSAp) antigen, whereby the fragment binds to the same antigen that is recognized by the antibody, comprising the framework (FR) regions of the light and heavy chain variable regions of a human antibody and the light and heavy chain constant regions of a human antibody, wherein the CDRs of the light chain variable region of the humanized anti-CSAp MAb or fragment thereof comprises CDR1 comprising an amino acid sequence of RSSQSIVHSNGNTYLE; CDR2 comprising an amino acid sequence of KVSNRFS and CDR3 comprising an amino acid sequence of FQGSRVPYT; and the CDRs of the heavy chain variable region of the humanized anti-CSAp MAb or fragment thereof comprises CDR1 comprising an amino acid sequence of EYVIT; CDR2 comprising an amino acid sequence of EIYPGSGSTSYNEKFK and CDR3 comprising an amino acid sequence of EDL.

2. The antibody or fragment thereof of claim 1, wherein the FRs of the light and heavy chain variable regions of said antibody or fragment thereof comprise at least one amino acid substituted from the corresponding FRs of the murine anti-CSAp antibody or fragment thereof.

3. The antibody or fragment thereof of claim 2, wherein said amino acid from said murine MAb is at least one amino acid selected from the group consisting of amino acid residue 5, 27, 30, 38, 40, 48, 66, 67, 74, 93, 94 and 103 of the murine heavy chain variable region of Fig. 10A; or wherein said murine amino acids are at least one amino acid selected from the group consisting of amino acid residue 37, 58 and 100 of the murine light chain variable region Fig. 10B.

4. The antibody or fragment thereof of claim 1, wherein said antibody or fragment thereof comprises a hMu-9 V_K amino acid sequence of Figure 11B.

5. The antibody or fragment thereof of claim 4, wherein said antibody or fragment thereof comprises a hMu-9 V_H amino acid sequence of Figure 11A.

6. The antibody or fragment thereof of claim 1, wherein said antibody or fragment thereof comprises a hMu-9V_H amino acid sequence of Figure 11 A.

7. The antibody or fragment thereof of claim 6, wherein said antibody or fragment thereof comprises a hMu-9 V_K amino acid sequence of Figure 11B.

8. The anti-CSAp antibodies or fragment thereof of any one of claims 1-7, wherein said fragment is selected from the group consisting of Fv, F(ab')2, Fab' and Fab.

9. A diagnostic/detection or therapeutic immunoconjugate comprising an antibody component comprising an anti-CSAp MAb or antigen-binding fragment thereof or an antibody fusion protein or fragment thereof of any one of claims 1-8, wherein said antibody component is bound to at least one diagnostic/detection agent or at least one therapeutic agent.

10. The diagnostic/detection immunoconjugate of claim 9, wherein said diagnostic/detection agent comprises at least one photoactive diagnostic/detection agent; in particular wherein said photoactive diagnostic agent comprises a chromogen or dye.

11. The diagnostic/detection immunoconjugate of claim 9, wherein said diagnostic/detection agent is a radionuclide with an energy between 20 and 2,000 keV; in particular wherein said radionuclide is a gamma-, beta- or a positron-emitting isotope; more particularly wherein said radionuclide is selected from the group consisting of 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, I-124, 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.

12. The diagnostic/detection immunoconjugate of claim 9, wherein said diagnostic agent is a contrast agent.

13. The diagnostic/detection immunoconjugate of claim 12, wherein said contrast agent is a paramagnetic ion; in particular wherein said paramagnetic ion comprises a metal selected from the group consisting of 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).

14. The diagnostic/detection immunoconjugate of claim 12, wherein said contrast agent is an ultrasound-enhancing agent, preferably wherein said ultrasound enhancing agent is a liposome that is conjugated to the humanized antibody or fragment thereof, more preferably wherein said liposome is gas filled.

15. The diagnostic/detection immunoconjugate of claim 12, wherein said contrast agent is a radiopaque compound; in particular wherein said radiopaque compound is selected from the group consisting of iodine compounds, barium compounds, gallium compounds and thallium compounds.

16. The diagnostic/detection immunoconjugate of claims 9-11, wherein said immunoconjugate is to be used in intraoperative, endoscopic, or intravascular tumor detection/diagnosis.

17. The therapeutic immunoconjugate of claim 9, wherein said therapeutic agent is selected from the group consisting of a radionuclide, boron, gadolinium or uranium atoms, an immunomodulator, a cytokine, a hormone, a hormone antagonist, an enzyme, an enzyme inhibitor, a photoactive therapeutic agent, a cytotoxic drug, a toxin, an angiogenesis inhibitor, a different antibody and a combination thereof.

18. The therapeutic immunoconjugate of claim 17, wherein said cytotoxic agent is a drug or a toxin; in particular wherein said drug is selected from the group consisting of antimitotic, alkylating, antimetabolite, angiogenesis-inhibiting, apoptotic , alkaloid, COX-2-inhibiting and antibiotic agents and combinations thereof; or wherein said drug is selected from the group consisting of nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, anthracyclines, taxanes, COX-2 inhibitors, pyrimidine analogs, purine analogs, antibiotics, enzymes, epipodophyllotoxins, platinum coordination complexes, vinca alkaloids, substituted ureas, methyl hydrazine derivatives, adrenocortical suppressants, hormone antagonists, enzyme inhibitors, endostatin, taxols, camptothecins, doxorubicins and their analogs, and a combination thereof or wherein said toxin is selected from the group consisting of plant, microbial, and animal toxins; in particular 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, diphtheria toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin.

19. The therapeutic immunoconjugate of claim 17, 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), a stem cell growth factor, erythropoietin, thrombopoietin, an antibody and a combination thereof; in particular 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 -γ, and said stem cell growth factor is designated "S1 factor"; in particular wherein said cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, interferon-γ, TNF-α and a combination thereof.

20. The therapeutic immunoconjugate of claim 17, wherein said immunomodulator is an antibody that is an agonist or antagonist of an immune factor; in particular wherein the antibody is against CD40.

21. The therapeutic immunoconjugate of claim 17, wherein said radionuclide is selected from the group consisting of an Auger emitter, a beta-emitter and an alpha-emitter.

22. A therapeutic immunoconjugate of claim 17, wherein said radionuclide is selected from the group consisting of P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, and Bi-213, Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, I-125, Ho-161, Os-189m, Ir-192, Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213, Fm-255 and combinations thereof.

23. The therapeutic immunoconjugate of claim 17, wherein said Boron atom is B-10; and/or said Gadolinium atom is Gd-157; and/or said Uranium atom is U-235.

24. The therapeutic immunoconjugate of claim 21, wherein said radionuclide has an energy between 20 and 10,000 keV.

25. The therapeutic immunoconjugate of claim 21, wherein said radionuclide is an Auger emitter and has an energy of less than 1000 keV, or wherein said radionuclide is a beta-emitter and has an energy between 20 and 5000 keV, or wherein said radionuclide is an alpha-emitter and has an energy between 2000 and 10,000 keV.

26. The therapeutic immunoconjugate of claim 17, wherein said photoactive therapeutic agent is a chromogen or dye.

27. The diagnostic or therapeutic immunoconjugate according to claim 9, wherein said diagnostic or therapeutic agent is bound to said MAb or fragment thereof by means of a carbohydrate moiety.

28. A multivalent, multispecific antibody or antigen-binding fragment thereof comprising one or more humanized monoclonal antibody or fragment thereof of claim 1 and one or more hapten binding sites having affinity towards hapten molecules.

29. The antibody or fragment thereof of claim 28, wherein said antibody or fragment thereof is humanized, or wherein said antibody or fragment thereof is chimerized, or wherein said antibody or fragment thereof is a human antibody.

30. The antibody or fragment thereof of claim 28 or 29, further comprising a diagnostic or therapeutic agent.

31. An antibody fusion protein or an antigen-binding fragment thereof comprising at least two anti-CSAp MAbs or fragments thereof, wherein said MAbs or fragments thereof are selected from said MAb or fragment thereof of any one of claims 1-30.

32. An antibody fusion protein or an antigen-binding fragment thereof comprising at least one first anti-CSAp MAb or fragment thereof of any one of claims 1-30 and at least one second MAb or fragment thereof, other than the MAb or fragment thereof of any one of claims 1-30.

33. The antibody fusion protein or fragment thereof of claim 32, wherein said second MAb or fragment thereof is a CD40 antibody.

34. The antibody fusion protein or fragment thereof of claim 31 or 32, further comprising a diagnostic or therapeutic agent conjugated to said fusion protein or fragment thereof.

35. The antibody fusion protein or fragment thereof of claim 32, wherein said second MAb, is a carcinoma-associated antibody; in particular wherein said carcinoma-associated antibody is selected from the group consisting of CEA, EGP-1, EGP-2, MUC1, MUC2, MUC3, MUC4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, BrE3, Le-Y, A3, KS-1, VEGF, other angiogenesis antibodies, anti-necrosis antibodies, and oncogene-product antibodies; or wherein said carcinoma-associated antibody binds to an antigen on a cancer selected from the group of gastrointestinal cancers and cancers of the ovary.

36. A therapeutically effective amount of an antibody or fragment according to any of claims 1-8 and 28 to 29, formulated in a pharmaceutically acceptable vehicle for use in a method of treating a malignancy in a subject, comprising the step of administering said formulation to said subject.

37. A therapeutically effective amount of a immunoconjugate or an antigen-binding fragment thereof of any one of claims 9, 17-21 and 30, formulated in a pharmaceutically acceptable vehicle for use in a method of treating a malignancy in a subject, comprising the step of administering said formulation to said subject.

38. A diagnostically effective amount of an antibody or an antigen-binding fragment thereof according to any of claims 1-8 and 28-29, formulated in a pharmaceutically acceptable vehicle for use in a method of diagnosing/detecting a malignancy in a subject, comprising the step of administering said formulation to said subject.

39. A diagnostically effective amount of a immunoconjugate or fragment thereof according to any of claims 9-16, and 30, formulated in a pharmaceutically acceptable vehicle for use in a method of diagnosing/treating a malignancy in a subject, comprising the step of administering said formulation to said subject.

40. A therapeutically or diagnostically effective amount of a fusion protein or fragment thereof of any one of claims 31-35, formulated in a pharmaceutically acceptable vehicle for use in a method of treating or diagnosing/detecting a malignancy in a subject, comprising the step of administering said formulation to said subject.

41. An antibody or fragment thereof according to any of claims 28-29 for use in a method of treating or diagnosing/detecting a malignancy in a subject, comprising (i) administering to a subject in need thereof the antibody or fragments thereof of any one of claims 28-29; (ii) waiting a sufficient amount of time for an amount of the non-binding protein to clear the subject's bloodstream; and (iii) administering to said subject a carrier molecule comprising a diagnostic agent, a therapeutic agent, or a combination thereof, that binds to a binding site of said antibody.

42. A DNA sequence comprising a nucleic acid encoding a anti-CSAp MAb or fragment thereof selected from the group consisting

(a) an anti-CSAp MAb or fragment thereof of any one of claims 1-30;
(b) an antibody fusion protein or fragment thereof comprising at least two of said MAbs or fragments thereof of any one of claims 1 to 30;
(c) an antibody fusion protein or fragment thereof comprising at least one first anti-CSAp MAb or fragment thereof comprising said MAb or fragment thereof of any one of claims 1-30 and at least one second MAb or fragment thereof, other than the MAb or fragment thereof of any one of claims 1-30; 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 claims 1-30 and at least one second MAb or fragment thereof, other than the MAb or fragment thereof of any one of claims 1-30 wherein said second MAb is selected from the group consisting of CEA, EGP-1, EGP-2, MUC-1, MUC-2, MUC-3, MUC-4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, BrE3, Le-Y, A3, KS-1, CD40, and VEGF antibody, and a combination thereof.

43. An expression vector comprising the DNA sequence of claim 42.

44. A host cell comprising the DNA sequence of claim 42.

45. An immunoconjugate according to claim 9 for use in a method of delivering a diagnostic/detection or therapeutic agent, or a combination thereof, to a target comprising (i) providing a composition that comprises said immunoconjugate and (ii) administering to a subject in need thereof said composition.

46. The immunoconjugate for use according to claim 45, wherein said diagnostic/detection agent comprises at least one photoactive diagnostic agent; in particular wherein said photoactive diagnostic agent comprises a chromogen or dye.

47. The immunoconjugate for use according to claim 45, wherein said diagnostic agent is a radionuclide with an energy between 20 and 2,000 keV; in particular wherein said radionuclide is a gamma-, beta- or a positron-emitting isotope; more particularly wherein said radionuclide is selected from the group consisting of 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, I-124, 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.

48. The immunoconjugate for use according to claim 45, wherein said diagnostic agent is a contrast agent.

49. The immunoconjugate for use according to claim 48, wherein said contrast agent is a paramagnetic ion; in particular wherein said paramagnetic ion is a metal comprising manganese, iron or gadolinium.

50. The immunoconjugate for use according to claim 48, wherein said contrast agent is an ultrasound enhancing agent; in particular wherein said ultrasound enhancing agent is a liposome that comprises the humanized antibody or fragment thereof; more particularly wherein said liposome is gas-filled.

51. The immunoconjugate for use according to claim 48, wherein said contrast agent is a radiopaque compound used in X-rays or computed tomography; in particular wherein said radiopaque compound is selected from the group consisting of iodine compounds, barium compounds, gallium compounds and thallium compounds; more particularly wherein said radiopaque compound is selected from the group consisting of 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.

52. The immunoconjugate for use according to claim 45, 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, a photoactive therapeutic agent, a cytotoxic agent, and a combination thereof; in particular wherein said photoactive therapeutic agent is a chromogen or dye.

53. The immunoconjugate for use according to claim 52, wherein said cytotoxic agent is a drug or a toxin; in particular wherein said drug is selected from the group consisting of antimitotic, alkylating, antimetabolite, antiangiogenic, apoptotic, anthracyclines, alkaloid, COX-2-inhibitor and antibiotic agents, and combinations thereof; or wherein said drug is selected from the group consisting of nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas, 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, hormones, hormone antagonists, endostatin, taxols, camptothecins, doxorubicins and their analogs, and a combination thereof; or wherein said toxin is selected from the group consisting of plant, microbial and animal toxin; in particular 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, diphtheria toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin.

54. The immunoconjugate for use according to claim 52, 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), a stem cell growth factor, erythropoietin, thrombopoietin, an antibody, and a combination thereof; in particular wherein said antibody is an anti-CD40 antibody or fragment thereof in particular 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 -γ, and said stem cell growth factor is designated "S1 factor"; in particular wherein said cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, interferon-γ, TNF-α and a combination thereof.

55. The immunoconjugate for use according to claim 52, wherein said radionuclide is selected from the group consisting of an Auger emitter, a β emitter and an α emitter; in particular wherein said radionuclide is an Auger emitter and has an energy of less than 1000 keV; or wherein said radionuclide is a β emitter and has an energy between 20 and 5000 keV; or wherein said radionuclide is an α emitter and has an energy between 2000 and 10,000 keV.

56. The immunoconjugate for use according to claim 52, wherein said radionuclide is selected from the group consisting of P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, and Bi-213, Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109 In-111, Sb-119, I-125, Ho-161, Os-189m, Ir-192, Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213, Fm-255 and combinations thereof.

57. The immunoconjugate for use according to claim 52, wherein said radionuclide has an energy between 20 and 10,000 keV.

58. An antibody or fragment thereof according to claim 28 or 29 for use in a method of delivering a diagnostic/detection agent, a therapeutic agent, or a combination thereof to a target, comprising: (i) administering to a subject said antibody or fragments; (ii) waiting a sufficient amount of time for an amount of the non-binding protein to clear the subject's blood stream; and (iii) 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 said antibody.

59. The antibody or fragment for use according to claim 58, wherein said carrier molecule binds to more than one binding site of the binding protein.

60. The antibody or fragment for use according to claim 58, wherein said diagnostic/detection agent or said therapeutic agent is selected from the group comprising isotopes, dyes, chromogens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, and metals.

61. A therapeutically effective amount of an antibody or fragment thereof or an antibody fusion protein or fragment thereof comprising at least two MAbs or fragments thereof for use in a method of treating a malignancy in a subject comprising administering said amount to said subject, wherein at least one anti-CSAp MAb or fragment thereof or fusion proteins or fragments thereof are any one of claims 1-35 formulated in a pharmaceutically suitable excipient.

62. A therapeutically effective amount of an antibody or fragment thereof comprising at least two MAbs or fragments thereof for use in a method of treating a malignancy in a subject comprising administering said amount to said subject, wherein said MAbs are selected from any one of claims 1-35, and formulated in a pharmaceutically suitable excipient.

63. The composition for use according to claim 62, further comprising a second Mab or fragment thereof not in any one of claims 1-35; in particular wherein said second Mab or fragment thereof is a naked Mab or fragment thereof, or wherein said second MAb or fragment thereof is selected from the group consisting of antibodies against BrE3, Le-Y, EGP-1, EGP-2, MUC-1, MUC-2, MUC-3, MUC-4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, A3, KS-1, CEA, VEGF, oncogene products, and an antibody to CD40 or another immunomodulator; in particular wherein said second MAb is immunoconjugated to a therapeutic or diagnostic/detection agent.

64. The composition for use according to claim 61, wherein said anti-CSAp antibody is administered parenterally; in particular wherein said anti-CSAp antibody is administered in a dosage of 20 to 2000 milligrams protein per dose; in particular wherein said dosage is repeatedly administered.

65. The composition for use according to claim 61, wherein said humanized anti-CSAp antibody constant and hinge regions comprise constant and hinge regions of a human IgG1.

66. The composition for use according to claim 61, wherein said anti-CSAp antibody or fragment thereof is administered before, in conjunction with, or after a second conjugated antibody reactive with a second tumor marker expressed by said malignancy is administered to said subject; or wherein said anti-CSAp antibody or fragment thereof is administered before, concurrently, or after at least one therapeutic or diagnostic/detection agent, in particular wherein said therapeutic or diagnostic/detection agent is conjugated to an antibody that targets a tumor marker that is expressed by said malignancy.

67. The composition for use according to claim 61, wherein a first binding site of the anti-CSAp antibody or fragment thereof is present in a multivalent, multispecific fusion protein or chemical conjugate and a second binding site is reactive with a tumor marker substance other than CSAp.

68. A diagnostically effective amount of a diagnostic/detecting conjugate comprising a anti-CSAp MAb or fragment thereof or an fusion protein or fragment thereof of any one of claims 1-35, wherein said anti-CSAp MAb or fragment thereof or fusion protein or fragment thereof is bound to at least one diagnostic/detection agent, formulated in a pharmaceutically suitable excipient, for use in a method of diagnosing or detecting a malignancy in a subject.

69. A therapeutically effective amount of a composition comprising a naked anti-CSAp MAb or fragment thereof or a naked antibody fusion protein or fragment thereof of any one of claims 1-35 for use in a method of treating a cancer cell in a subject comprising (i) administering said amount to said subject, (ii) formulating said naked CSAp MAb or fragment thereof or antibody fusion protein or fragment thereof in a pharmaceutically suitable excipient.

70. The composition for use according to claim 69, wherein said composition further comprises a second naked antibody or fragment thereof not in any one of claims 1-9, 28-29, and 31-35, in particular wherein said second antibody or fragment thereof is selected from the group consisting of antibodies to CEA, EGP-1, EGP-2, MUC-1, MUC-2, MUC-3, MUC-4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, BrE3, Le-Y, A3, KS-1, CD40, VEGF and other angiogenesis factors, and oncogene products; or wherein said composition further comprises a second naked antibody or fragment thereof of any one of claims 1-9, 28-29, and 31-35; or wherein said composition further comprises a second antibody or fragment thereof not in anyone of claims 1-35.

71. The composition for use according to claim 69, wherein said naked anti CSA antibody is administered parenterally; in particular wherein said naked anti-CSAp antibody is administered in a dosage of 20 to 2000 milligrams protein per dose; more particularly wherein said dosage is repeatedly administered.

72. The composition for use according to claim 69, wherein said humanized naked anti-CSAp antibody constant and hinge regions comprise constant and hinge regions of a human IgG1.

73. The composition for use according to claim 69, wherein said naked anti-CSAp antibody is administered before, in conjunction with, or after a second naked antibody reactive with a second tumor marker expressed by said malignancy is administered to said subject; or wherein said naked anti-CSAp antibody is administered before, concurrently or after a therapeutic or diagnostic/detection agent.

74. The composition for use according to claims 69 to73 wherein said naked anti-CSAp antibody is the naked antibody that binds to colon-specific antigen-p mucin (CSAp).

75. A method of diagnosing or detecting a malignancy in a subject comprising performing an in vitro diagnosis assay on a specimen from said subject with a composition comprising a naked anti-CSAp MAb or fragment thereof or a naked antibody fusion protein or fragment thereof of any one of claims 1-35.

76. The method of claim 75, wherein said malignancy is a carcinoma; in particular wherein (i) said carcinoma is a gastrointestinal cancer, more particularly wherein said carcinoma is colorectal or pancreatic cancer; or (ii) wherein said carcinoma is ovarian cancer.

77. The method of claim 75, wherein said in vitro diagnosis assay is selected from the group consisting of immunoassays, RT-PCR and immunohistochemistry; in particular wherein (i) said diagnosis assay is RT-PCR or immunoassays; more particularly wherein said specimen is body fluid or a tissue or cell population; or (ii) wherein said diagnostic assay is immunohistochemistry or immunocytochemistry.

78. The method of claim 77, wherein said specimen is a cell aliquot or a tissue.

79. The method of any one of claims 35-41 and 45-78 wherein said subject is a mammal; in particular wherein said subject is a human; a domestic pet; or wherein said subject is selected from the group consisting of a horse, dot, and cat.

80. A bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, wherein said one arm that specifically binds a targeted tissue is the antibody as defined in any one of claims 1 to 8 for use in a method of treating or identifying diseased tissues in a subject, comprising:

(a) administering said antibody or antibody fragment to said subject;
(b) optionally, administering to said subject a clearing composition, and allowing said composition to clear non-localized antibodies or antibody fragments from circulation;
(c) administering to said subject a first targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bispecific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents; and
(d) when said therapeutic agent is an enzyme, further administering to said subject

(1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site; or
(2) a drug which is capable of being detoxified in said subject form an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of

increasing the toxicity of said drug at the target site, or
(3) a prodrug which is activated in said subject through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or
(4) a second targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site.

81. The antibody or antibody fragment for use according to claim 80, wherein said targetable conjugate comprises at least two HSG haptens.

82. The antibody or antibody fragment for use according to claim 80, further comprising, when said first targetable conjugate comprises a prodrug, administering a second targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and an enzyme capable of converting said prodrug to a drug or of reconverting a detoxified intermediate of said drug to a toxic form.

83. The antibody or antibody fragment for use according to claim 80, wherein said diagnostic/detection agent is a radionuclide.

84. The antibody or antibody fragment for use according to claim 80, wherein said diagnostic/detection agent is a radionuclide with an energy between 20 and 2,000 keV.

85. The antibody or antibody fragment for use according to claim 83, wherein said diagnostic/detection agent emits 25-600 keV gamma particles and/or positrons; or wherein said radionuclide is a gamma-, beta- or a positron-emitting isotope; in particular wherein said radionuclide is selected from the group consisting of 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, I-124, 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.

86. The antibody or antibody fragment for use according to claim 80, wherein said diagnostic/detection agent comprises at least one photoactive diagnostic agent; in particular wherein said photoactive diagnostic/ detection agent comprises a chromogen or dye.

87. The antibody or antibody fragment for use according to claim 80, wherein said diagnostic/detection agent is a radiopaque compound; in particular wherein said radiopaque compound is selected from the group consisting of iodine compounds, barium compounds, gallium compounds and thallium compounds; in particular wherein said radiopaque compound is selected from the group consisting of 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.

88. The antibody or antibody fragment for use according to claim 80, wherein said diagnostic/detection agent is a contrast agent.

89. The antibody or antibody fragment for use according to claim 88, wherein said contrast agent is a paramagnetic ion; in particular wherein said paramagnetic ion comprises a metal selected from the group consisting of manganese, iron and gadolinium.

90. The antibody or antibody fragment for use according to claim 88, wherein said contrast agent is an ultrasound enhancing agent; in particular said ultrasound enhancing agent is a liposome that is conjugated to the humanized antibody or fragment thereof; more particularly wherein said liposome is gas filled.

91. The antibody or antibody fragment for use according to claim 80, wherein said therapeutic agent is selected from the group consisting of a radionuclide, boron, gadolinium or uranium atoms, an immunomodulator, a hormone, a hormone antagonist, an enzyme, and enzyme inhibitor, a photoactive therapeutic agent, a cytotoxic agent, an angiogenesis inhibitor, and a combination thereof.

92. The antibody or antibody fragment for use according to claim 91, wherein said cytotoxic agent is a drug, prodrug or a toxin; in particular wherein said prodrug is selected from the group consisting of epirubicin glucuronide, CPT-11, etoposide glucuronide, daunomicin glucuronide and doxorubicin glucuronide; in particular wherein said toxin is selected from the group consisting of a plant toxin, an animal toxin and a microbial toxin; or wherein said toxin is selected from the group consisting of ricin, abrin, ribonuclease (RNase), DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin; in particular wherein said drug is selected from the group consisting of antimitotic, alkylating, anti-metabolite, angiogenesis-inhibiting, apoptotic, alkaloid, COX-2-inhibiting and antibiotic agents and combinations thereof; or wherein said drug is selected from the group consisting of nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, anthracyclines, taxanes, COX-2 inhibitors, pyrimidine analogs, purine analogs, antibiotics, enzymes, epipodophyllotoxins, platinum coordination complexes, vinca alkaloids, substituted ureas, methyl hydrazine derivatives, adrenocortical suppressants, hormones, hormone antagonists endostatin, taxols, camptothecins, doxorubicins and their analogs, and a combination thereof.

93. The antibody or antibody fragment for use according to claim 91, 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), a stem cell growth factor, erythropoietin, thrombopoietin, an antibody agonist or antagonist to an immunomodulator, and a combination thereof; in particular 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 -γ, and said stem cell growth factor is designated "S1 factor."

94. The antibody or antibody fragment for use according to claim 93, wherein said cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, interferon-γ, TNF-α and a combination thereof.

95. The antibody or antibody fragment for use according to claim 91, wherein said radionuclide is selected from the group consisting of an Auger emitter, a β emitter and an α emitter; or wherein said radionuclide is selected from the group consisting of P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, and Bi-213, Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, I-125, Ho-161, Os-189m, Ir-192, Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213, Fm-255 and combinations thereof.

96. The antibody or antibody fragment for use according to claim 91, wherein said Boron atom is B-10, and/or wherein said Gadolinium Atom is Gd-157, and/or wherein said Uranium atom is U-235.

97. The antibody or antibody fragment for use according to claim 95, wherein said radionuclide has an energy between 20 and 10,000 keV; or wherein said radionuclide is an Auger emitter and has an energy of less than 1000 keV; or wherein said radionuclide is a β emitter and has an energy between 20 and 5000 keV; or wherein said radionuclide is an a emitter and has an energy between 2000 and 10,000 keV.

98. The antibody or antibody fragment for use according to claim 80, wherein said targetable conjugate comprises one or more radioactive isotopes useful for killing diseased tissue; in particular wherein said targetable conjugate comprises 10B atoms, and said method further comprises the step of irradiating said boron atoms localized at said diseased tissue, thereby effecting BNCT of said diseased tissue; or wherein the targetable conjugate comprises one or more agents for photodynamic therapy, in particular wherein said agent for photodynamic therapy is a photosensitizer, more particularly wherein said photosensitizer is selected from the group consisting of benzoporphyrin monoacid ring A (BPD-MA), tin etiopurpurin (SnET2), sulfonated aluminum phthalocyanine (AlSPc) and lutetium texaphyrin (Lutex).

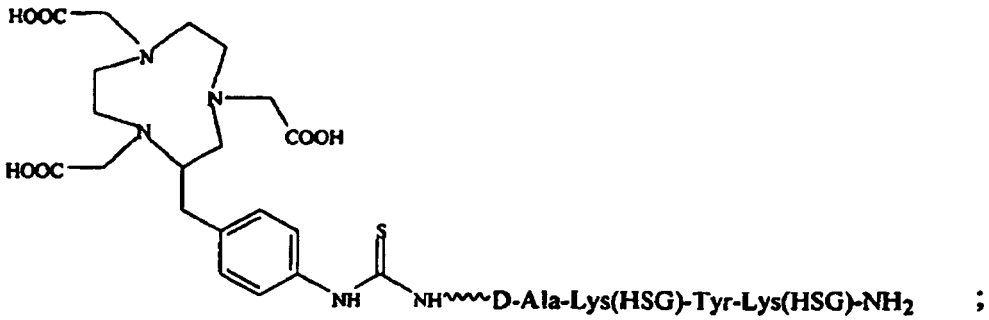
99. The antibody or antibody fragment for use according to claim 80, wherein said targeted tissue is a tumor; in particular wherein said tumor produces or is associated with colon-specific antigen-p (CSAp).

100. The antibody or antibody fragment for use according to claim 80, wherein said antibody or fragment thereof that binds to colon-specific antigen-p mucin (CSAp) comprises the Fv of the MAb of any one of claims 1 to 8.

101. The antibody or antibody fragment for use according to claim 80, wherein said bispecific antibody is a fusion protein; in particular wherein the fusion protein is trivalent, and incorporates the Fv of an antibody reactive with CSAp.

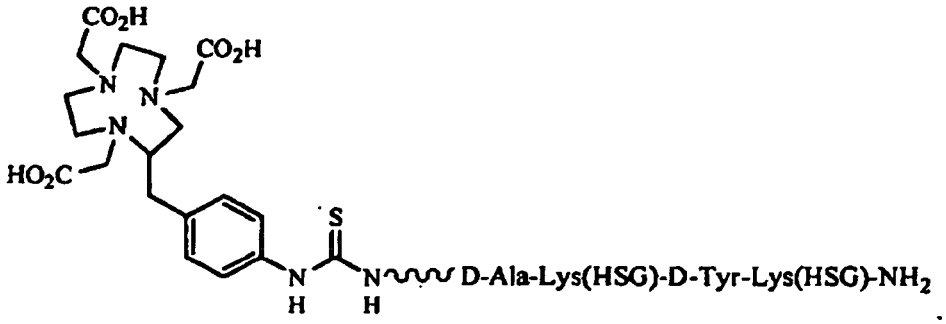
102. An effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, wherein said one arm that specifically binds a targeted tissue is an antibody or fragment thereof as defined in any one of claims 1 to 8 and 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)-NH2;
(iii) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(iv)



;

and
(v)



for use in a method for detecting or treating tumors expressing CSAp in a mammal, comprising administering said antibody or antibody fragment and administering said conjugate.

103. The substances for use according to claim 102, further comprising administering to said subject a clearing composition, and allowing said composition to clear non-localized antibodies or antibody fragments from circulation.

104. A kit useful for treating or identifying diseased tissues in a subject comprising:

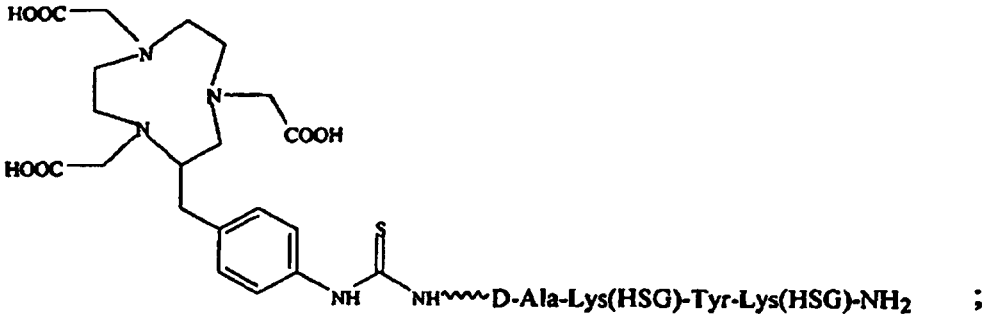
(a) a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, wherein said one arm that specifically binds a targeted tissue is an antibody or fragment thereof as defined in any one of claims 1 to 8;
(b) a first targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents; and
(c) optionally, a clearing composition useful for clearing non-localized antibodies and antibody fragments; and
(d) optionally, when said therapeutic agent conjugated to said first targetable conjugate is an enzyme,

(1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site; or
(2) a drug which is capable of being detoxified in said subject to form an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or

(3) a prodrug which is activated in said subject through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or
(4) a second targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site.

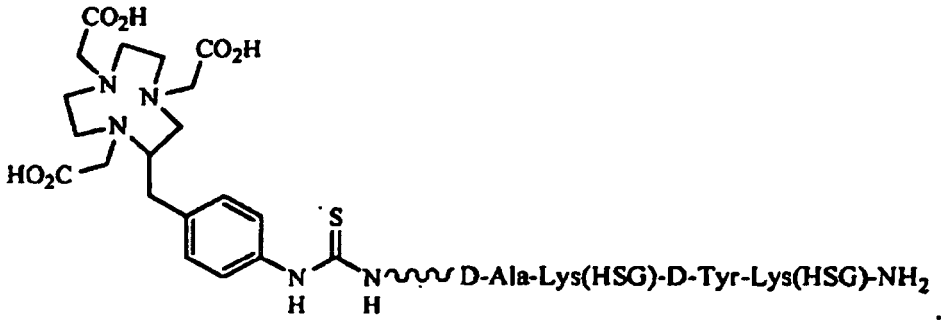
105. The kit of claim 104, wherein said targetable conjugate is selected from the group consisting of:

(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)



;

and
(v)



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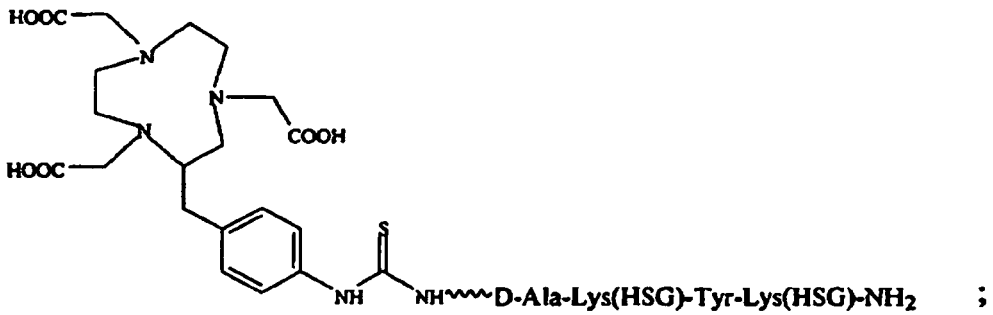
106. An in vitro method of screening for a targetable conjugate comprising:

(a) contacting said targetable construct with a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds said targetable conjugate to give a mixture, wherein said one arm that specifically binds a targeted tissue is an antibody or fragment thereof as defined in any one of claims 1 to 8; and
(b) optionally incubating said mixture; and
(c) analyzing said mixture.

107. An effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, wherein said one arm that specifically binds a targeted tissue is an antibody or fragment thereof as defined in any one of claims 1 to 8; for use in a method for imaging malignant tissue or cells in a mammal expressing CSAp, comprising:

(a) administering the 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-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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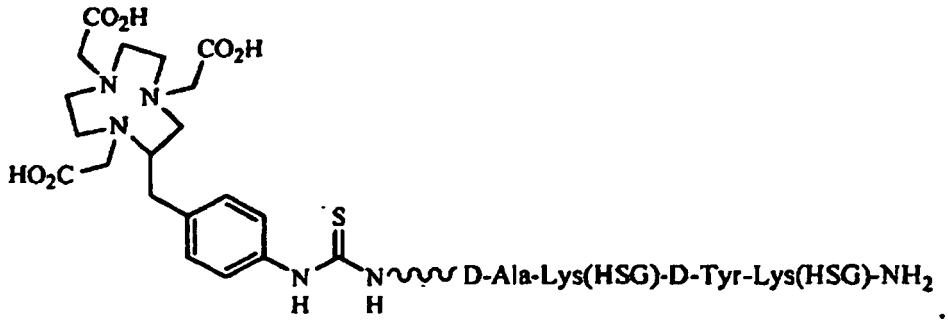

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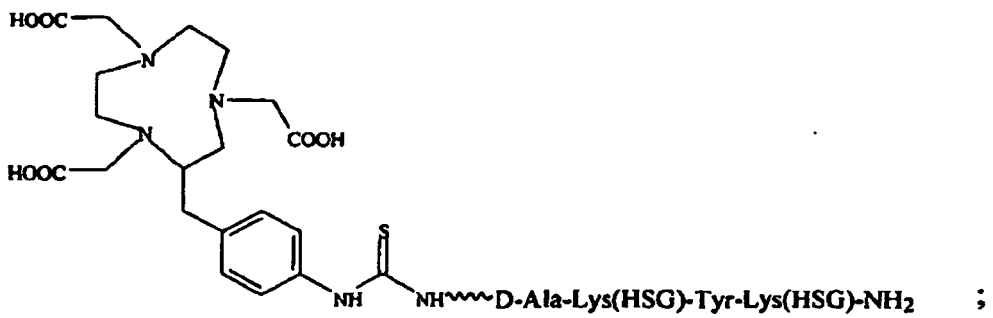
108. An effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue expressing CSAp and at least one other arm that specifically binds a targetable conjugate, wherein said one arm that specifically binds a targeted tissue is an antibody or fragment thereof as defined in any one of claims 1 to 8 for use in a method of intraoperatively identifying/disclosing diseased tissues expressing CSAp, in a subject, comprising:

(a) administering the 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-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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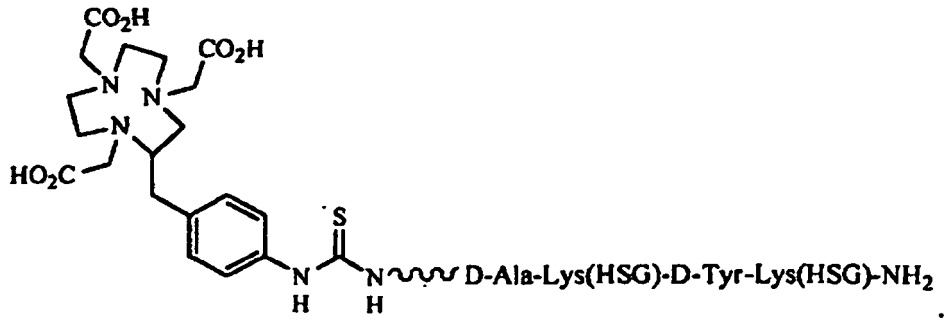
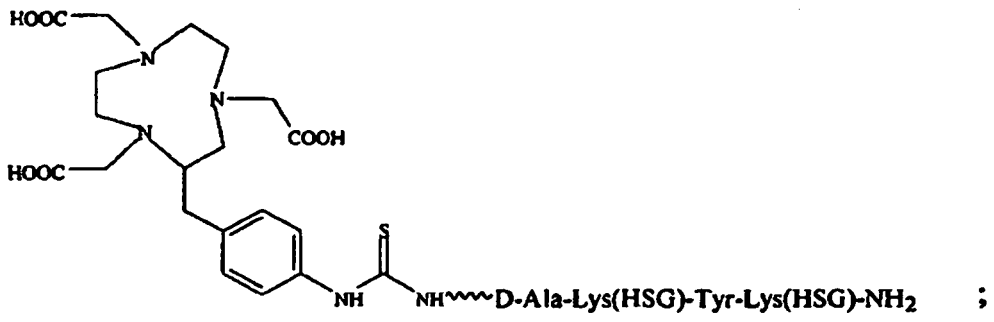


109. An effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue expressing CSAp and at least one other arm that specifically binds a targetable conjugate wherein said one arm that specifically binds a targeted tissue is an antibody or fragment thereof as defined in any one of claims 1 to 8 for use in a method for the endoscopic identification of diseased tissues expressing CSAp, in a subject, comprising:

(a) administering the 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-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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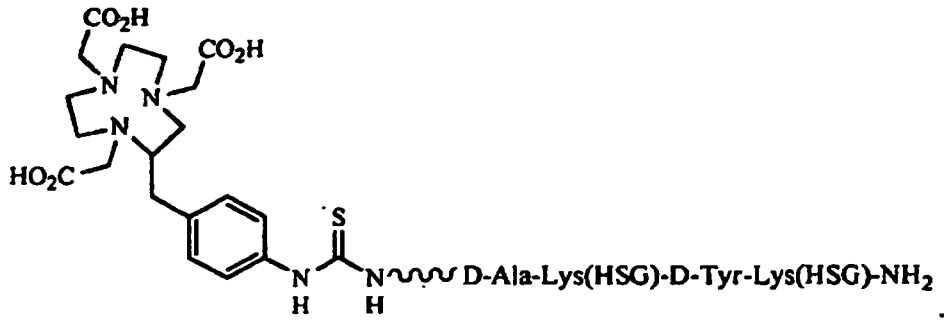
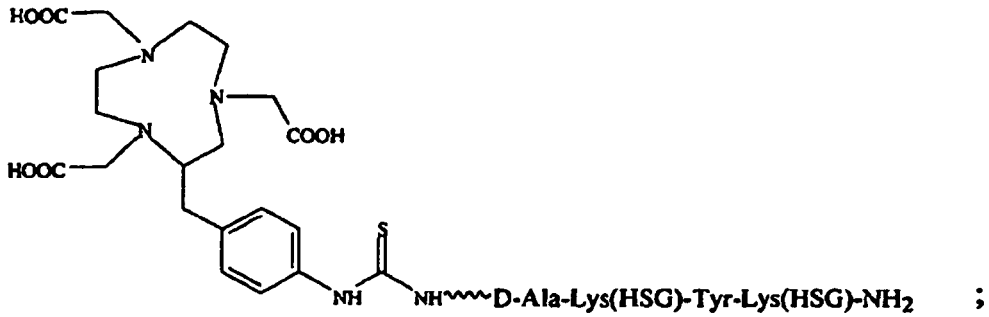


110. An effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue expressing CSAp and at least one other arm that specifically binds a targetable conjugate wherein

said one arm that specifically binds a targeted tissue is an antibody or fragment thereof as defined in any one of claims 1 to 8 for use in a method for the intravascular identification of diseased tissues expressing CSAp, in a subject, comprising:

(a) administering the 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-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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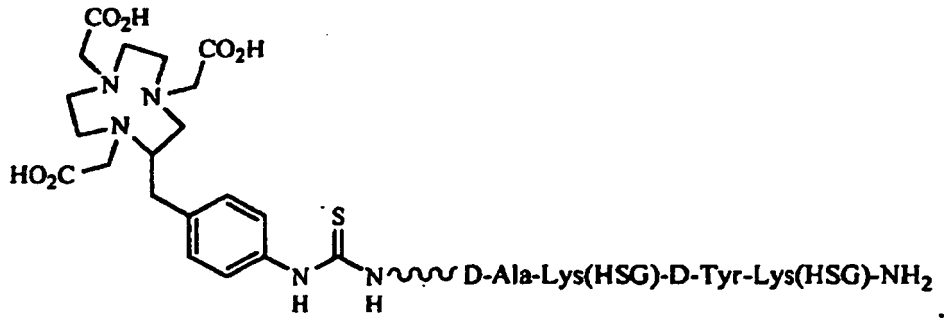

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111. A bispecific antibody $F(ab)_2$ or $F(ab')_2$ fragment, wherein the bispecific antibody or fragment has a first antibody binding site which specifically binds to a CSAp antigen as defined in any one of claims 1 to 8, and has a second antibody binding site which specifically binds to a hapten for use in a method of detection of lesions during an endoscopic, laparoscopic, intravascular catheter, or surgical procedure, wherein the method comprises:

(a) injecting a subject who is to undergo such a procedure with the bispecific antibody $F(ab)_2$ or $F(ab')_2$ fragment, 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.

112. The method of claim 111, wherein said hapten is labeled with a diagnostic radioisotope, a MRI image-enhancing agent or a fluorescent label.

113. An effective amount of an immunoconjugate or fragment thereof as defined in any one of the preceding claims for use in a method for close-range lesion detection, during an operative, intravascular, laparoscopic, or endoscopic

procedure, wherein the method comprises:

(a) injecting a subject to such a procedure parenterally with the effective amount of the immunoconjugate or fragment thereof,
(b) conducting the procedure within 48 hours of the injection;
(c) scanning the accessed interior of the subject at dose 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.

114. The substances for use according to of claim 113, wherein said immunoconjugate or fragment thereof comprises a radioisotope that emits at an energy of 20-1,000 keV; in particular wherein the radioisotope is selected from the group consisting of technetium-99m, iodine-125, iodine-131, iodine-123, indium-111, fluorine-18, gallium 68 and gallium-67M; or wherein the immunoconjugate or fragment thereof comprises a non-isotopic agent, in particular wherein said non-isotopic agent is a photoactive agent; more particularly wherein said photoactive agent is a fluorescent agent.

115. A bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate comprising at least two HSG haptens, whereby the at least one other arm that specifically binds a targeted tissue is an antibody or a fragment thereof as defined in any one of claims 1 to 8 for use in a method of treating or identifying diseased tissues in a subject, comprising:

(a) administering said antibody or antibody fragment to said subject;
(b) optionally, administering to said subject a clearing composition, and allowing said composition to clear non-localized antibodies or antibody fragments from circulation;
(c) administering to said subject a targetable conjugate which comprises a carrier portion which comprises or bears at least two HSG haptens and may comprise a diagnostic or therapeutic cation, and/or one or more chelated or chemically bound therapeutic or diagnostic agents, or enzymes; and
(d) when said targetable conjugate comprises an enzyme, further administering to said subject

(1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site; or
(2) a drug which is capable of being detoxified in said subject to form an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or
(3) a prodrug which is activated in said subject through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site.

116. The antibody or antibody fragment according to claim 115, wherein said diagnostic agent emits 25-600 keV gamma particles and/or positrons; in particular wherein said diagnostic agent is selected from the group consisting of 18F, 52Fe, 62Cu, 64Cu, 67Cu, 67Ga, 68Ga, 86Y, 89Zr, 94mTc, 94Tc, 99mTc, 111In, 123I, 124I, 125I, and 131I.

117. The antibody or antibody fragment for use according to claim 115, wherein said therapeutic agent is a drug, prodrug or toxin; in particular wherein said prodrug is selected from the group consisting of epirubicin glucuronide, CPT-11, etoposide glucuronide, daunomicin glucuronide and doxorubicin glucuronide; in particular wherein said toxin is selected from the group consisting of ricin, abrin, ribonuclease, DNase I, *Staphylococcal* enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, *Pseudomonas* exotoxin, and *Pseudomonas* endotoxin.

118. The antibody or antibody fragment for use according to claim 115 further comprising a therapeutic nuclide; in particular wherein said therapeutic nuclide is selected from the group consisting of 32P, 33P, 47Sc, 64Cu, 67Cu, 67Ga, 90Y, 111Ag, 111In, 125I, 131I, 142Pr, 153Sm, 161Tb, 166Dy, 166Ho, 177Lu, 186Re, 188Re, 189Re, 212Pb, 212Bi, 213Bi, 211At, 223Ra and 225Ac.

119. The antibody or antibody fragment for use according to claim 115, wherein said targetable conjugate comprises one or more radioactive isotopes useful for killing diseased tissue; or wherein said targetable conjugate comprises 10B atoms, and said method further comprises the step of irradiating said boron atoms localized at said diseased tissue, thereby effecting BNCT of said diseased tissue.

120. The antibody or antibody fragment for use according to claim 115, wherein said targetable conjugate comprises one or more toxins; in particular wherein said toxin is selected from the group consisting of ricin, abrin, ribonuclease, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin.

121. The antibody or antibody fragment for use according to claim 115, wherein said targetable conjugate comprises one or more drugs; or wherein said targetable conjugate comprises one or more prodrugs; in particular wherein said prodrug is selected from the group consisting of epirubicin glucuronide, CPT-11, etoposide glucuronide, daunomicin glucuronide and doxorubicin glucuronide.

122. The antibody or antibody fragment for use according to claim 115, wherein said targetable conjugate comprises one or more diagnostic agents useful for detecting diseased tissue, in particular wherein the diagnostic agent is selected from the group consisting of 118F, 52Fe, 62Cu, 64Cu, 67Cu, 67Ga, 68Ga, 86Y, 89Zr, 94mTc, 94Tc, 99mTc, 111In, 123I, 124I, 125I, and 131I; in particular wherein said radioactive isotope is used to perform positron-emission tomography (PET).

123. The antibody or antibody fragment for use according to claim 115, wherein said targetable conjugate comprises one or more image enhancing agents for use in magnetic resonance imaging (MRI), in particular wherein said enhancing agent is selected from the group consisting of Mn, Fe and Gd; or wherein the targetable conjugate comprises one or more agents for photodynamic therapy, in particular wherein said agent for photodynamic therapy is a photosensitizer, more particularly wherein said photosensitizer is selected from the group consisting of benzo-porphyrin monoacid ring A (BPD-MA), tin etiopurpurin (SnET2), sulfonated aluminum phthalocyanine (AlSPc) and lutetium texaphyrin (Lutex).

124. The antibody or antibody fragment for use according to claim 115, wherein (i) said at least one arm that specifically binds a targeted tissue is a monoclonal antibody or a fragment of a monoclonal antibody; (ii) said at least one other arm that specifically binds a targetable conjugate is a monoclonal antibody or a fragment of a monoclonal antibody; (iii) said at least one arm that specifically binds a targeted tissue is a human, chimeric or humanized antibody or a fragment of a human, chimeric or humanized antibody; or wherein (iv) said at least one other arm that specifically binds a targetable conjugate is a human, chimeric or humanized antibody or a fragment of a human, chimeric or humanized antibody.

125. The antibody or antibody fragment for use according to claim 115, wherein said bi-specific antibody or antibody fragment further comprises a therapeutic nuclide, in particular wherein said therapeutic radionuclide is selected from the group consisting of 32P, 33P, 47Sc, 64Cu, 67Cu, 67Ga, 90Y, 111Ag, 111In, 125I, 131I, 142Pr, 153Sm, 161Tb, 166Dy, 166Ho, 177Lu, 186Re, 188Re, 189Re, 212Pb, 212Bi, 213Bi, 211At, 223Ra and 225Ac.

126. The antibody or antibody fragment for use according to claim 115, wherein said targetable conjugate comprises doxorubicin, SN-38, etoposide, methotrexate, 6-mercaptopurine or etoposide phosphate.

127. The antibody or antibody fragment for use according to claim 115, wherein said targeted tissue is a tumor; in particular wherein said tumor produces or is associated with colon-specific antigen-p (CSAp); more particularly wherein the bispecific antibody comprises the Fv of MAb Mu9 and the Fv of MAb 679; even more particularly wherein Mu9 and/or 679 are chimerized or humanized, or wherein Mu9 and/or 679 are human Mu9 and 679, or wherein the bispecific antibody comprises one or more of the CDRs of Mu9; or wherein the bispecific antibody comprises one or more of the CDRs of 679.

128. The antibody or antibody fragment for use according to claim 127, wherein the bispecific antibody is a fusion protein.

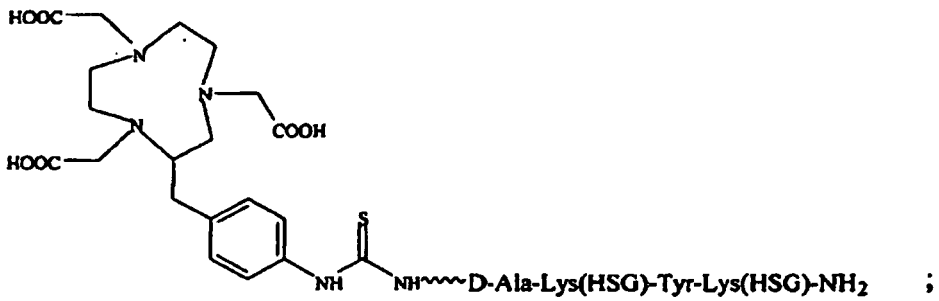
129. The antibody or antibody fragment for use according to claim 127, wherein the tumor produces carcinoembryonic antigen (CEA); in particular wherein the bispecific antibody comprises the Fv of MAb MN14 and the Fv of MAb 679; more particularly wherein MN14, and/or 679 are chimerized or humanized; or wherein MN14, and/or 679 are human MN14 and 679; or wherein the bispecific antibody comprises one or more of the CDRs of MN14; or wherein the bispecific antibody comprises one or more of the CDRs of 679; or wherein the bispecific antibody is a fusion protein, in particular wherein the fusion protein is trivalent, and incorporates the Fv of an antibody reactive with CSAp.

130. The antibody or antibody fragment for use according to claim 129, wherein the bispecific antibody incorporates a Class III anti-CEA antibody and the Fv of 679.

131. An effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate for use in a method for detecting or treating target cells or tissues in a mammal, comprising:

administering said antibody or antibody fragment to said mammal;
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells or tissues or on a molecule produced by or associated therewith, whereby such moiety is the CSAp antigen and wherein said one arm that specifically binds a targeted tissue is the antibody or a fragment thereof as defined in any one of claims 1 to 8; and
administering a targetable conjugate selected from the group consisting of

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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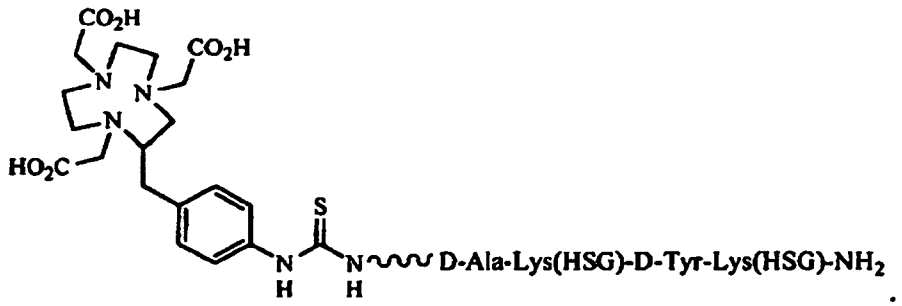

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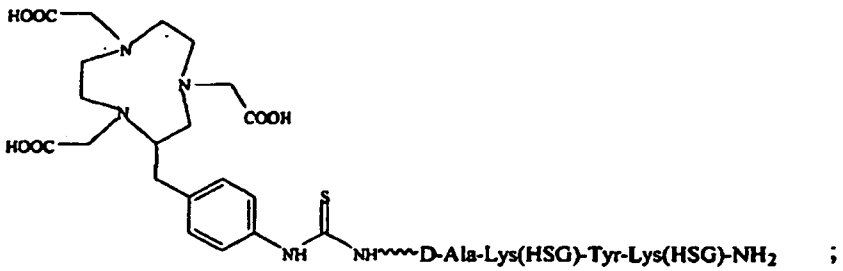
132. A bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate for use in a method of treating or identifying diseased tissues in a subject, comprising:

administering said antibody or antibody fragment to said subject;
optionally, administering to said subject a clearing composition, and allowing said composition to clear non-localized antibodies or antibody fragments from circulation; and
administering to said subject a targetable conjugate selected from the group consisting of:

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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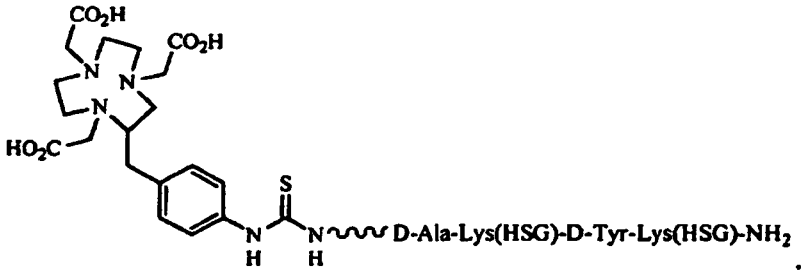

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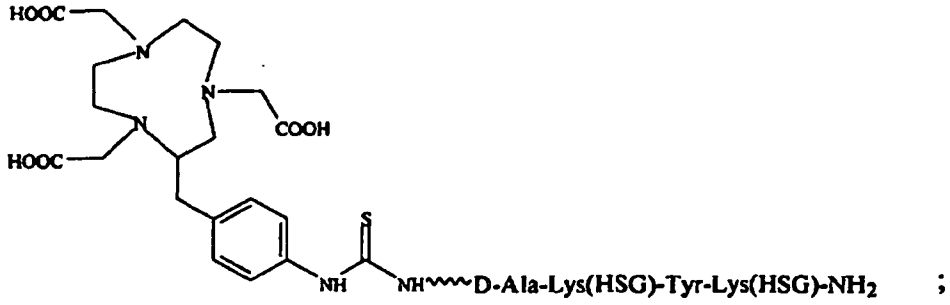
wherein the antibody or antibody fragment is an antibody or antibody fragment according to any of claims 1 to 8.

133. A kit useful for treating or identifying diseased tissues in a subject comprising:

(a) a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, whereby the arm that specifically binds the targeted tissue is an antibody or a fragment thereof as defined in any one of claims 1 to 8, and the conjugate is 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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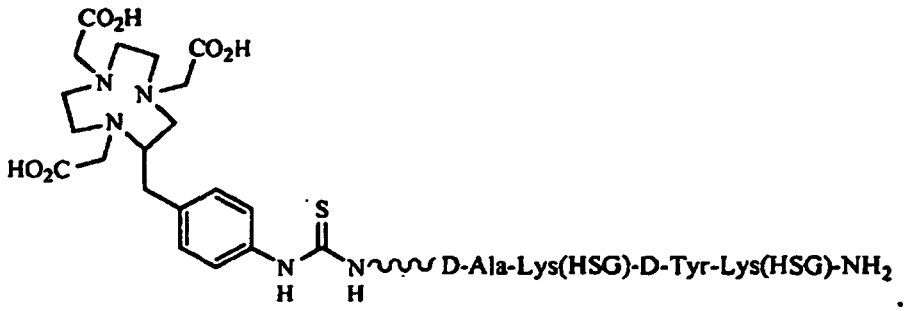


(b) a targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents, or enzymes; and
(c) optionally, a clearing composition useful for clearing non-localized antibodies and antibody fragments; and
(d) optionally, when said first targetable conjugate comprises an enzyme,

(1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site; or
(2) a drug which is capable of being detoxified in said subject to form an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or
(3) a prodrug which is activated in said subject through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site.

134. An in vitro method of screening for a targetable conjugate comprising:

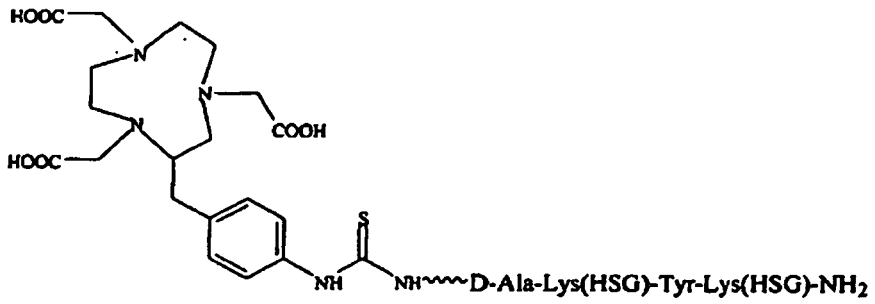
contacting said targetable construct with a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds said targetable conjugate to give a mixture;
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or on a molecule produced by or associated therewith, whereby the moiety is the CSAp antigen and wherein said at least one arm that specifically binds a targeted tissue is an antibody or a fragment thereof as defined in any one of claims 1 to 8; and
optionally incubating said mixture; and
analyzing said mixture.

135. The method of claim 134, wherein said analysis comprises an analytical method selected from the group consisting of FABMS, high-field NMR or size-exclusion HPLC.

136. An effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or on a molecule produced by or associated therewith, whereby the moiety is the CSAp antigen and wherein said at least one arm that specifically binds a targeted tissue is an antibody or a fragment thereof as defined in any one of claims 1 to 8 for use in a method for imaging normal tissue in a mammal, comprising:

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

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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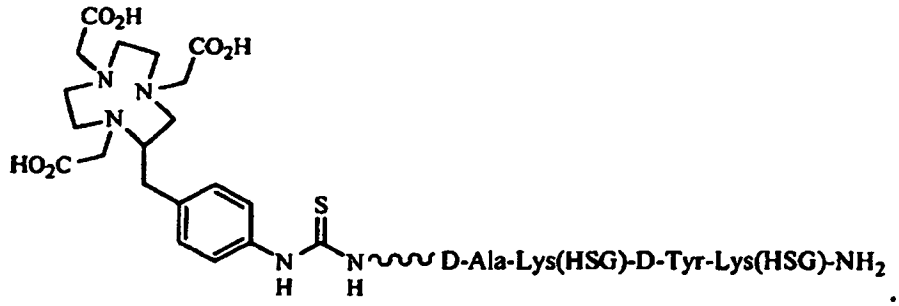

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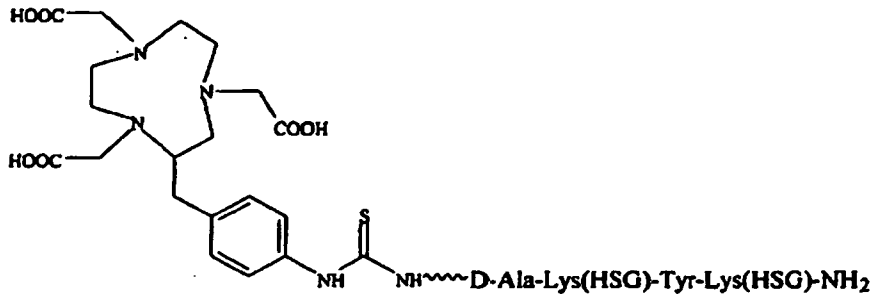
137. The substance for use according to claim 136, wherein said normal tissue is tissue from the ovary, thymus, parathyroid or spleen.

138. An effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or on a molecule produced by or associated therewith, whereby the moiety is the CSAp antigen and wherein said at least one arm that specifically binds a targeted tissue is an antibody or a fragment thereof as defined in any one of claims 1 to 8; for use in a method of intraoperatively identifying diseased tissues, in a subject, comprising:

administering the effective amount of the bispecific antibody or antibody fragment and
administering a targetable conjugate selected from the group consisting of

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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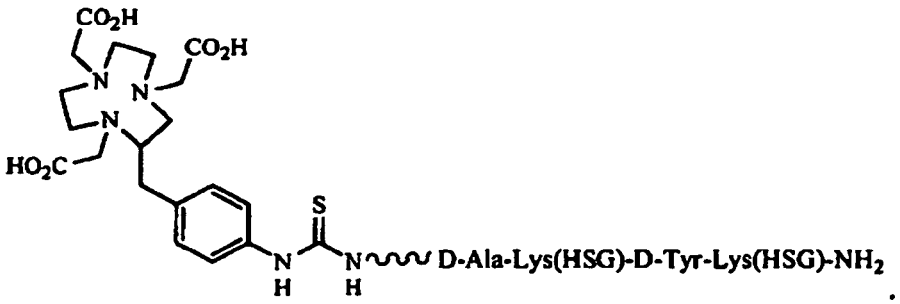
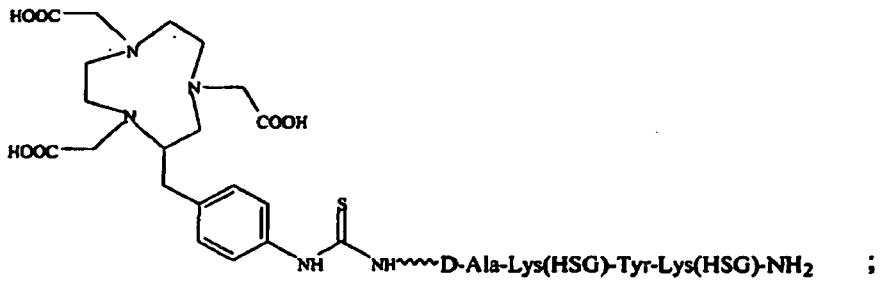


139. An effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or on a molecule produced by or associated therewith, whereby the moiety is the CSAp antigen and wherein said at least one arm that specifically binds a targeted tissue is an antibody or a fragment thereof as defined in any one of claims 1 to 8; for use in a method for the endoscopic identification of diseased tissues, in a subject, comprising:

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

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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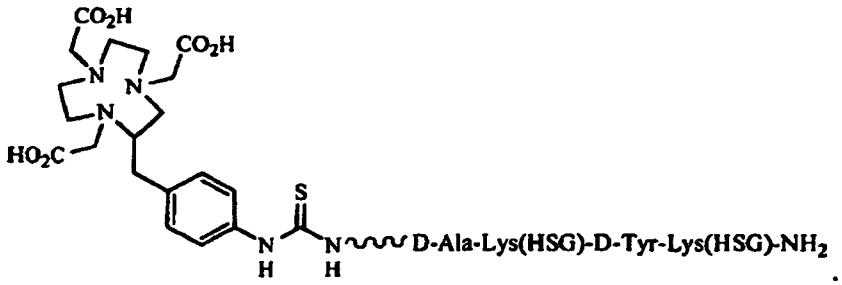
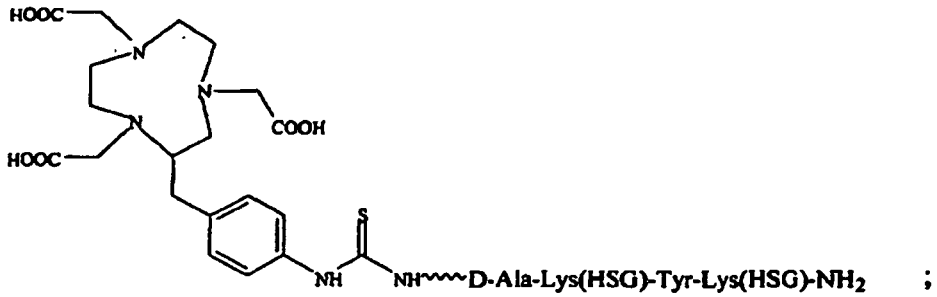


140. An effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith, whereby the moiety is the CSAp antigen and wherein said at least one arm that specifically binds a targeted tissue is an antibody or a fragment thereof as defined in any one of claims 1 to 8; for use in a method for the intravascular identification of diseased tissues, in a subject, comprising:

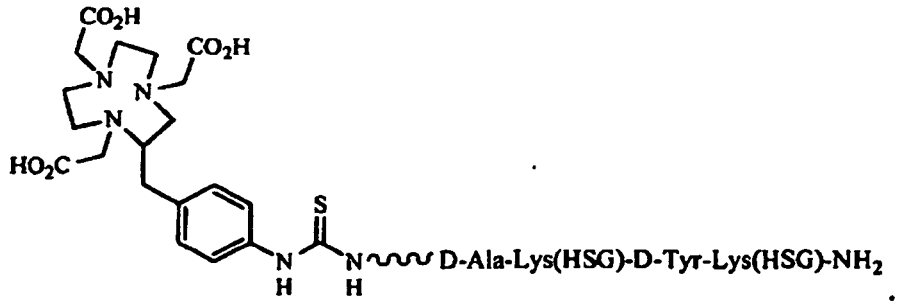
administering the effective amount of the bispecific antibody or antibody fragment; and
administering a targetable conjugate selected from the group consisting of

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



;

and
(e)



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141. The bispecific antibody or antibody fragment, the effective amount of the bispecific antibody or antibody fragment, the kit or the method of any one of claims 131, 132, 133, 134, 136, 138, 139 or 140 wherein said targetable conjugate further comprises a diagnostic agent selected from the group consisting of ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, ^{94m}Tc, ^{94}Tc, ^{99m}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154\text{-}158}Gd$ and ^{175}Lu; in particular wherein said targetable conjugate further comprises a therapeutic nuclide selected from the group consisting of ^{32}P, ^{33}P, ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{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.

Patentansprüche

1. Humanisierter monoklonaler (MAb) Antikörper oder Fragment davon, der/das an Colon-spezifisches Antigen-p-Mucin (CSAp)-Antigen bindet, wobei das Fragment an das gleiche Antigen bindet, das durch den Antikörper erkannt wird, umfassend die Gerüstregionen (FR) der variablen Regionen einer leichten und einer schweren Kette eines humanen Antikörpers und die konstanten Regionen einer leichten und einer schweren Kette eines humanen Antikörpers, wobei die CDRs der variablen Region einer leichten Kette des humanisierten anti-CSAp MAb oder Fragments davon die CDR1 umfassend eine Aminosäuresequenz RSSQSIVHSNGNTYLE; die CDR2 umfassend eine Aminosäuresequenz KVSNRFS und die CDR3 umfassend eine Aminosäuresequenz FQGSRVPYT umfassen; und die CDRs der variable Region einer schweren Kette des humanisierten anti-CSAp MAb oder Fragments davon die CDR1 umfassend eine Aminosäuresequenz EYVIT; die CDR2 umfassend eine Aminosäuresequenz EIYPGSGSTSYNEKFK und die CDR3 umfassend eine Aminosäuresequenz EDL umfassen.

2. Antikörper oder Fragment davon nach Anspruch 1, wobei die FRs der variablen Regionen einer leichten und einer schweren Kette des Antikörpers oder Fragments davon wenigstens eine Aminosäure umfassen, die gegenüber den

entsprechenden FRs des murinen anti-CSAp Antikörpers oder Fragments davon ausgetauscht ist.

3. Antikörper oder Fragment davon nach Anspruch 2, wobei die Aminosäure von dem murinen MAb wenigstens eine Aminosäure ist, die ausgewählt ist aus der Gruppe bestehend aus Aminosäurerest 5, 27, 30, 38, 40, 48, 66, 67, 74, 93, 94 und 103 der variablen Region einer schweren murinen Kette von Fig. 10A; oder wobei die murinen Aminosäuren wenigstens eine Aminosäure sind, die ausgewählt ist aus der Gruppe bestehend aus Aminosäurerest 37, 58 und 100 der variablen Region einer leichten murinen Kette von Fig. 10B.

4. Antikörper oder Fragment davon nach Anspruch 1, wobei der Antikörper oder Fragment davon eine hMu-9 V_K Aminosäuresequenz von Figur 11 B umfasst.

5. Antikörper oder Fragment davon nach Anspruch 4, wobei der Antikörper oder Fragment davon eine hMu-9 V_H Aminosäuresequenz von Figur 11A umfasst.

6. Antikörper oder Fragment davon nach Anspruch 1, wobei der Antikörper oder Fragment davon eine hMu-9V_H Aminosäuresequenz von Figur 11 A umfasst.

7. Antikörper oder Fragment davon nach Anspruch 6, wobei der Antikörper oder Fragment davon eine hMu-9 V_K Aminosäuresequenz von Figur 11B umfasst.

8. Anti-CSAp-Antikörper oder Fragment davon nach einem der Ansprüche 1-7, wobei das Fragment ausgewählt ist aus der Gruppe bestehend aus Fv, F(ab')2, Fab' und Fab.

9. Diagnostisches/Nachweis- oder therapeutisches Immunkonjugat umfassend eine Antikörperkomponente umfassend einen anti-CSAp-MAb oder Antigen-bindendes Fragment davon oder ein Antikörperfusionsprotein oder Fragment davon nach einem der Ansprüche 1-8, wobei die Antikörperkomponente an wenigstens ein diagnostisches/Nachweis-Agens oder wenigstens ein therapeutisches Agens gebunden ist.

10. Diagnostisches/Nachweis-Immunkonjugat nach Anspruch 9, wobei das diagnostische/Nachweis-Agens wenigstens ein photoaktives diagnostisches/Nachweis-Agens umfasst; wobei das photoaktive diagnostische Agens bevorzugterweise ein Chromogen oder einen Farbstoff umfasst.

11. Diagnostisches/Nachweis-Immunkonjugat nach Anspruch 9, wobei das diagnostische/Nachweis-Agens ein Radionuklid mit einer Energie zwischen 20 und 2,000 keV ist; wobei das Radionuklid bevorzugterweise ein gamma-, beta- oder ein Positron-emittierendes Isotop ist; wobei das Radionuklid bevorzugtererweise ausgewählt ist aus der Gruppe bestehend aus 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, I-124, 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 und Tl-201.

12. Diagnostisches/Nachweis-Immunkonjugat nach Anspruch 9, wobei das diagnostische Agens ein Kontrastmittel ist.

13. Diagnostisches/Nachweis-Immunkonjugat nach Anspruch 12, wobei das Kontrastmittel ein paramagnetisches Ion ist; wobei das paramagnetische Ion bevorzugterweise ein Metall umfasst, das ausgewählt ist aus der Gruppe bestehend aus 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) und Erbium (III).

14. Diagnostisches/Nachweis-Immunkonjugat nach Anspruch 12, wobei das Kontrastmittel ein Ultraschall-verstärkendes Mittel ist, wobei das Ultraschall-verstärkende Mittel bevorzugterweise ein Liposom ist, das mit einem humanisierten Antikörper oder Fragment davon konjugiert ist, wobei das Liposome bevorzugtererweise gasgefüllt ist.

15. Diagnostisches/Nachweis-Immunkonjugat nach Anspruch 12, wobei das Kontrastmittel eine strahlendichte Verbindung ist; wobei bevorzugterweise die strahlendichte Verbindung ausgewählt ist aus der Gruppe bestehend aus Jodverbindungen, Bariumverbindungen, Galliumverbindungen und Thalliumverbindungen.

16. Diagnostisches/Nachweis-Immunkonjugat nach den Ansprüchen 9-11, wobei das Immunkonjugat zum/zur intraoperativen, endoskopischen oder intravaskulären Tumor-Nachweis/Diagnose verwendet werden soll.

17. Therapeutisches Immunkonjugat nach Anspruch 9, wobei das therapeutische Agens ausgewählt ist aus der Gruppe

bestehend aus einem Radionuklid, Bor-, Gadolinium-, oder Uranatomen, einem Immunmodulator, einem Zytokin, einem Hormon, einem Hormonantagonisten, einem Enzym, einem Enzyminhibitor, einem photoaktiven therapeutischen Agens, einem zytotoxischen Wirkstoff, einem Toxin, einem Angiogeneseinhibitor, einem anderen Antikörper und einer Kombination davon.

18. Therapeutisches Immunkonjugat nach Anspruch 17, wobei das zytotoxische Agens ein Wirkstoff oder ein Toxin ist; wobei bevorzugterweise der Wirkstoff ausgewählt ist aus der Gruppe bestehend aus antimitotischen, alkylierenden, antimetabolischen, angiogeneseinhibierenden, apoptotischen, alkaloiden, COX-2-inhibierenden und antibiotischen Agenzien und Kombinationen davon; oder der Wirkstoff ausgewählt ist aus der Gruppe bestehend aus Stickstoffsenfgasen, Ethyleniminderivaten, Alkylsulfonaten, Nitrosoharnstoffen, Triazenen, Folsäureanalogen, Anthrazyklinen, Taxanen, COX-2-Inhibitoren, Pyrimidinanalogen, Purinanalogen, Antibiotika, Enzymen, Epipodophyllotoxinen, Platinkoordinationskomplexen, Vincaalkaloiden, substituierten Harnstoffen, Methylhydrazinderivaten, adrenocorticalen Suppressoren, Hormonantagonisten, Enzyminhibitoren, Endostatin, Taxolen, Camptothecinen, Doxorubicinen und deren Analogen, und einer Kombination davon oder wobei das Toxin ausgewählt ist aus der Gruppe bestehend aus pflanzlichen, mikrobiellen und tierischen Toxinen; wobei bevorzugterweise das Toxin ausgewählt ist aus der Gruppe bestehend aus Ricin, Abrin, Alphatoxin, Saporin, Ribonuklease (RNase), DNase I, *Staphylokokken*-Enterotoxin-A, antivirales Pokeweed-Protein, Gelonin, Diphtherietoxin, *Pseudomonas*-Exotoxin, und *Pseudomonas*-Endotoxin.

19. Therapeutisches Immunkonjugat nach Anspruch 17, wobei der Immunmodulator ausgewählt ist aus der Gruppe bestehend aus einem Zytokin, einem Stammzellwachstumsfaktor, einem Lymphotoxin, einem hämatopoietischen Faktor, einem Kolonie-stimulierenden Faktor (CSF), einem Interferon (IFN), einem Stammzellwachstumsfaktor, Erythropoietin, Thrombopoietin, einem Antikörper und einer Kombination davon; wobei bevorzugterweise das Lymphotoxin Tumor-Nekrose-Faktor (TNF) ist, der hämatopoietische Faktor ein Interleukin (IL) ist, der Kolonie-stimulierenden 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 der als "S 1 Faktor" bezeichnete ist; wobei bevorzugterweise das Zytokin ausgewählt ist aus der Gruppe bestehend aus IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, Interferon-γ, TNF-α und einer Kombination davon.

20. Therapeutisches Immunkonjugat nach Anspruch 17, wobei der Immunmodulator ein Antikörper ist, der ein Agonist oder Antagonist eines Immunfaktors ist; wobei bevorzugterweise der Antikörper gegen CD40 ist.

21. Therapeutisches Immunkonjugat nach Anspruch 17, wobei das Radionuklid ausgewählt ist aus der Gruppe bestehend aus einem Auger-Emitter, einem beta-Emitter und einem alpha-Emitter.

22. Therapeutisches Immunkonjugat nach Anspruch 17, wobei das Radionuklid ausgewählt ist aus der Gruppe bestehend aus P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, und Bi-213, Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, I-125, Ho-161, Os-189m, Ir-192, Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213, Fm-255 und Kombinationen davon.

23. Therapeutisches Immunkonjugat nach Anspruch 17, wobei das Boronatom B-10 ist; und/oder das Gadoliniumatom Gd-157 ist; und/oder das Uranatom U-235 ist.

24. Therapeutisches Immunkonjugat nach Anspruch 21, wobei das Radionuklid eine Energie zwischen 20 und 10.000 keV aufweist.

25. Therapeutisches Immunkonjugat nach Anspruch 21, wobei das Radionuklid ein Auger-Emitter ist und eine Energie von weniger als 1000 keV aufweist, oder wobei das Radionuklid ein beta-Emitter ist und eine Energie zwischen 20 und 5000 keV aufweist, oder wobei das Radionuklid ein alpha-Emitter ist und eine Energie zwischen 2000 und 10.000 keV aufweist.

26. Therapeutisches Immunkonjugat nach Anspruch 17, wobei das photoaktive therapeutische Agens ein Chromogen oder ein Farbstoff ist.

27. Diagnostisches oder therapeutisches Immunkonjugat nach Anspruch 9, wobei das diagnostische oder therapeutische Agens an den MAb oder Fragment davon mittels eines Kohlenhydrat-Teils gebunden ist.

28. Multivalenter, multi-spezifischer Antikörper oder Antigen-bindendes Fragment davon umfassend einen oder mehrere humanisierte(n) monoklonale(n) Antikörper oder Fragment davon nach Anspruch 1 und eine oder mehrere Haptenbindende Stellen mit Affinität zu.

29. Antikörper oder Fragment davon nach Anspruch 28, wobei der Antikörper oder Fragment davon humanisiert ist, oder wobei der Antikörper oder Fragment davon chimärisiert ist, oder wobei der Antikörper oder Fragment davon ein humaner Antikörper ist.

30. Antikörper oder Fragment davon nach Anspruch 28 oder 29, weiter umfassend ein diagnostisches oder therapeutisches Agens.

31. Antikörperfusionsprotein oder ein Antigen-bindendes Fragment davon umfassend wenigstens zwei anti-CSAp-MAbs oder Fragmente davon, wobei die MAbs oder Fragmente davon ausgewählt sind von dem MAb oder Fragment davon nach einem der Ansprüche 1-30.

32. Antikörperfusionsprotein oder ein Antigen-bindendes Fragment davon umfassend wenigstens einen ersten anti-CSAp-MAb oder Fragment davon nach einem der Ansprüche 1-30 und wenigstens einen zweiten MAb oder Fragment davon, der verschieden ist von dem MAb oder Fragment davon nach einem der Ansprüche 1-30.

33. Antikörperfusionsprotein oder Fragment davon nach Anspruch 32, wobei der zweite MAb oder Fragment davon ein CD40-Antikörper ist.

34. Antikörperfusionsprotein oder Fragment davon nach Anspruch 31 oder 32, weiter umfassend ein diagnostisches oder therapeutisches Agens, das mit dem Fusionsprotein oder Fragment davon konjugiert ist.

35. Antikörperfusionsprotein oder Fragment davon nach Anspruch 32, wobei der zweite MAb ein Karzinom-assoziierter Antikörper ist; bevorzugterweise wobei der Karzinom-assoziierter Antikörper ausgewählt ist aus der Gruppe bestehend aus CEA, EGP-1, EGP-2, MUC1, MUC2, MUC3, MUC4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, BrE3, Le-Y, A3, KS-1, VEGF, anderen Angiogeneseantikörpern, Anti-Nekroseantikörpern und Onkogenproduktantikörpern; oder bevorzugterweise der Karzinom-assoziierter Antikörper an ein Antigen auf einem Karzinom bindet, das ausgewählt ist aus der Gruppe von gastrointestinalen Karzinomen und Karzinomen der Eierstöcke.

36. Therapeutisch wirksame Menge eines Antikörpers oder Fragments nach einem der Ansprüche 1-8 und 28 bis 29, formuliert in einem pharmazeutisch akzeptablen Vehikel zur Verwendung in einem Verfahren zur Behandlung einer Malignität in einem Lebewesen, umfassend den Schritt Verabreichen der Formulierung an das Lebewesen.

37. Therapeutisch wirksame Menge eines Immunkonjugats oder eines Antigen-bindenden Fragments davon nach einem der Ansprüche 9, 17-21 und 30, formuliert in einem pharmazeutisch akzeptablen Vehikel zur Verwendung in einem Verfahren zur Behandlung einer Malignität in einem Lebewesen, umfassend den Schritt Verabreichen der Formulierung an das Lebewesen.

38. Diagnostisch wirksame Menge eines Antikörpers oder eines Antigen-bindenden Fragments davon nach einem der Ansprüche 1-8 und 28-29, formuliert in einem pharmazeutisch akzeptablen Vehikel zur Verwendung in einem Verfahren zum Diagnostizieren/Nachweisen einer Malignität in einem Lebewesen, umfassend den Schritt Verabreichen der Formulierung an das Lebewesen.

39. Diagnostisch wirksame Menge eines Immunkonjugats oder Fragments davon nach einem der Ansprüche 9-16, und 30, formuliert in einem pharmazeutisch akzeptablen Vehikel zur Verwendung in einem Verfahren zum Diagnostizieren/Behandeln einer Malignität in einem Lebewesen, umfassend den Schritt Verabreichen der Formulierung an das Lebewesen.

40. Therapeutisch oder diagnostisch wirksame Menge eines Fusionsproteins oder Fragments davon nach einem der Ansprüche 31-35, formuliert in einem pharmazeutisch akzeptablen Vehikel zur Verwendung in einem Verfahren zur Behandlung oder zum Diagnostizieren/Nachweisen einer Malignität in einem Lebewesen, umfassend den Schritt Verabreichen der Formulierung an das Lebewesen.

41. Antikörper oder Fragment davon nach einem der Ansprüche 28-29 zur Verwendung in einem Verfahren zur Behandlung oder zum Diagnostizieren/Nachweisen einer Malignität in einem Lebewesen, umfassend (i) Verabreichen

des Antikörpers oder von Fragmenten davon nach einem der Ansprüche 28-29 an ein Lebewesen, das dessen bedarf; (ii) Warten für eine ausreichenden Zeitspanne bis eine Menge des nicht-bindenden Proteins den Blutstrom des Lebewesens geklärt hat; und (iii) Verabreichen eines Trägermoleküls, das an eine Bindungsstelle des Antikörpers bindet, an das Lebewesen, wobei das Trägermolekül ein diagnostisches Agens, ein therapeutisches Agens oder eine Kombination davon umfasst.

42. DNA-Sequenz umfassend eine Nukleinsäure, die für einen anti-CSAp-MAb oder Fragment davon kodiert, der ausgewählt ist aus der Gruppe bestehend aus

(a) einem anti-CSAp-MAb oder Fragment davon nach einem der Ansprüche 1-30;
(b) einem Antikörperfusionsprotein oder Fragment davon umfassend wenigstens zwei der MAbs oder Fragmente davon nach einem der Ansprüche 1 bis 30;
(c) einem Antikörperfusionsprotein oder Fragment davon umfassend wenigstens einen ersten anti-CSAp-MAb oder Fragment davon umfassend den MAb oder Fragment davon nach einem der Ansprüche 1-30 und wenigstens einen zweiten MAb oder Fragment davon, der verschieden ist von dem MAb oder Fragment davon nach einem der Ansprüche 1-30; und
(d) einem Antikörperfusionsprotein oder Fragment davon umfassend wenigstens einen ersten MAb oder Fragment davon umfassend den MAb oder Fragment davon nach einem der Ansprüche 1-30 und wenigstens einen zweiten MAb oder Fragment davon, der verschieden ist von dem MAb oder Fragment davon nach einem der Ansprüche 1-30, wobei der zweite MAb ausgewählt ist aus der Gruppe bestehend aus CEA, EGP-1, EGP-2, MUC-1, MUC-2, MUC-3, MUC-4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, BrE3, Le-Y, A3, KS-1, CD40, und VEGF-Antikörper und einer Kombination davon.

43. Expressionsvektor umfassend die DNA-Sequenz nach Anspruch 42.

44. Wirtszelle umfassend die DNA-Sequenz nach Anspruch 42.

45. Immunkonjugat nach Anspruch 9 zur Verwendung in einem Verfahren zum Abgeben eines diagnostischen/Nachweis- oder therapeutischen Agens, oder einer Kombination davon, an ein Ziel umfassend (i) Bereitstellen einer Zusammensetzung, die das Immunkonjugat umfasst, und (ii) Verabreichen der Zusammensetzung an ein Lebewesen, das dessen bedarf.

46. Immunkonjugat zur Verwendung nach Anspruch 45, wobei das diagnostische/Nachweis-Agens wenigstens ein photoaktives diagnostisches Agens umfasst; bevorzugterweise wobei das photoaktive diagnostische Agens ein Chromogen oder einen Farbstoff umfasst.

47. Immunkonjugat zur Verwendung nach Anspruch 45, wobei das diagnostische Agens ein Radionuklid mit einer Energie zwischen 20 und 2.000 keV ist; bevorzugterweise wobei das Radionuklid ein gamma-, beta- oder ein Positron-emittierendes Isotop ist; bevorzugterweise wobei das Radionuklid ausgewählt ist aus der Gruppe bestehend aus 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, I-124, Cr-51, Co-57, Co-58, Fe-59, Cu-67, Ga-67, Se-75, Ru-97, Tc-99m, In-111, In-114m, 1-123, 1-125, I-131, Yb-169, Hg-197 und T1-201.

48. Immunkonjugat zur Verwendung nach Anspruch 45, wobei das diagnostische Agens ein Kontrastmittel ist.

49. Immunkonjugat zur Verwendung nach Anspruch 48, wobei das Kontrastmittel ein paramagnetisches Ion ist; wobei das paramagnetische Ion bevorzugterweise ein Metall umfassend Mangan, Eisen oder Gadolinium ist.

50. Immunkonjugat zur Verwendung nach Anspruch 48, wobei das Kontrastmittel ein Ultraschall-verstärkendes Mittel ist; bevorzugterweise wobei das Ultraschall-verstärkende Mittel ein Liposom ist, das den humanisierten Antikörper oder Fragment davon umfasst; bevorzugtererweise wobei das Liposom gasgefüllt ist.

51. Immunkonjugat zur Verwendung nach Anspruch 48, wobei das Kontrastmittel eine in Röntgenstrahlung oder Computertomographie verwendete strahlendichte Verbindung, ist; bevorzugterweise wobei die strahlendichte Verbindung ausgewählt ist aus der Gruppe bestehend aus Jodverbindungen, Bariumverbindungen, Galliumverbindungen und Thalliumverbindungen; bevorzugtererweise wobei die strahlendichte Verbindung ausgewählt ist aus der Gruppe bestehend aus Barium, Diatrizoat, ethyliodisiertes Öl, Galliumzitrat, Iocarminsäure, Iocetaminsäure, Iodamid, Iodipamid, Iodoxaminsäure, Iogulamid, Iohexol, Iopamidol, Iopanoinsäure, Ioproceminsäure, Iosefaminsäure, Ioserin-

säure, Iosulamide Meglumine, Iosemetinsäure, Iotasul, Iotetrinsäure, Iothalaminsäure, Iotroxinsäure, Ioxaglinsäure, Ioxotrizoinsäure, Ipodat, Meglumin, Metrizamid, Metrizoat, Propyliodon und Thallium(I)-chlorid.

52. Immunkonjugat zur Verwendung nach Anspruch 45, wobei das therapeutische Agens ausgewählt ist aus der Gruppe bestehend aus einem Radionuklid, einem Immunmodulator, einem Hormon, einem Hormonantagonist, einem Enzym, einem Enzyminhibitor, einem photoaktiven therapeutischen Agens, einem zytotoxischen Wirkstoff und einer Kombination davon; bevorzugterweise wobei das photoaktive therapeutischen Agens ein Chromogen oder Farbstoff ist.

53. Immunkonjugat zur Verwendung nach Anspruch 52, wobei das zytotoxische Agens ein Wirkstoff oder ein Toxin ist; bevorzugterweise wobei der Wirkstoff ausgewählt ist aus der Gruppe bestehend aus antimitotischen, alkylierenden, antimetabolischen, antiangiogenetischen, apoptotischen, anthrazyklinen, alkaloiden, COX-2-inhibierenden und antibiotischen Agenzien und Kombinationen davon; oder der Wirkstoff ausgewählt ist aus der Gruppe bestehend aus Stickstoffsenfgasen, Ethyleniminderivaten, Alkylsulfonaten, Nitrosoharnstoffen, Triazenen, Folsäureanalogen, Anthrazyklinen, Taxanen, COX-2-Inhibitoren, Pyrimidinanalogen, Purinanalogen, Antibiotika, Enzymen, Enzyminhibitoren, Epipodophyllotoxinen, Platinkoordinationskomplexen, Vincaalkaloiden, substituierten Harnstoffen, Methylhydrazinderivaten, adrenocorticalen Suppressoren, Hormonen, Hormonantagonisten, Endostatin, Taxolen, Camptothecinen, Doxorubicinen und deren Analogen, und einer Kombination davon; oder das Toxin ausgewählt ist aus der Gruppe bestehend aus pflanzlichem, mikrobiellem und tierischem Toxin; bevorzugterweise wobei das Toxin ausgewählt ist aus der Gruppe bestehend Ricin, Abrin, Alphatoxin, Saporin, Ribonuklease (RNase), DNase I, *Staphylokokken*-Enterotoxin-A, antivirales Pokeweed-Protein, Gelonin, Diphtherietoxin, *Pseudomonas-Exotoxin,* und *Pseudomonas-Endotoxin.*

54. Immunkonjugat zur Verwendung nach Anspruch 52, wobei der Immunmodulator ausgewählt ist aus der Gruppe bestehend aus einem Zytokin, einem Stammzellwachstumsfaktor, einem Lymphotoxin, einem hämatopoietischen Faktor, einem Kolonie-stimulierenden Faktor (CSF), einem Interferon (IFN), einem Stammzellwachstumsfaktor, Erythropoietin, Thrombopoietin, einem Antikörper und einer Kombination davon; bevorzugterweise wobei der Antikörper ein anti-CD40 Antikörper oder Fragment davon ist, bevorzugterweise wobei das Lymphotoxin Tumor-Nekrose-Faktor (TNF) ist, der hämatopoietische Faktor ein Interleukin (IL) ist, der Kolonie-stimulierenden 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 der als "S1 Faktor" bezeichnete ist; bevorzugterweise wobei das Zytokin ausgewählt ist aus der Gruppe bestehend aus IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, Interferon -γ, TNF-α und einer Kombination davon.

55. Immunkonjugat zur Verwendung nach Anspruch 52, wobei das Radionuklid ausgewählt ist aus der Gruppe bestehend einem Auger-Emitter, einem β-Emitter und einem α-Emitter; wobei bevorzugterweise das Radionuklid ein Auger-Emitter ist und eine Energie von weniger als 1000 keV aufweist; oder das Radionuklid ein β-Emitter ist und eine Energie zwischen 20 und 5000 keV aufweist; oder das Radionuklid ein α-Emitter ist und eine Energie zwischen 2000 und 10.000 keV aufweist.

56. Immunkonjugat zur Verwendung nach Anspruch 52, wobei das Radionuklid ausgewählt ist aus der Gruppe bestehend aus P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, und Bi-213, Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109 In-111, Sb-119, I-125, Ho-161, Os-189m, Ir-192, Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213, Fm-255 und Kombinationen davon.

57. Immunkonjugat zur Verwendung nach Anspruch 52, wobei das Radionuklid eine Energie zwischen 20 und 10.000 keV aufweist.

58. Antikörper oder Fragment davon nach Anspruch 28 oder 29 zur Verwendung in einem Verfahren zum Abgeben eines diagnostischen/Nachweis-Agens, eines therapeutischen Agens, oder einer Kombination davon, an ein Ziel umfassend: (i) Verabreichen des Antikörpers oder der Fragmente an ein Lebewesen; (ii) Warten für eine ausreichenden Zeitspanne bis eine Menge des nicht-bindenden Proteins den Blutstrom des Lebewesens geklärt hat; und (iii) Verabreichen eines Trägermoleküls, das an eine Bindungsstelle des Antikörpers bindet, an das Lebewesen, wobei das Trägermolekül ein diagnostisches/Nachweis-Agens, ein therapeutisches Agens oder eine Kombination davon umfasst.

59. Antikörper oder Fragment zur Verwendung nach Anspruch 58, wobei das Trägermolekül an mehr als eine Bindungsstelle des bindenden Proteins bindet.

60. Antikörper oder Fragment zur Verwendung nach Anspruch 58, wobei das diagnostische/Nachweis-Agens oder das therapeutische Agens ausgewählt ist aus der Gruppe umfassend Isotope, Farbstoffe, Chromogene, Kontrastmittel, Wirkstoffe, Toxine, Zytokine, Enzyme, Enzyminhibitoren, Hormone, Hormonantagonisten, Wachstumsfaktoren, Radionuklide und Metalle.

61. Therapeutisch wirksame Menge eines Antikörpers oder Fragments davon oder eines Antikörperfusionsproteins oder Fragments davon umfassend wenigstens zwei MAbs oder Fragmente davon zur Verwendung in einem Verfahren zur Behandlung einer Malignität in einem Lebewesen, umfassend Verabreichen der Menge an das Lebewesen, wobei wenigstens ein anti-CSAp-MAb oder Fragment davon oder Fusionsproteine oder Fragmente davon (ein) solcher/solches nach Ansprüchen 1-35 sind, formuliert in einem pharmazeutisch geeigneten Bindemittel.

62. Therapeutisch wirksame Menge eines Antikörpers oder Fragments davon umfassend wenigstens zwei MAbs oder Fragmente davon zur Verwendung in einem Verfahren zur Behandlung einer Malignität in einem Lebewesen, umfassend Verabreichen der Menge an das Lebewesen, wobei die MAbs ausgewählt sind aus einem jeden gemäß den Ansprüchen 1-35, und formuliert sind in einem pharmazeutisch geeigneten Bindemittel.

63. Zusammensetzung zur Verwendung nach Anspruch 62, weiter umfassend einen zweiten MAb oder Fragment davon, der kein MAb oder Fragment davon nach einem der Ansprüche 1-35 ist; bevorzugterweise wobei der zweite MAb oder Fragment davon ein nackter MAb oder Fragment davon ist, oder der zweite MAb oder Fragment davon ausgewählt ist aus der Gruppe bestehend aus Antikörpern gegen BrE3, Le-Y, EGP-1, EGP-2, MUC-1, MUC-2, MUC-3, MUC-4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, A3, KS-1, CEA, VEGF, Onkogenprodukte und einem Antikörper gegen CD40 oder einem weiteren Immunmodulator; bevorzugtererweise wobei der zweite MAb immunkonjugiert ist an ein therapeutisches oder diagnostisches/Nachweis-Agens.

64. Zusammensetzung zur Verwendung nach Anspruch 61, wobei der anti-CSAp-Antikörper parenteral verabreicht wird; bevorzugterweise wobei der anti-CSAp-Antikörper in einer Dosierung von 20 bis 2000 Milligramm Protein pro Dosis verabreicht wird; bevorzugtererweise wobei die Dosierung wiederholt verabreicht wird.

65. Zusammensetzung zur Verwendung nach Anspruch 61, wobei die konstanten Regionen und Gelenksregionen des humanisierten anti-CSAp-Antikörpers konstante Regionen und Gelenksregionen eines humanen IgG1 umfassen.

66. Zusammensetzung zur Verwendung nach Anspruch 61, wobei der anti-CSAp-Antikörper oder Fragment davon verabreicht wird vor, zusammen mit oder nach Verabreichen eines zweiten konjugierten Antikörpers an das Lebewesen, wobei der zweite konjugierte Antikörper reaktiv ist mit einem zweiten Tumormarker, der von der Malignität exprimiert wird; oder wobei der anti-CSAp-Antikörper oder Fragment davon verabreicht wird vor, gleichzeitig oder nach wenigstens einem therapeutischen oder diagnostischen/Nachweis-Agens, bevorzugterweise wobei das therapeutische oder diagnostische/Nachweis-Agens konjugiert ist an einen Antikörper, der einen Tumormarker targetiert, der von der Malignität exprimiert wird.

67. Zusammensetzung zur Verwendung nach Anspruch 61, wobei eine erste Bindungsstelle des anti-CSAp-Antikörpers oder Fragments davon in einem multivalenten, multi-spezifischen Fusionsprotein oder chemischen Konjugat vorhanden ist und eine zweite Bindungsstelle mit einer Tumormarkersubstanz, die verschieden ist von CSAp, reaktiv ist.

68. Diagnostisch wirksame Menge eines diagnostischen/Nachweis-Konjugats umfassend einen anti-CSAp-MAb oder Fragment davon oder ein Fusionsprotein oder Fragment davon nach einem der Ansprüche 1-35, wobei der anti-CSAp-MAb oder Fragment davon oder Fusionsprotein oder Fragment davon an wenigstens ein diagnostisches/Nachweis-Agens gebunden ist, formuliert in einem pharmazeutisch geeigneten Bindemittel, zur Verwendung in einem Verfahren zur Diagnose oder zum Nachweisen einer Malignität in einem Lebewesen.

69. Therapeutisch wirksame Menge einer Zusammensetzung umfassend einen nackten anti-CSAp-MAb oder Fragment davon oder ein nacktes Antikörperfusionsprotein oder Fragment davon nach einem der Ansprüche 1-35 zur Verwendung in einem Verfahren zur Behandlung einer Krebszelle in einem Lebewesen, umfassend (i) Verabreichen der Menge an das Lebewesen, (ii) Formulieren des nackten CSAp-MAb oder Fragments davon oder Antikörperfusionsproteins oder Fragments davon in einem pharmazeutisch geeigneten Bindemittel.

70. Zusammensetzung zur Verwendung nach Anspruch 69, wobei die Zusammensetzung weiter umfasst einen zweiten nackten Antikörper oder Fragment davon, der/das verschieden ist von dem nach einem der Ansprüche 1-9, 28-29 und 31-35, bevorzugterweise wobei der zweite Antikörper oder Fragment davon ausgewählt ist aus der Gruppe bestehend aus Antikörpern gegen CEA, EGP-1, EGP-2, MUC-1, MUC-2, MUC-3, MUC-4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, BrE3, Le-Y, A3, KS-1, CD40, VEGF und anderen Angiogenesefaktoren, und Onkogenprodukten; oder wobei die Zusammensetzung weiter einen zweiten nackten Antikörper oder Fragment davon nach einem der Ansprüche 1-9, 28-29 und 31-35 umfasst; oder wobei die Zusammensetzung weiter einen zweiten Antikörper oder Fragment davon umfasst, der verschieden ist von einem Antikörper oder Fragment davon nach einem der Ansprüche 1-35.

71. Zusammensetzung zur Verwendung nach Anspruch 69, wobei der nackte anti-CSA-Antikörper parenteral verabreicht wird; bevorzugterweise wobei der nackte anti-CSAp-Antikörper in einer Dosierung von 20 bis 2000 Milligramm Protein pro Dosis verabreicht wird; bevorzugtererweise wobei die Dosis wiederholt verabreicht wird.

72. Zusammensetzung zur Verwendung nach Anspruch 69, wobei die konstanten Regionen und Gelenksregionen des humanisierten nackten anti-CSAp-Antikörpers konstante Regionen und Gelenksregionen eines humanen IgG1 umfassen.

73. Zusammensetzung zur Verwendung nach Anspruch 69, wobei der nackte anti-CSAp-Antikörper verabreicht wird vor, zusammen mit oder nach Verabreichen eines zweiten nackten Antikörpers an das Lebewesen, wobei der zweite nackte Antikörper reaktiv ist mit einem zweiten Tumormarker, der von der Malignität exprimiert wird; oder wobei der nackte anti-CSAp-Antikörper vor, gleichzeitig oder nach wenigstens einem therapeutischen oder diagnostischen/ Nachweis-Agens verabreicht wird.

74. Zusammensetzung zur Verwendung nach den Ansprüchen 69 bis 73 wobei der nackte anti-CSAp-Antikörper der nackte Antikörper ist, der an Colon-spezifisches Antigen-p-Mucin (CSAp) bindet.

75. Verfahren zum Diagnostizieren oder Nachweisen einer Malignität in einem Lebewesen umfassend Durchführen eines in vitro Diagnoseassays an einer Probe von dem Lebewesen mit einer Zusammensetzung umfassend einen nackten anti-CSAp-MAb oder Fragment davon oder ein nacktes Antikörperfusionsprotein oder Fragment davon nach einem der Ansprüche 1-35.

76. Verfahren nach Anspruch 75, wobei die Malignität ein Karzinom ist; bevorzugterweise wobei (i) das Karzinom ein gastrointestinales Karzinom ist, bevorzugtererweise wobei das Karzinom ein kolorektales oder Pankreas-Karzinom ist; oder (ii) wobei das Karzinom ein Eierstock-Karzinom ist.

77. Verfahren nach Anspruch 75, wobei der in vitro Diagnoseassay ausgewählt ist aus der Gruppe bestehend aus Immunoassays, RT-PCR und Immunhistochemie; bevorzugterweise wobei (i) der Diagnoseassay RT-PCR oder Immunoassays ist; bevorzugtererweise wobei die Probe Körperflüssigkeit oder ein Gewebe oder eine Zellpopulation ist; oder (ii) wobei der diagnostische Assay Immunhistochemie oder Immunzytochemie ist.

78. Verfahren nach Anspruch 77, wobei die Probe ein Zellaliquot oder ein Gewebe ist.

79. Verfahren nach einem der Ansprüche 35-41 und 45-78, wobei das Lebewesen ein Säugetier ist; bevorzugterweise wobei das Lebewesen ein Mensch; ein Haustier ist; oder wobei das Lebewesen ausgewählt ist aus der Gruppe bestehend aus einem Pferd, einem Hund und einer Katze.

80. Bi-spezifischer Antikörper oder Antikörperfragment mit wenigstens einem Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einen anderen Arm, der ein targetierbares Konjugat spezifisch bindet, wobei der eine Arm, der ein targetiertes Gewebe spezifisch bindet, der Antikörper nach einem der Ansprüche 1 bis 8 ist, zur Verwendung in einem Verfahren zum Behandeln oder Identifizieren von erkranktem Gewebe in einem Lebewesen, umfassend:

(a) Verabreichen des Antikörpers oder Antikörperfragments an das Lebewesen;
(b) optional, Verabreichen an das Lebewesen einer klärenden Zusammensetzung und Erlauben, dass die Zusammensetzung nicht-lokalisierte Antikörper oder Antikörperfragmente aus dem Kreislauf klärt;
(c) Verabreichen eines ersten targetierbaren Konjugates an das Lebewesen, das einen Trägerteil umfasst, der wenigstens ein Epitop, das durch den wenigstens einen anderen Arm des bi-spezifischen Antikörpers oder

Antikörperfragments erkennbar ist, umfasst oder trägt, und ein oder mehrere konjugierte therapeutische oder diagnostische Agenzien; und
(d) wenn das therapeutische Agens ein Enzym ist, weiter Verabreichen an das Lebewesen

(1) eines Wirkstoff-Vorläufers, wenn das Enzyme in der Lage ist, den Wirkstoff-Vorläufer an der Zielstelle in einen Wirkstoff umzuwandeln; oder
(2) eines Wirkstoffes, der in der Lage ist, in dem Lebewesen entgiftet zu werden, um ein Zwischenprodukt geringerer Toxizität zu bilden, wenn das Enzym in der Lage ist, das entgiftete Zwischenprodukt in eine toxische Form rückzuwandeln, und, deshalb, die Toxizität des Wirkstoffes an der Zielstelle zu erhöhen, oder
(3) eines Wirkstoff-Vorläufers, der in dem Lebewesen durch natürliche Prozesse aktiviert wird und durch Umwandlung in ein Zwischenprodukt geringerer Toxizität einer Entgiftung unterworfen wird, wenn das Enzym in der Lage ist, das entgiftete Zwischenprodukt in eine toxische Form rückzuwandeln, und, deshalb, die Toxizität des Wirkstoffes an der Zielstelle zu erhöhen, oder
(4) eines zweiten targetierbaren Konjugates, das einen Trägerteil umfasst, der wenigstens ein Epitop umfasst oder trägt, das durch den wenigstens einen anderen Arm des bi-spezifischen Antikörpers oder Antikörperfragments erkennbar ist, und eines Wirkstoff-Vorläufers, wenn das Enzym in der Lage ist, den Wirkstoff-Vorläufer an der Zielstelle in einen Wirkstoff umzuwandeln.

81. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 80, wobei das targetierbare Konjugat wenigstens zwei HSG-Haptene umfasst.

82. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 80, weiter umfassend, wenn das erste targetierbare Konjugat einen Wirkstoff-Vorläufer umfasst, Verabreichen eines zweiten targetierbaren Konjugates, das einen Trägerteil umfasst, der wenigstens ein Epitop umfasst oder trägt, das durch den wenigstens einen anderen Arm des bi-spezifischen Antikörpers oder Antikörperfragments erkennbar ist, und eines Enzyms, das in der Lage ist den Wirkstoff-Vorläufer in einen Wirkstoff umzuwandeln oder ein entgiftetes Zwischenprodukt des Wirkstoffs in eine toxische Form rückzuwandeln.

83. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 80, wobei das diagnostische/Nachweis-Agens ein Radionuklid ist.

84. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 80, wobei das diagnostische/Nachweis-Agens ein Radionuklid mit einer Energie zwischen 20 und 2,000 keV ist.

85. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 83, wobei das diagnostische/Nachweis-Agens 25-600 keV Gammapartikel und/oder Positronen emittiert; oder wobei das Radionuklid ein gamma-, beta- oder ein Positron-emittierendes Isotop ist; bevorzugterweise wobei das Radionuklid ausgewählt ist aus der Gruppe bestehend aus 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, I-124, Cr-51, Co-57, Co-58, Fe-59, Cu-67, Ga-67, Se-75, Ru-97, Tc-99m, In-111, In-114m, 1-123, I-125, I-131, Yb-169, Hg-197 und TI-201.

86. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 80, wobei das diagnostische/Nachweis-Agens wenigstens ein wenigstens ein photoaktives diagnostisches Agens umfasst; bevorzugterweise wobei das diagnostische/Nachweis-Agens ein Chromogen oder einen Farbstoff umfasst.

87. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 80, wobei das diagnostische/Nachweis-Agens eine strahlendichte Verbindung ist; bevorzugterweise wobei die strahlendichte Verbindung ausgewählt ist aus der Gruppe bestehend aus Jodverbindungen, Bariumverbindungen, Galliumverbindungen und Thalliumverbindungen; bevorzugterweise wobei die strahlendichte Verbindung ausgewählt ist aus der Gruppe bestehend aus Barium, Diatrizoat, ethyliodisiertes Öl, Galliumzitrat, Iocarminsäure, Iocetaminsäure, Iodamid, Iodipamid, Iodoxaminsäure, Iogulamid, Iohexol, Iopamidol, Iopanoinsäure, Ioproceminsäure, Iosefaminsäure, Ioserinsäure, Iosulamide Meglumine, Iosemetinsäure, Iotasul, Iotetrinsäure, Iothalaminsäure, Iotroxinsäure, Ioxaglinsäure, Ioxotrizoinsäure, Ipodat, Meglumin, Metrizamid, Metrizoat, Propyliodon und Thalliumchlorid.

88. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 80, wobei Das diagnostische/Nachweis-Agens ein Kontrastmittel ist.

89. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 88, wobei das Kontrastmittel ein paramagne-

tisches Ion ist; bevorzugterweise wobei das paramagnetische Ion ein Metall umfasst, das ausgewählt ist aus der Gruppe bestehend aus Mangan, Eisen und Gadolinium.

90. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 88, wobei das Kontrastmittel ein Ultraschall-verstärkendes Mittel ist; bevorzugterweise das Ultraschall-verstärkende Mittel ein Liposom ist, das mit dem humanisierten Antikörper oder Fragment davon konjugiert ist; bevorzugterweise wobei das Liposom gasgefüllt ist.

91. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 80, wobei das therapeutische Agens ausgewählt ist aus der Gruppe bestehend aus einem Radionuklid, Bor-, Gadolinium- oder Uran-Atom, einem Immunmodulator, einem Hormon, einem Hormonantagonisten, einem Enzym, einem Enzyminhibitor, einem photoaktiven therapeutischen Agens, einem zytotoxischen Wirkstoff, einem Angiogeneseinhibitor und einer Kombination davon.

92. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 91, wobei der zytotoxische Wirkstoff ein Wirkstoff, ein Wirkstoff-Vorläufer oder ein Toxin ist; bevorzugterweise wobei der Wirkstoff-Vorläufer ausgewählt ist aus der Gruppe bestehend aus Epirubicinglucuronid, CPT-11, Etoposidglucuronid, Daunomicinglucuronid und Doxorubicinglucuronid; bevorzugterweise wobei das Toxin ausgewählt ist aus der Gruppe bestehend aus einem pflanzlichen Toxin, einem tierischen Toxin und einem mikrobiellen; oder wobei das Toxin ausgewählt ist aus der Gruppe bestehend aus Ricin, Abrin, Ribonuklease (RNase), DNase I, *Staphylokokken*-Enterotoxin-A, antivirales Pokeweed-Protein, Gelonin, Diphtherietoxin, *Pseudomonas-Exotoxin,* und *Pseudomonas*-Endotoxin; bevorzugterweise wobei der Wirkstoff ausgewählt ist aus der Gruppe bestehend aus antimitotischen, alkylierenden, antimetabolischen, angiogeneseinhibierenden, apoptotischen, alkaloiden, COX-2-inhibierenden und antibiotischen Agenzien und Kombinationen davon; oder wobei der Wirkstoff ausgewählt ist aus der Gruppe bestehend aus Stickstoffsenfgasen, Ethyleniminderivaten, Alkylsulfonaten, Nitrosoharnstoffen, Triazenen, Folsäureanalogen, Anthrazyklinen, Taxanen, COX-2-Inhibitoren, Pyrimidinanalogen, Purinanalogen, Antibiotika, Enzymen, Epipodophyllotoxinen, Platinkoordinationskomplexen, Vincaalkaloiden, substituierten Harnstoffen, Methylhydrazinderivaten, adrenocorticalen Suppressoren" Hormonen, Hormonantagonisten, Endostatin, Taxolen, Camptothecinen, Doxorubicinen und deren Analogen, und einer Kombination davon.

93. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 91, wobei der Immunmodulator ausgewählt ist aus der Gruppe bestehend aus einem Zytokin, einem Stammzellwachstumsfaktor, einem Lymphotoxin, einem hämatopoietischen Faktor, einem Kolonie-stimulierenden Faktor (CSF), einem Interferon (IFN), einem Stammzellwachstumsfaktor, Erythropoietin, Thrombopoietin, einem Antikörperagonist oder -antagonist eines Immunmodulators, und einer Kombination davon; bevorzugterweise wobei das Lymphotoxin Tumor-Nekrose-Faktor (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 der als "S 1 Faktor" bezeichnete ist.

94. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 93, wobei das Zytokin ausgewählt ist aus der Gruppe bestehend aus IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, Interferon-γ, TNF-α und einer Kombination davon.

95. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 91, wobei das Radionuklid ausgewählt ist aus der Gruppe bestehend aus einem Auger-Emitter, einem β-Emitter und einem α-Emitter; oder wobei das Radionuklid ausgewählt ist aus der Gruppe bestehend aus P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, und Bi-213, Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, 1-125, Ho-161, Os-189m, Ir-192, Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213, Fm-255 und Kombinationen davon.

96. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 91, wobei das Boratom B-10 ist und/oder wobei das Gadoliniumatom Gd-157 ist und/oder wobei das Uranatom U-235 ist.

97. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 95, wobei das Radionuklid eine Energie zwischen 20 und 10.000 keV aufweist; oder wobei das Radionuklid ein Auger-Emitter ist und eine Energie von weniger als 1000 keV aufweist; oder wobei das Radionuklid ein β-Emitter ist und eine Energie zwischen 20 und 5000 keV aufweist; oder wobei das Radionuklid ein α-Emitter ist und eine Energie zwischen 2000 und 10.000 keV aufweist.

98. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 80, wobei das targetierbare Konjugat ein oder mehrere radioaktive(s) Isotop(e) umfasst, das/die geeignet ist/sind, um erkranktes Gewebe abzutöten; bevorzug-

terweise wobei das targetierbare Konjugat 10B Atome umfasst, und das Verfahren weiter den Schritt umfasst Bestrahlen der Boratome, die an dem erkrankten Gewebe lokalisiert sind, wobei eine BNCT des erkrankten Gewebes bewirkt wird; oder wobei das targetierbare Konjugat ein oder mehrere Agenzien zur photodynamischen Therapie umfasst, bevorzugterweise wobei das Agens zur photodynamischen Therapie ein Photosensibilisator ist, bevorzugterweise wobei der Photosensibilisator ausgewählt ist aus der Gruppe bestehend aus Benzoporphyrin-Monosäure (BPD-MA), Zinn Etiopurpurin (SnET2), sulfoniertes Aluminum-Phthalcyanin (AlSPc) und Lutetium-Texaphyrin (Lutex).

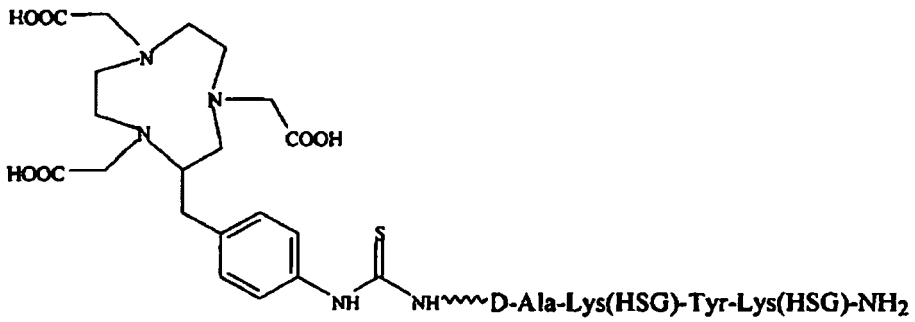
99. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 80, wobei das targetierte Gewebe ein Tumor ist; bevorzugterweise wobei der Tumor Colon-spezifisches Antigen-p (CSAp) produziert oder mit Colon-spezifischem Antigen-p (CSAp) assoziiert ist.

100. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 80, wobei der Antikörper oder Fragment davon, der an Colon-spezifisches Antigen-p-Mucin (CSAp) bindet, den Fv des MAb nach einem der Ansprüche 1 bis 8 umfasst.

101. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 80, wobei der bi-spezifische Antikörper ein Fusionsprotein ist; bevorzugterweise wobei das Fusionsprotein trivalent ist und den Fv eines Antikörpers enthält, der reaktiv ist mit CSAp.

102. Wirksame Menge eines bi-spezifischen Antikörpers oder Antikörperfragments umfassend wenigstens einen Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einen anderen Arm, der ein targetierbares Konjugat spezifisch bindet, wobei der eine Arm, der ein targetiertes Gewebe spezifisch bindet, ein Antikörper oder Fragment davon nach einem der Ansprüche 1 bis 8 ist, und eines targetierbaren Konjugates ausgewählt aus der Gruppe bestehend aus:

(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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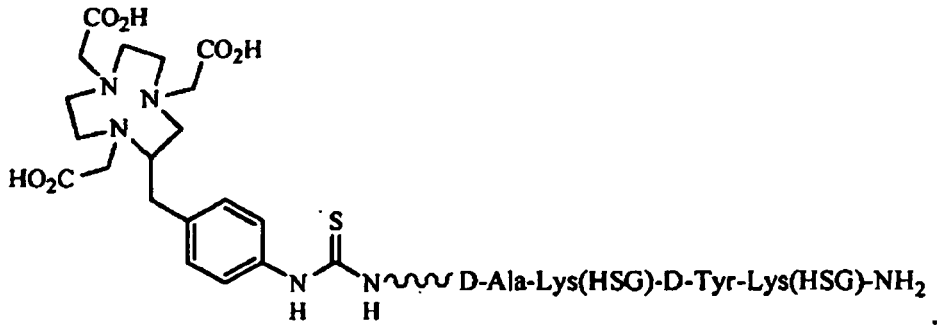


zur Verwendung in einem Verfahren zum Nachweisen oder Behandeln von Tumoren, die CSAp exprimieren in einem Säugetier, umfassend Verabreichen des Antikörpers oder Antikörperfragments und Verabreichen des Konjugats.

103. Substanzen zur Verwendung nach Anspruch 102, weiter umfassend Verabreichen einer klärende Zusammensetzung an das Lebewesen, und Erlauben, dass die Zusammensetzung nicht-lokalisierte Antikörper oder Antikörperfragmente aus dem Kreislauf klärt.

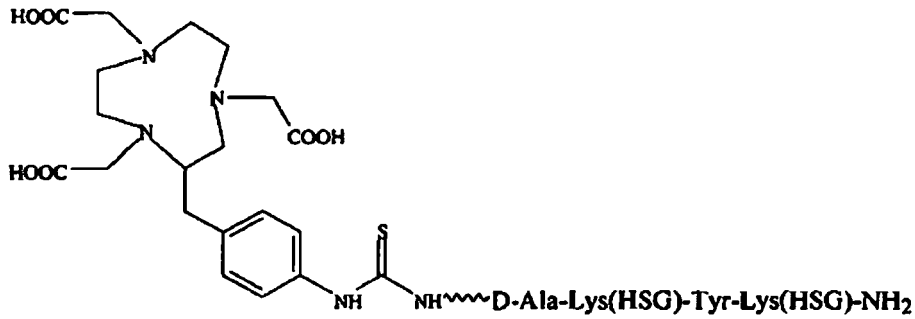
104. Kit geeignet zum Behandeln oder Identifizieren erkrankter Gewebe in einem Lebewesen umfassend:

(a) einen bi-spezifischen Antikörper oder Antikörperfragment mit wenigstens einem Arm, der spezifische ein targetiertes Gewebe bindet, und wenigstens einen anderen Arm, der spezifisch ein targetierbares Konjugat bindet, wobei der eine Arm, der spezifisch ein targetiertes Gewebe bindet, ein Antikörper oder Fragment davon nach einem der Ansprüche 1 bis 8 ist;
(b) ein erstes targetierbares Konjugat, das einen Trägerteil umfasst, der wenigstens ein Epitop umfasst oder trägt, das durch den wenigstens einen anderen Arm des bi-spezifischen Antikörpers oder Antikörperfragmentes erkennbar ist, und ein oder mehrere konjugierte therapeutische oder diagnostische Agenzien; und
(c) optional, eine klärende Zusammensetzung, die geeignet ist zum Klären nichtlokalisierter Antikörper und Antikörperfragmente; und
(d) optional, wenn das therapeutische Agens, das an das erste targetierbare Konjugat konjugiert, ist ein Enzym ist,

(1) einen Wirkstoff-Vorläufer, wenn das Enzym in der Lage ist, den Wirkstoff-Vorläufer an der Zielstelle in einen Wirkstoff umzuwandeln; oder
(2) einen Wirkstoff, der in der Lage ist, in dem Lebewesen entgiftet zu werden, um ein Zwischenprodukt geringerer Toxizität zu bilden, wenn das Enzym in der Lage ist, das entgiftete Zwischenprodukt in eine toxische Form rückzuwandeln und, deshalb, die Toxizität des Wirkstoffs an der Zielstelle zu erhöhen, oder
(3) einen Wirkstoff-Vorläufer, der in dem Lebewesen durch natürliche Prozesse aktiviert wird und durch Umwandlung in ein Zwischenprodukt geringerer Toxizität einer Entgiftung unterworfen wird, wenn das Enzym in der Lage ist, das entgiftete Zwischenprodukt in eine toxische Form rückzuwandeln, und, deshalb, die Toxizität des Wirkstoffes an der Zielstelle zu erhöhen, oder
(4) ein zweites targetierbares Konjugat, das einen Trägerteil umfasst, der wenigstens ein Epitop umfasst oder trägt, das durch den wenigstens einen anderen Arm des bi-spezifischen Antikörpers oder Antikörperfragments erkennbar ist, und einen Wirkstoff-Vorläufer, wenn das Enzym in der Lage ist, den Wirkstoff-Vorläufer an der Zielstelle in einen Wirkstoff umzuwandeln.

105. Kit nach Anspruch 104, wobei das targetierbare Konjugat ausgewählt ist aus der Gruppe bestehend aus:

(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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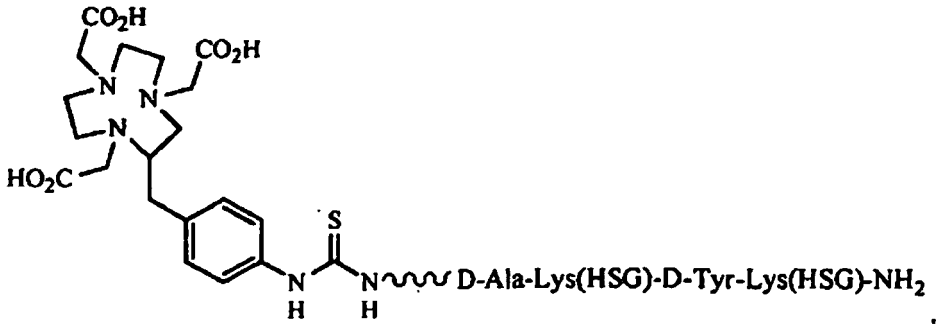

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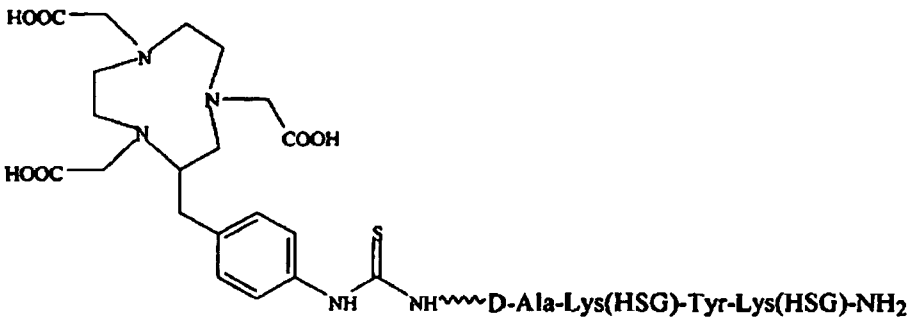
106. In vitro-Verfahren zum Screenen eines targetierbaren Konjugats umfassend:

(a) in Kontakt bringen des targetierbaren Konstrukts mit einem bi-spezifischen Antikörper oder Antikörperfragment mit wenigstens einem Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einen anderen Arm, der das targetierbares Konjugat spezifisch bindet, um eine Mischung zu ergeben, wobei der eine Arm, der spezifisch an ein targetiertes Gewebe bindet, ein Antikörper oder Fragment davon nach einem der Ansprüche 1 bis 8 ist; und
(b) optional, Inkubieren der Mischung; und
(c) Analysieren der Mischung.

107. Wirksame Menge eines bi-spezifischen Antikörpers oder Antikörperfragments umfassend wenigstens einen Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einen anderen Arm, der ein targetierbares Konjugat spezifisch bindet, wobei der eine Arm, der ein targetiertes Gewebe spezifisch bindet, ein Antikörper oder Fragment davon nach einem der Ansprüche 1 bis 8 ist; zur Verwendung in einem Verfahren zur bildlichen Darstellung von malignen Geweben oder Zellen in einem Säugetier, das/die CSAp exprimiert, umfassend:

(a) Verabreichen der wirksamen Menge des bi-spezifische Antikörpers oder Antikörperfragments und
(b) Verabreichen eines targetierbaren Konjugats, das ausgewählt ist aus der Gruppe bestehend aus

(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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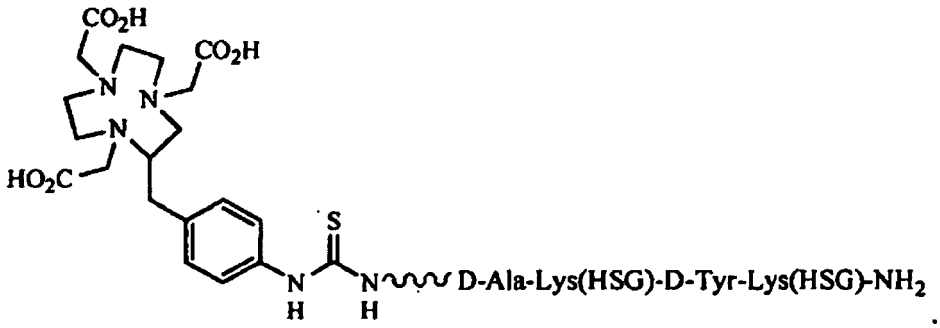

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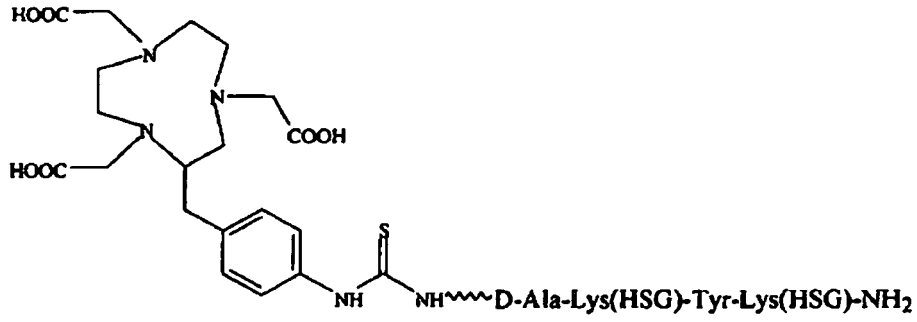
108. Wirksame Menge eines bi-spezifischen Antikörpers oder Antikörperfragments umfassend wenigstens einen Arm, der ein targetiertes Gewebe, das CSAp exprimiert, spezifisch bindet, und wenigstens einen anderen Arm, der ein targetierbares Konjugat spezifisch bindet, wobei der eine Arm, der ein targetiertes Gewebe spezifisch bindet, ein Antikörper oder Fragment davon nach einem der Ansprüche 1 bis 8 ist, zur Verwendung in einem Verfahren zum intraoperativen Identifizieren/Offenlegen erkrankter Gewebe, die CSAp exprimieren, in einem Lebewesen, umfassend:

(a) Verabreichen der wirksamen Menge des bi-spezifischen Antikörpers oder Antikörperfragments und
(b) Verabreichen eines targetierbaren Konjugats, das ausgewählt ist aus der Gruppe bestehend aus

(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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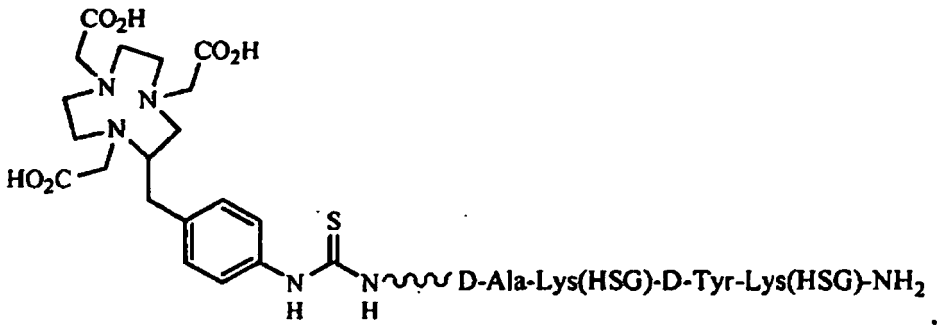
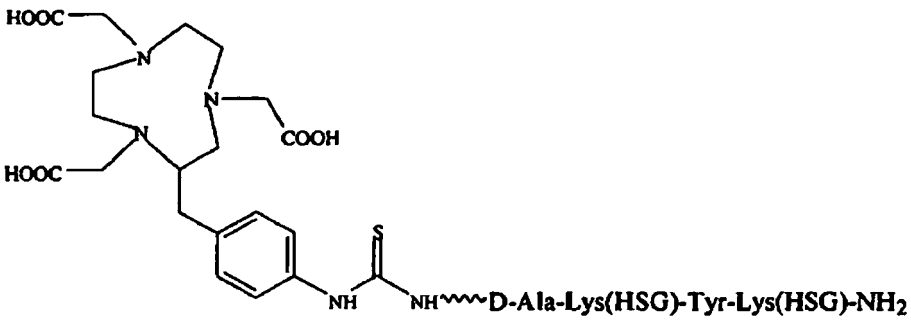


109. Wirksame Menge eines bi-spezifischen Antikörpers oder Antikörperfragments umfassend wenigstens einen Arm, der ein targetiertes Gewebe, das CSAp exprimiert, spezifisch bindet, und wenigstens einen anderen Arm, der ein targetierbares Konjugat spezifisch bindet, wobei der eine Arm, der ein targetiertes Gewebe spezifisch bindet, ein Antikörper oder Fragment davon nach einem der Ansprüche 1 bis 8 ist, zur Verwendung in einem Verfahren für die endoskopische Identifizierung erkrankter Gewebe, die CSAp exprimieren, in einem Lebewesen, umfassend:

(a) Verabreichen der wirksamen Menge des bi-spezifischen Antikörpers oder Antikörperfragments und
(b) Verabreichen eines targetierbaren Konjugats, das ausgewählt ist aus der Gruppe bestehend aus

(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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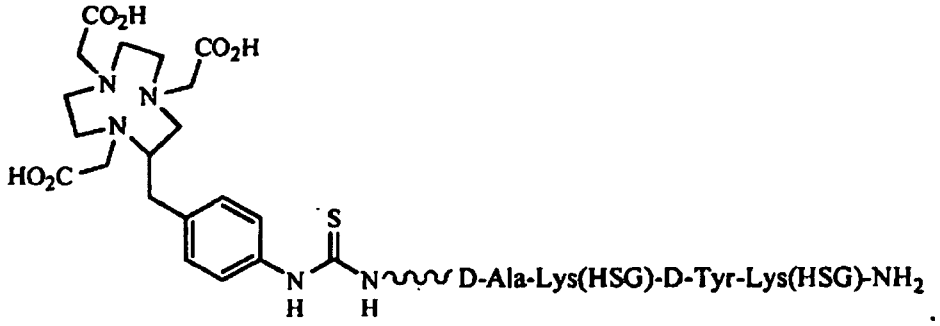
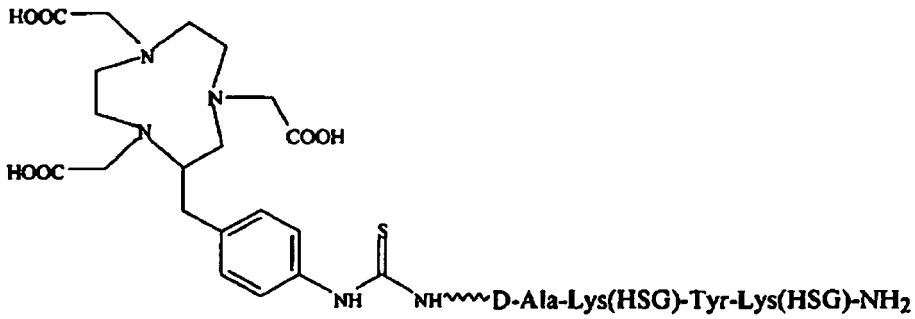


110. Wirksame Menge eines bi-spezifischen Antikörpers oder Antikörperfragments umfassend wenigstens einen Arm, der ein targetiertes Gewebe, das CSAp exprimiert, spezifisch bindet, und wenigstens einen anderen Arm, der ein targetierbares Konjugat spezifisch bindet, wobei der eine Arm, der ein targetiertes Gewebe spezifisch bindet, ein Antikörper oder Fragment davon nach einem der Ansprüche 1 bis 8 ist, zur Verwendung in einem Verfahren zur intravaskulären Identifikation erkrankter Gewebe, die CSAp exprimieren, in einem Lebewesen, umfassend:

(a) Verabreichen der wirksamen Menge des bi-spezifischen Antikörpers oder Antikörperfragments und
(b) Verabreichen eines targetierbaren Konjugats, das ausgewählt ist aus der Gruppe bestehend aus

(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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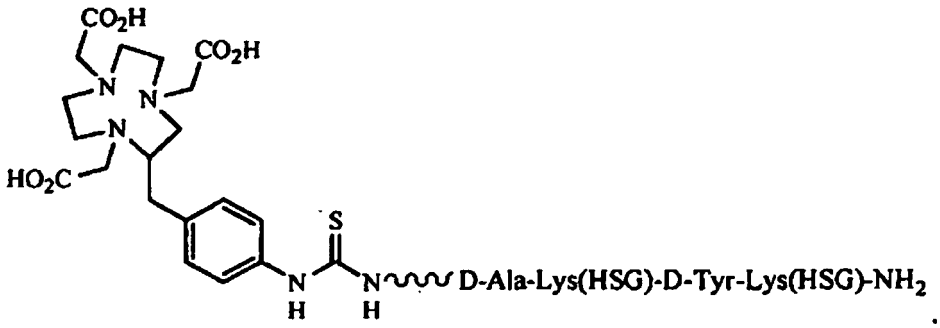

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111. Bi-spezifisches Antikörper $F(ab)_2$- oder $F(ab')_2$-Fragment, wobei der bi-spezifische Antikörper oder das Fragment eine erste Antikörperbindungsstelle, die an ein CSAp-Antigen spezifisch bindet, wie in einem der Ansprüche 1 bis 8 definiert, und eine zweite Antikörperbindungsstelle, die an ein Hapten spezifisch bindet, aufweist, zur Verwendung in einem Verfahren zum Nachweis von Läsionen während eines endoskopischen, laparoskopischen, intravaskulären Katheter- oder chirurgischen Eingriffs, wobei das Verfahren umfasst:

(a) Injizieren des bi-spezifischen Antikörper $F(ab)_2$- oder $F(ab')_2$-Fragments in ein Lebewesen, an dem ein derartiger Eingriff vorgenommen werden soll, und Erlauben, dass sich das Antikörperfragment an Zielstellen ansammelt;
(b) optional Klären nicht-targetierter Antikörperfragmente unter Verwendung eines galaktosylierten anti-idiotypischen Klärmittels, wenn das bi-spezifische Fragment innerhalb von etwa 24 Stunden nach der Injektion nicht weitgehend aus dem Kreislauf geklärt ist, und Injizieren eines bivalenten markierten Haptens, das sich schnell an der Zielstelle lokalisiert und durch die Nieren geklärt wird;
(c) Nachweisen des Vorhandenseins des Haptens durch ortsnahen Nachweis erhöhter Niveaus von angesammelter Markierung an den Zielstellen mit Nachweismittel innerhalb von 48 Stunden nach der ersten Injektion, und Durchführen des Eingriffs, wobei der Nachweis ohne die Verwendung eines Kontrastmittel oder Subtrak-

tionsagens durchgeführt wird.

112. Verfahren nach Anspruch 111, wobei das Hapten markiert ist mit einem diagnostischen Radioisotop, einem MRI-Bild-verstärkenden Agens oder einer fluoreszierenden Markierung.

113. Wirksame Menge eines Immunkonjugats oder Fragments davon wie in einem der vorhergehenden Ansprüche definiert, zur Verwendung in einem Verfahren zum ortsnahen Nachweis von Läsionen während eines operativen, intravaskulären, laparoskopischen oder endoskopischen Eingriffs, wobei das Verfahren umfasst:

(a) parenterales Injizieren der wirksamen Menge des Immunkonjugats oder Fragmentes davon in ein Lebewesen, das einem derartigen Eingriff unterzogen wird,
(b) Durchführen des Eingriffs innerhalb von 48 Stunden nach der Injektion;
(c) ortsnahes Abtasten des Inneren des Lebewesens, zu dem man sich Zugang verschafft hat, mit einem Nachweismittel zum Nachweisen des Vorhandenseins des markierten Antikörpers oder Fragments davon; und
(d) Lokalisieren der Ansammlungsstellen des markierten Antikörpers oder Fragments davon durch Nachweisen erhöhter Niveaus des markierten Antikörpers oder Fragments davon an solchen Stellen mit dem Nachweismittel.

114. Substanzen zur Verwendung nach Anspruch 113, wobei das Immunkonjugat oder Fragment davon ein Radioisotop umfasst, das eine Energie von 20-1.000 keV emittiert; bevorzugterweise wobei das Radioisotop ausgewählt ist aus der Gruppe bestehend aus Technetium-99m, Jod-125, Jod-131, Jod -123, Indium-111, Fluor-18, Gallium-68 und Gallium-67M; oder wobei das Immunkonjugat oder Fragment davon ein Nicht-Isotopen-Agens umfasst, bevorzugterweise wobei das Nicht-Isotopen-Agens ein photoaktives Agens ist; bevorzugtererweise wobei das photoaktive Agens ein fluoreszierendes Agens ist.

115. Bi-spezifischer Antikörper oder Antikörperfragment mit wenigstens einem Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einem anderen Arm, der ein targetierbares Konjugat spezifisch bindet, das wenigstens zwei HSG-Haptene umfasst, wobei der wenigstens eine andere Arm, ein targetiertes Gewebe spezifisch bindet, ein Antikörper oder ein Fragment davon nach einem der Ansprüche 1 bis 8 ist, zur Verwendung in einem Verfahren zum Behandeln oder Identifizieren erkrankter Gewebe in einem Lebewesen umfassend:

(a) Verabreichen des Antikörpers oder Antikörperfragments an das Lebewesen;
(b) optional, Verabreichen an das Lebewesen einer klärenden Zusammensetzung, und Erlauben, dass die Zusammensetzung nicht-lokalisierte Antikörper oder Antikörperfragmente aus dem Kreislauf klärt;
(c) Verabreichen eines targetierbaren Konjugats an das Lebewesen, das einen Trägerteil umfasst, der wenigstens zwei HSG-Haptene umfasst oder trägt, und ein diagnostisches oder therapeutisches Kation und/oder ein oder mehrere Chelat- oder chemisch-gebundene therapeutische oder diagnostische Agenzien oder Enzyme umfassen kann; und
(d) wenn das targetierbare Konjugat eine Enzym umfasst, weiter Verabreichen an das Lebewesen

(1) eines Wirkstoff-Vorläufers, wenn das Enzym in der Lage ist, den Wirkstoff-Vorläufer an der Zielstelle in einen Wirkstoff umzuwandeln; oder
(2) eines Wirkstoffs, der in der Lage ist, in dem Lebewesen entgiftet zu werden, um ein Zwischenprodukt geringerer Toxizität zu bilden, wenn das Enzym in der Lage ist, das entgiftete Zwischenprodukt in eine toxische Form rückzuwandeln und, deshalb, die Toxizität des Wirkstoffs an der Zielstelle zu erhöhen, oder
(3) eines Wirkstoff-Vorläufers, der in dem Lebewesen durch natürliche Prozesse aktiviert wird und durch Umwandlung in ein Zwischenprodukt geringerer Toxizität einer Entgiftung unterworfen wird, wenn das Enzym in der Lage ist, das entgiftete Zwischenprodukt in eine toxische Form rückzuwandeln, und, deshalb, die Toxizität des Wirkstoffes an der Zielstelle zu erhöhen.

116. Antikörper oder Antikörperfragment nach Anspruch 115, wobei das diagnostische Agens 25-600 keV gamma-Partikel und/oder Positronen emittiert; bevorzugterweise wobei das diagnostische Agens ausgewählt ist aus der Gruppe bestehend aus 18F, 52Fe, 62Cu, 64Cu, 67Cu, 67Ga, 68Ga, 86Y, 89Zr, 94mTc, 94Tc, 99mTc, 111In, 123I, 124I, 125I und 131I.

117. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 115, wobei das therapeutische Agens ein Wirkstoff, Wirkstoff-Vorläufer oder Toxin ist; bevorzugterweise wobei der Wirkstoff-Vorläufer ausgewählt ist aus der Gruppe bestehend aus Epirubicinglucuronid, CPT-11, Etoposidglucuronid, Daunomicinglucuronid und Doxorubicinglucuronid; bevorzugterweise wobei das Toxin ausgewählt ist aus der Gruppe bestehend aus Ricin, Abrin, Ri-

bonuklease, DNase I, *Staphylokokken*-Enterotoxin-A, antivirales Pokeweed-Protein, Gelonin, Diphtherietoxin, *Pseudomonas-Exotoxin* und *Pseudomonas-Endotoxin.*

118. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 115, weiter umfassend ein therapeutisches Nuklid; bevorzugterweise wobei das therapeutische Nuklid ausgewählt ist aus der Gruppe bestehend aus 32P, 33P, 47Sc, 64Cu, 67Cu, 67Ga, 90Y, 111Ag, 111In, 125I, 131I, 142Pr, 153Sm, 161Tb, 166Dy, 166Ho, 177Lu, 186Re, 188Re, 189Re, 212Pb, 212Bi, 213Bi, 211At, 223Ra und 225Ac.

119. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 115, wobei das targetierbare Konjugat ein oder mehrere radioaktive Isotope umfasst, die geeignet sind, um erkranktes Gewebe abzutöten; oder wobei das targetierbare Konjugat 10B-Atome umfasst, und das Verfahren weiter den Schritt umfasst Bestrahlen der Boratome, die an dem erkrankten Gewebe lokalisiert sind, wodurch eine BNCT des erkrankten Gewebes bewirkt wird.

120. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 115, wobei das targetierbare Konjugat ein oder mehrere Toxine umfasst; bevorzugterweise wobei das Toxin ausgewählt ist aus der Gruppe bestehend aus Ricin, Abrin, Ribonuklease, DNase I, *Staphylokokken*-Enterotoxin-A, antivirales Pokeweed-Protein, Gelonin, Diphtherietoxin, *Pseudomonas*-Exotoxin und *Pseudomonas-Endotoxin.*

121. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 115, wobei das targetierbare Konjugat ein oder mehrere Wirkstoffe umfasst; oder wobei das targetierbare Konjugat ein oder mehrere Wirkstoff-Vorläufer umfasst; bevorzugterweise wobei der Wirkstoff-Vorläufer ausgewählt ist aus der Gruppe bestehend aus Epirubicinglucuronid, CPT-11, Etoposidglucuronid, Daunomicinglucuronid und Doxorubicinglucuronid.

122. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 115, wobei das targetierbare Konjugat ein oder mehrere diagnostische Agenzien umfasst, die geeignet sind zum Nachweisen erkrankten Gewebes, bevorzugterweise wobei das diagnostische Agens ausgewählt ist aus der Gruppe bestehend aus 118F, 52Fe, 62Cu, 64Cu, 67Cu, 67Ga, 68Ga, 86Y, 89Zr, 94mTc, 94Tc, 99mTc, 111In, 123I, 124I, 125I und 131I; bevorzugterweise wobei das radioaktive Isotop verwendet wird, um Positronen-Emissions-Tomographie (PET) durchzuführen.

123. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 115, wobei das targetierbare Konjugat ein oder mehrere Bild-Verstärkungsmittel zur Verwendung in der Magnetresonanztomographie (MRI) umfasst, bevorzugterweise wobei das Verstärkungsmittel ausgewählt ist aus der Gruppe bestehend aus Mn, Fe und Gd; oder wobei das targetierbare Konjugat ein oder mehrere Agenzien zur photodynamischen Therapie umfasst, bevorzugterweise wobei das Agens zur photodynamischen Therapie ein Photosensibilisator ist, bevorzugtererweise wobei der Photosensibilisator ausgewählt ist aus der Gruppe bestehend aus Benzoporphyrin-Monosäure-Ring A (BPD-MA), Zinn-Etiopurpurin (SnET2), sulfoniertem Aluminum-Phthalcyanin (AlSPc) und Lutetium-Texaphyrin (Lutex).

124. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 115, wobei (i) der wenigstens eine Arm, der ein targetiertes Gewebe spezifisch bindet, ein monoklonaler Antikörper oder ein Fragment eines monoklonalen Antikörpers ist; (ii) der wenigstens eine anderen Arm, der ein targetierbares Konjugat spezifisch bindet, ein monoklonaler Antikörper oder ein Fragment eines monoklonalen Antikörpers ist; (iii) der wenigstens eine Arm, der ein targetiertes Gewebe spezifisch bindet, ein humaner, chimärer oder humanisierter Antikörper oder ein Fragment eines humanen, chimären oder humanisierten Antikörpers ist; oder wobei (iv) der wenigstens eine andere Arm, der ein targetierbares Konjugat spezifisch bindet, ein humaner, chimärer oder humanisierter Antikörper oder ein Fragment eines humanen, chimären oder humanisierten Antikörpers ist.

125. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 115, wobei der bi-spezifische Antikörper oder Antikörperfragment weiter umfasst ein therapeutisches Nuklid, bevorzugterweise wobei das therapeutische Radionuklid ausgewählt ist aus der Gruppe bestehend aus 32P, 33P, 47Sc, 64Cu, 67Cu, 67Ga, 90Y, 111Ag, 111In, 1251, 1311, 142Pr, 153Sm, 161Tb, 166Dy, 166Ho, 177Lu, 186Re, 188Re, 189Re, 212Pb, 212Bi, 213Bi, 211At, 223Ra und 225Ac.

126. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 115, wobei das targetierbare Konjugat Doxorubicin, SN-38, Etoposid, Methotrexat, 6-Mercaptopurin oder Etoposidphosphat umfasst.

127. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 115, wobei das targetierte Gewebe ein Tumor ist; bevorzugterweise wobei der Tumor Colon-spezifisches Antigen-p (CSAp) produziert oder assoziiert ist mit Colon-spezifischem Antigen-p (CSAp); bevorzugtererweise wobei der bi-spezifische Antikörper den Fv von MAb Mu9 und

den Fv von MAb 679 umfasst; noch bevorzugterweweise wobei Mu9 und/oder 679 chimärisiert oder humanisiert sind, oder wobei Mu9 und/oder 679 humaner Mu9 und 679 sind, oder wobei der bi-spezifische Antikörper ein oder mehrere der CDRs von Mu9 umfasst; oder wobei der bi-spezifische Antikörper ein oder mehrere der CDRs von 679 umfasst.

128. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 127, wobei der bi-spezifische Antikörper ein Fusionsprotein ist.

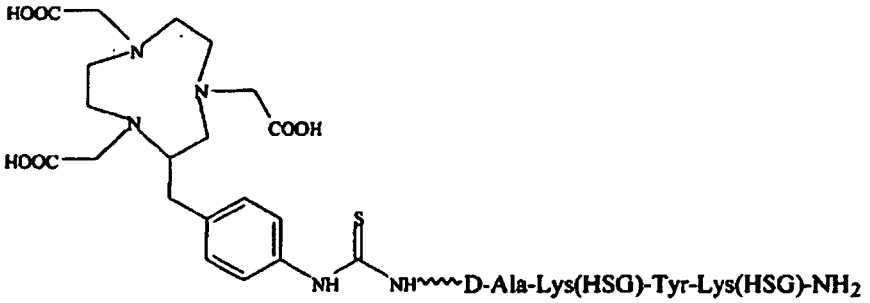
129. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 127, wobei der Tumor carcinoembryonales Antigen (CEA) produziert; bevorzugterweise wobei der bi-spezifische Antikörper den Fv von MAb MN 14 und den Fv von MAb 679 umfasst; bevorzugtererweise wobei MN14 und/oder 679 chimärisiert oder humanisiert sind; oder wobei MN14 und/oder 679 humaner MN14 und 679 sind; oder wobei der bi-spezifische Antikörper ein oder mehrere der CDRs von MN14 umfasst; oder wobei der bi-spezifische Antikörper ein oder mehrere der CDRs von 679 umfasst; oder wobei der bi-spezifische Antikörper ein Fusionsprotein ist, bevorzugterweise wobei das Fusionsprotein trivalent ist und den Fv eines Antikörpers enthält, der reaktiv ist mit CSAp.

130. Antikörper oder Antikörperfragment zur Verwendung nach Anspruch 129, wobei der bi-spezifische Antikörper einen Klasse III anti-CEA-Antikörper und den Fv von 679 enthält.

131. Wirksame Menge eines bi-spezifischen Antikörpers oder Antikörperfragments umfassend wenigstens einen Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einen anderen Arm, der ein targetierbares Konjugat spezifisch bindet, zur Verwendung in einem Verfahren zum Nachweisen oder Behandeln von Zielzellen oder -Geweben in einem Säugetier, umfassend:

Verabreichen des Antikörpers oder Antikörperfragments an das Säugetier;
wobei der wenigstens eine Arm in der Lage ist, einen komplementären bindenden Teil auf den Zielzellen oder -Geweben oder auf einem Molekül, das durch diese produziert wurde oder mit diesen assoziiert ist, zu binden, wobei ein solcher Teil das CSAp-Antigen ist und wobei der eine Arm, der ein targetiertes Gewebe spezifisch bindet, der Antikörper oder ein Fragment davon nach einem der Ansprüche 1 bis 8 ist; und
Verabreichen eines targetierbaren Konjugats, das ausgewählt ist aus der Gruppe bestehend aus

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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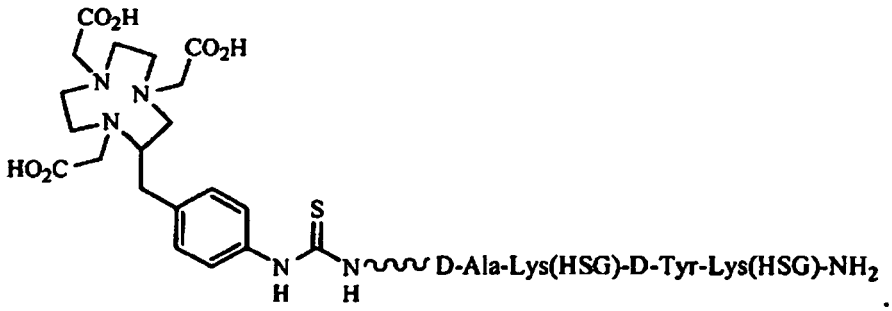
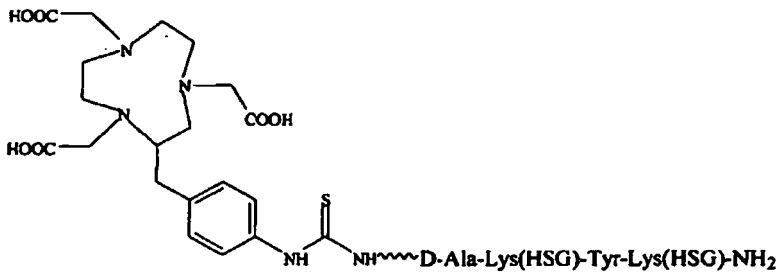


132. Bi-spezifischer Antikörper oder Antikörperfragment mit wenigstens einem Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einem anderen Arm, der ein targetierbares Konjugat spezifisch bindet, zur Verwendung in einem Verfahren zum Behandeln oder Identifizieren erkrankter Gewebe in einem Lebewesen umfassend:

Verabreichen des Antikörpers oder Antikörperfragments an das Lebewesen;
optional, Verabreichen einer klärenden Zusammensetzung an das Lebewesen, und Erlauben, dass die Zusammensetzung nicht-lokalisierte Antikörper oder Antikörperfragmente aus dem Kreislauf klärt; und
Verabreichen an das Lebewesen eines targetierbaren Konjugats, das ausgewählt ist aus der Gruppe bestehend aus:

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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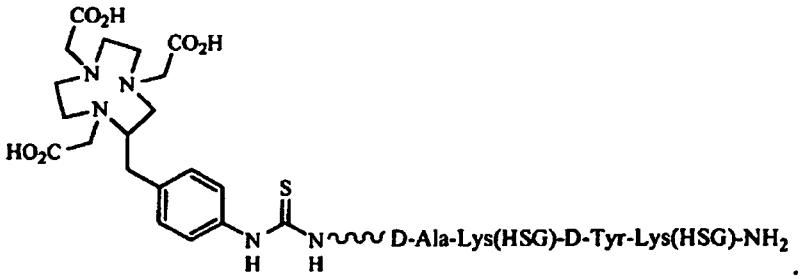
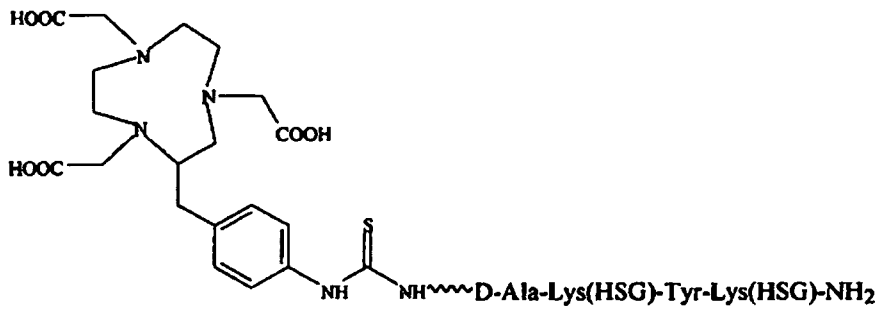


wobei der Antikörper oder das Antikörperfragment ein Antikörper oder Antikörperfragment nach einem der Ansprüche 1 bis 8 ist.

133. Kit geeignet zum Behandeln oder Identifizieren erkrankter Gewebe in einem Lebewesen umfassend:

(a) einen bi-spezifischen Antikörper oder Antikörperfragment mit wenigstens einem Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einem anderen Arm, der ein targetierbares Konjugat spezifisch bindet, wobei der Arm, der spezifisch das targetierte Gewebe bindet, ein Antikörper oder ein Fragment davon nach einem der Ansprüche 1 bis 8 ist, und das Konjugat ausgewählt ist aus der Gruppe bestehend aus

(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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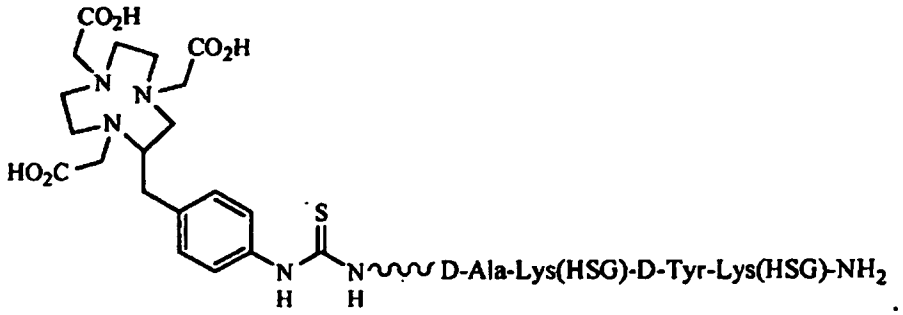


(b) ein targetierbares Konjugat, das einen Trägerteil umfasst, der wenigstens ein Epitop umfasst oder trägt, das durch den wenigstens einen anderen Arm des bi-spezifischen Antikörpers oder Antikörperfragmentes erkennbar ist, und ein oder mehrere konjugierte therapeutische oder diagnostische Agenzien oder Enzyme und
(c) optional, eine klärende Zusammensetzung, die geeignet ist zum Klären nichtlokalisierter Antikörper und Antikörperfragmente; und
(d) optional, wenn das erste targetierbare Konjugat ein Enzym umfasst,

(1) einen Wirkstoff-Vorläufer, wenn das Enzym in der Lage ist, den Wirkstoff-Vorläufer an der Zielstelle in einen Wirkstoff umzuwandeln; oder
(2) einen Wirkstoff, der in der Lage ist, in dem Lebewesen entgiftet zu werden, um ein Zwischenprodukt geringerer Toxizität zu bilden, wenn das Enzym in der Lage ist, das entgiftete Zwischenprodukt in eine toxische Form rückzuwandeln und, deshalb, die Toxizität des Wirkstoffs an der Zielstelle zu erhöhen, oder
(3) einen Wirkstoff-Vorläufer, der in dem Lebewesen durch natürliche Prozesse aktiviert wird und durch Umwandlung in ein Zwischenprodukt geringerer Toxizität einer Entgiftung unterworfen wird, wenn das Enzym in der Lage ist, das entgiftete Zwischenprodukt in eine toxische Form rückzuwandeln, und, deshalb, die Toxizität des Wirkstoffes an der Zielstelle zu erhöhen.

134. In vitro-Verfahren zum Screenen eines targetierbaren Konjugats umfassend:

in Kontakt bringen des targetierbaren Konstrukts mit einem bi-spezifischen Antikörper oder Antikörperfragment mit wenigstens einem Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einem anderen Arm,

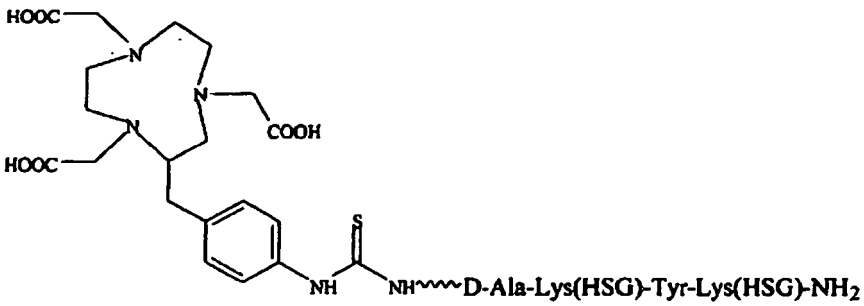
der das targetierbare Konjugat spezifisch bindet, um eine Mischung herzustellen;
wobei der wenigstens eine Arm in der Lage ist einen komplementären bindenden Teil auf den Zielzellen, -geweben oder auf einem Molekül, das durch diese produziert ist oder mit diesen assoziiert ist, zu binden, wobei der Teil das CSAp-Antigen ist und wobei der wenigstens eine Arm, der ein targetiertes Gewebe spezifisch bindet, ein Antikörper oder ein Fragment davon nach einem der Ansprüche 1 bis 8 ist; und
optional, Inkubieren der Mischung; und
Analysieren der Mischung.

135. Verfahren nach Anspruch 134, wobei die Analyse ein analytisches Verfahren umfasst, das ausgewählt ist aus der Gruppe bestehend aus FABMS, Hochfeld-NMR oder Größenausschluss-HPLC.

136. Wirksame Menge eines bi-spezifischen Antikörpers oder Antikörperfragments umfassend wenigstens einen Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einen anderen Arm, der ein targetierbares Konjugat spezifisch bindet;
wobei der wenigstens eine Arm in der Lage ist, einen komplementären bindenden Teil auf den Zielzellen, -geweben oder auf einem Molekül, das durch diese produziert ist oder mit diesen assoziiert ist, zu binden, wobei der Teil das CSAp-Antigen ist und wobei der wenigstens eine Arm, der ein targetiertes Gewebe spezifisch bindet, ein Antikörper oder ein Fragment davon nach einem der Ansprüche 1 bis 8 ist, zur Verwendung in einem Verfahren zur bildlichen Darstellung normaler Gewebe in einem Säugetier umfassend:

Verabreichen der wirksamen Menge des bi-spezifischen Antikörpers oder Antikörperfragments; und
Verabreichen eines targetierbaren Konjugats, das ausgewählt ist aus der Gruppe bestehend aus

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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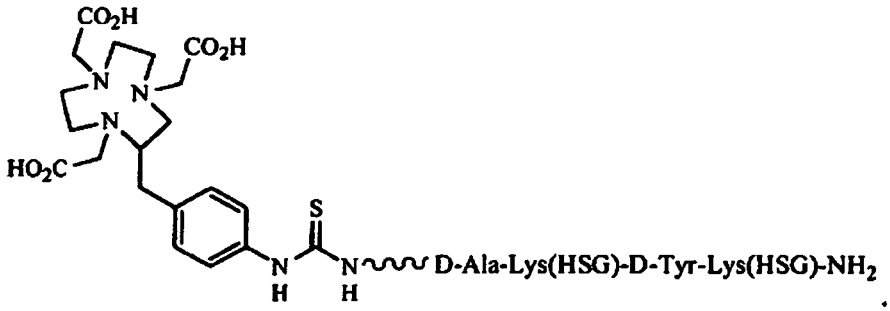

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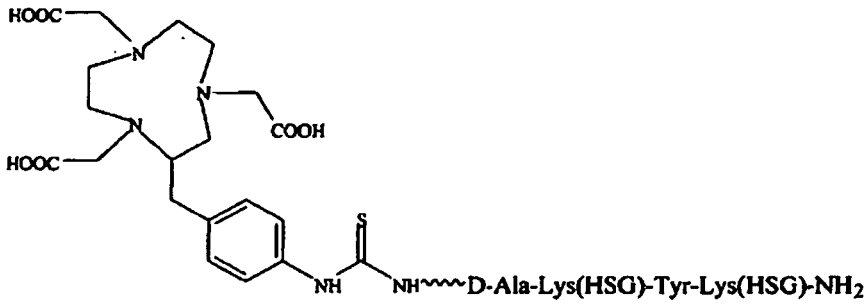
137. Substanz zur Verwendung nach Anspruch 136, wobei das normale Gewebe Gewebe aus dem Eierstock, dem

Thymus, der Nebenschilddrüse oder der Milz ist.

138. Wirksame Menge eines bi-spezifischen Antikörpers oder Antikörperfragments umfassend wenigstens einen Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einen anderen Arm, der ein targetierbares Konjugat spezifisch bindet;
wobei der wenigstens eine Arm in der Lage ist, einen komplementären bindenden Teil auf den Zielzellen, -geweben oder auf einem Molekül, das durch diese produziert ist oder mit diesen assoziiert ist, zu binden, wobei der Teil das CSAp-Antigen ist, und wobei der wenigstens eine Arm, der ein targetiertes Gewebe spezifisch bindet, ein Antikörper oder ein Fragment davon nach einem der Ansprüche 1 bis 8 ist, zur Verwendung in einem Verfahren zum intraoperativen Identifizieren erkrankter Gewebe in einem Lebewesen umfassend:

Verabreichen der wirksame Menge des bi-spezifischen Antikörpers oder Antikörperfragments und Verabreichen eines targetierbaren Konjugats, das ausgewählt ist aus der Gruppe bestehend aus

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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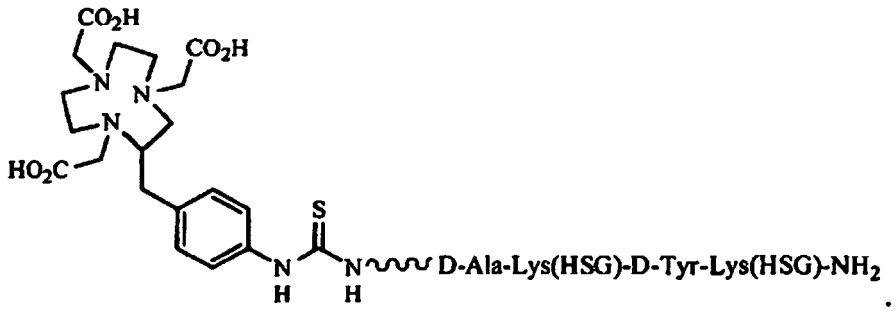

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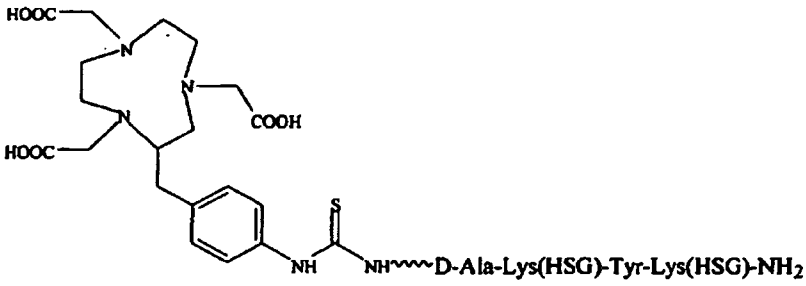
139. Wirksame Menge eines bi-spezifischen Antikörpers oder Antikörperfragments umfassend wenigstens einen Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einen anderen Arm, der ein targetierbares Konjugat spezifisch bindet;
wobei der wenigstens eine Arm in der Lage ist, einen komplementären bindenden Teil auf den Zielzellen, -geweben oder auf einem Molekül, das durch diese produziert ist oder mit diesen assoziiert ist, zu binden, wobei der Teil das CSAp-Antigen ist, und wobei der wenigstens eine Arm, der ein targetiertes Gewebe spezifisch bindet, ein Antikörper oder ein Fragment davon nach einem der Ansprüche 1 bis 8 ist; zur Verwendung in einem Verfahren für die endoskopische Identifikation erkrankter Gewebe in einem Lebewesen umfassend:

Verabreichen der wirksame Menge des bi-spezifischen Antikörpers oder Antikörperfragments; und Verabreichen eines targetierbaren Konjugats, das ausgewählt ist aus der Gruppe bestehend aus

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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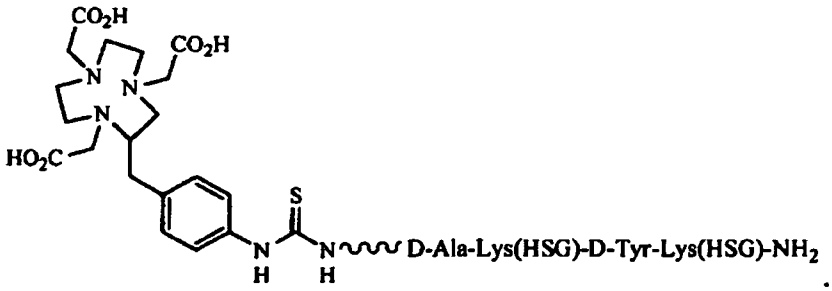

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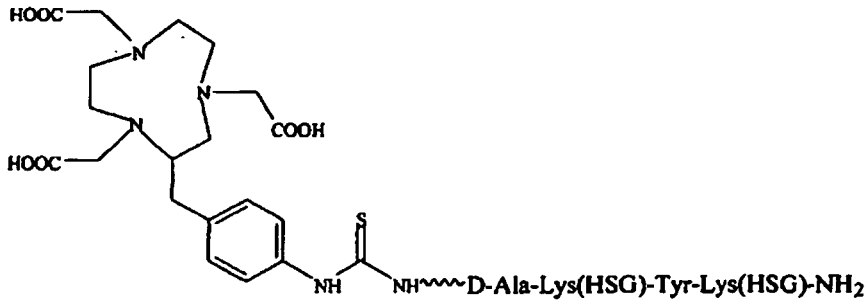
140. Wirksame Menge eines bi-spezifischen Antikörpers oder Antikörperfragments umfassend wenigstens einen Arm, der ein targetiertes Gewebe spezifisch bindet, und wenigstens einen anderen Arm, der ein targetierbares Konjugat spezifisch bindet;
wobei der wenigstens eine Arm in der Lage ist, einen komplementären bindenden Teil auf den Zielzellen, -geweben oder -Pathogen oder auf einem Molekül, das durch diese produziert ist oder mit diesen assoziiert ist, zu binden, wobei der Teil das CSAp-Antigen ist, und wobei der wenigstens eine Arm, der ein targetiertes Gewebe spezifisch bindet, ein Antikörper oder ein Fragment davon nach einem der Ansprüche 1 bis 8 ist, zur Verwendung in einem Verfahren für die intravaskuläre Identifikation erkrankter Gewebe in einem Lebewesen umfassend:

Verabreichen der wirksame Menge des bi-spezifischen Antikörpers oder Antikörperfragments; und
Verabreichen eines targetierbaren Konjugats, das ausgewählt ist aus der Gruppe bestehend aus

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;
(d)



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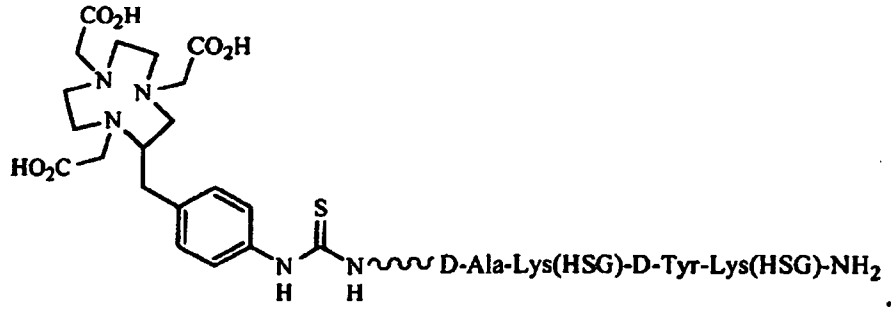

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141. Bi-spezifischer Antikörper oder Antikörperfragment, die wirksame Menge des bi-spezifischen Antikörpers oder Antikörperfragments, das Kit oder das Verfahren nach einem der Ansprüche 131, 132, 133, 134, 136, 138, 139 oder 140, wobei das targetierbare Konjugat weiter umfasst ein diagnostisches Agens, das ausgewählt ist aus der Gruppe bestehend aus ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, ^{94m}Tc, ^{94}Tc, ^{99m}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154\text{-}158}Gd$ und ^{175}Lu; bevorzugterweise wobei das targetierbare Konjugat weiter umfasst ein therapeutisches Nuklid, das ausgewählt ist aus der Gruppe bestehend aus ^{32}P, ^{33}P, ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{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.

Revendications

1. Anticorps monoclonal humanisé (MAb) ou fragment de celui-ci qui se lie à l'antigène de mucine d'antigène p spécifique au côlon (CSAp), de telle manière que le fragment se lie au même antigène qui est reconnu par l'anticorps, comprenant les régions de charpente (FR) des régions variables de chaîne légère et lourde d'un anticorps humain et les régions constantes de chaîne légère et lourde d'un anticorps humain, les CDR de la région variable de chaîne légère du MAb anti-CSAp humanisé ou un fragment de celui-ci comprennent CDR1 comprenant une séquence d'acides aminés de RSSQSIVHSNGNTYLE ; CDR2 comprenant une séquence d'acides aminés de KVSNRFS et CDRS comprenant une séquence d'acides aminés de FQGSRVPYT ; et les CDR de la région variable de chaîne lourde du MAb anti-CSAp humanisé ou fragment de celui-ci comprend CDR1 comprenant une séquence d'acides aminés de EYVIT ; CDR2 comprenant une séquence d'acides aminés de EIYPGSGSTSYNEKFK et CDR3 comprenant une séquence d'acides aminés de EDL.

2. Anticorps ou fragment de celui-ci de la revendication 1, les FR des régions variables de chaîne légère et lourde dudit anticorps ou fragment de celui-ci comprenant au moins un acide aminé substitué des FR correspondants de l'anticorps anti-CSAp murin ou fragment de celui-ci.

3. Anticorps ou fragment de celui-ci de la revendication 2, ledit acide aminé dudit MAb murin étant au moins un acide aminé choisi dans le groupe constitué des résidus d'acide aminé 5, 27, 30, 38, 40, 48, 66, 67, 74, 93, 94 et 103 de la région variable de chaîne lourde murine de la figure 10A ; ou dans lequel lesdits acides aminés murins sont au

moins un acide aminé choisi dans le groupe constitué des résidus d'acide aminé 37, 58 et 100 de la région variable de chaîne légère murine de la figure 10B.

4. Anticorps ou fragment de celui-ci de la revendication 1, ledit anticorps ou fragment de celui-ci comprenant une séquence d'acides aminés hMu-9 V_K de la figure 11B.

5. Anticorps ou fragment de celui-ci de la revendication 4, ledit anticorps ou fragment de celui-ci comprenant une séquence d'acides aminés hMu-9 V_H de la figure 11A.

6. Anticorps ou fragment de celui-ci de la revendication 1, ledit anticorps ou fragment de celui-ci comprenant une séquence d'acides aminés hMu-9 V_H de la figure 11A.

7. Anticorps ou fragment de celui-ci de la revendication 6, ledit anticorps ou fragment de celui-ci comprenant une séquence d'acides aminés hMu-9 V_K de la figure 11B.

8. Anticorps anti-CSAp ou fragment de ceux-ci de l'une quelconque des revendications 1 à 7, ledit fragment étant choisi dans le groupe constitué de Fv, F(ab')2, Fab' et Fab.

9. Immunoconjugué de diagnostic/détection ou thérapeutique comprenant un composant d'anticorps comprenant un MAb anti-CSAp ou un fragment de liaison d'antigène de celui-ci ou une protéine de fusion d'anticorps ou un fragment de celle-ci de l'une quelconque des revendications 1 à 8, ledit composant d'anticorps étant lié à au moins un agent de diagnostic/détection ou au moins un agent thérapeutique.

10. Immunoconjugué de diagnostic/détection de la revendication 9, ledit agent de diagnostic/détection comprenant au moins un agent de diagnostic/détection photoactif ; en particulier, l'agent de diagnostic photoactif comprenant un chromogène ou un colorant.

11. Immunoconjugué de diagnostic/détection de la revendication 9, ledit agent de diagnostic/détection étant un radionucléide avec une énergie comprise entre 20 et 2 000 keV ; en particulier ledit radionucléide étant un isotope émetteur gamma, bêta ou de positons ; plus particulièrement ledit radionucléide étant choisi dans le groupe constitué de 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, I-124, Cr-51, Co-57, Co-58, Fe-59, Cu-67, Ga-67, Se-75, Ru-97, Tc-99m, In-111, In-114m, 1-123, 1-125, I-131, Yb-169, Hg-197, et Tl-201.

12. Immunoconjugué de diagnostic/détection de la revendication 9, ledit agent de diagnostic étant un agent de contraste.

13. Immunoconjugué de diagnostic/détection de la revendication 12, ledit agent de contraste étant un ion paramagnétique ; en particulier ledit ion paramagnétique comprenant un métal choisi dans le groupe constitué du chrome (III), du manganèse (II), du fer (III), du fer (II), du cobalt (II), du nickel (II), du cuivre (II), du néodyme (III), du samarium (III), de l'ytterbium (III), du gadolinium (III), du vanadium (II), du terbium (III), du dysprosium (III), de l'holmium (III) et de l'erbium (III).

14. Immunoconjugué de diagnostic/détection de la revendication 12, ledit agent de contraste étant un agent accentuant les ultrasons, de préférence ledit agent accentuant les ultrasons étant un liposome qui est conjugué à l'anticorps humanisé ou un fragment de celui-ci, plus préférablement, ledit liposome étant rempli de gaz.

15. Immunoconjugué de diagnostic/détection de la revendication 12, ledit agent de contraste étant un composé radio-opaque ; en particulier, ledit composé radio-opaque étant choisi dans le groupe constitué de composés d'iode, de composés de baryum, de composés de gallium et de composés de thallium.

16. Immunoconjugué de diagnostic/détection des revendications 9 à 11, ledit immunoconjugué étant destiné à être utilisé en détection/diagnostic de tumeur intraopératoire, endoscopique, ou intravasculaire.

17. Immunoconjugué thérapeutique de la revendication 9, ledit agent thérapeutique étant choisi dans le groupe constitué d'un radionucléide, d'atomes de bore, de gadolinium ou d'uranium, d'un immunomodulateur, d'une cytokine, d'une hormone, d'un antagoniste d'hormone, d'une enzyme, d'un inhibiteur d'enzyme, d'un agent thérapeutique photoactif, d'un médicament cytotoxique, d'une toxine, d'un inhibiteur d'angiogenèse, d'un anticorps différent et d'une combinaison de ceux-ci.

18. Immunoconjugué thérapeutique de la revendication 17, ledit agent cytotoxique étant un médicament ou une toxine ; en particulier ledit médicament étant choisi dans le groupe constitué d'agents antimitotiques, d'alkylation, antimétabolites, inhibiteurs d'angiogenèse, apoptotiques, alcaloïdes, inhibiteurs de COX-2 et antibiotiques et des combinaisons de ceux-ci ; ou ledit médicament étant choisi dans le groupe constitué de moutardes azotées, dérivés d'éthylénimine, alkylsulfonates, nitroso-urées, triazènes, analogues d'acide folique, anthracyclines, taxanes, inhibiteurs de COX-2, analogues de pyrimidine, analogues de purine, antibiotiques, enzymes, épipodophyllotoxines, complexes de coordination de platine, alcaloïdes de pervenche, urées substituées, dérivés de méthylhydrazine, suppresseurs corticosurrénaux, antagonistes d'hormone, inhibiteurs d'enzyme, endostatine, taxols, camptothécines, doxorubicines et leurs analogues, et une combinaison de ceux-ci ou ladite toxine étant choisie dans le groupe constitué de toxines végétales, microbiennes, et animales ; en particulier ladite toxine étant choisie dans le groupe constitué de la ricine, l'abrine, la toxine alpha, la saporine, une ribonucléase (RNase), une DNase I, l'entérotoxine staphylococcique A, la protéine antivirale de phytolaque, la gélonine, la toxine diphtérique, l'exotoxine de *Pseudomonas,* et l'endotoxine de *Pseudomonas.*

19. Immunoconjugué thérapeutique de la revendication 17, ledit immunomodulateur étant choisi dans le groupe constitué d'une cytokine, un facteur de croissance de cellule souche, une lymphotoxine, un facteur hématopoïétique, un facteur stimulant les colonies (CSF), un interféron (IFN), un facteur de croissance de cellule souche, l'érythropoïétine, la thrombopoïétine, un anticorps et une combinaison de ceux-ci ; en particulier, ladite lymphotoxine étant un facteur de nécrose tumorale (TNF), ledit facteur hématopoïétique étant une interleukine (IL), ledit facteur stimulant les colonies étant le facteur stimulant les colonies de granulocytes (G-CSF) ou le facteur stimulant les colonies de granulocytes macrophages (GM-CSF), ledit interféron étant les interférons α, β ou γ, ledit facteur de croissance de cellule souche étant appelé « facteur S1 » ; en particulier ladite cytokine étant choisie dans le groupe constitué d'IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, interféron γ, TNF-α et une combinaison de ceux-ci.

20. Immunoconjugué thérapeutique de la revendication 17, ledit immunomodulateur étant un anticorps qui est un agoniste ou antagoniste d'un facteur immunitaire ; en particulier l'anticorps étant contre CD40.

21. Immunoconjugué thérapeutique de la revendication 17, ledit radionucléide étant choisi dans le groupe constitué d'un émetteur Auger, un émetteur bêta et un émetteur alpha.

22. Immunoconjugué thérapeutique de la revendication 17, ledit radionucléide étant choisi dans le groupe constitué de P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, 1-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, et Bi-213, Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, 1-125, Ho-161, Os-189m, Ir-192, Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213, Fm-255 et des combinaisons de ceux-ci.

23. Immunoconjugué thérapeutique de la revendication 17, ledit atome de bore étant B-10 ; et/ou ledit atome de gadolinium étant Gd-157 ; et/ou ledit atome d'uranium étant U-235.

24. Immunoconjugué thérapeutique de la revendication 21, ledit radionucléide ayant une énergie comprise entre 20 et 10 000 keV.

25. Immunoconjugué thérapeutique de la revendication 21, ledit radionucléide étant un émetteur Auger et ayant une énergie inférieure à 1000 keV, ou ledit radionucléide étant un émetteur bêta et ayant une énergie comprise entre 20 et 5000 keV, ou ledit radionucléide étant un émetteur alpha et ayant une énergie comprise entre 2000 et 10 000 keV.

26. Immunoconjugué thérapeutique de la revendication 17, ledit agent thérapeutique photoactif étant un chromogène ou un colorant.

27. Immunoconjugué diagnostique ou thérapeutique selon la revendication 9, ledit agent de diagnostic ou thérapeutique étant lié audit MAb ou fragment de celui-ci au moyen d'un fragment glucidique.

28. Anticorps multivalent, multispécifique ou fragment de liaison d'antigène de celui-ci comprenant un ou plusieurs anticorps monoclonaux humanisés ou un fragment de ceux-ci de la revendication 1 et un ou plusieurs sites de liaison d'haptène ayant une affinité pour des molécules d'haptène.

29. Anticorps ou fragment de celui-ci de la revendication 28, ledit anticorps ou fragment de celui-ci étant humanisé, ou ledit anticorps ou fragment de celui-ci étant chimérisé, ou ledit anticorps ou fragment de celui-ci étant un anticorps humain.

30. Anticorps ou fragment de celui-ci de la revendication 28 ou 29, comprenant en outre un agent de diagnostic ou thérapeutique.

31. Protéine de fusion d'anticorps ou fragment de liaison d'antigène de celui-ci comprenant au moins deux MAb anti-CSAp ou fragments de ceux-ci, lesdits MAb ou fragments de ceux-ci étant choisis parmi ledit MAb ou fragment de celui-ci de l'une quelconque des revendications 1 à 30.

32. Protéine de fusion d'anticorps ou fragment de liaison d'antigène de celui-ci comprenant au moins un premier MAb anti-CSAp ou un fragment de celui-ci de l'une quelconque des revendications 1 à 30 et au moins un deuxième MAb ou un fragment de celui-ci, autre que le MAb ou fragment de celui-ci de l'une quelconque des revendications 1 à 30.

33. Protéine de fusion d'anticorps ou fragment de celle-ci de la revendication 32, ledit deuxième MAb ou fragment de celui-ci étant un anticorps anti-CD40.

34. Protéine de fusion d'anticorps ou fragment de celle-ci de la revendication 31 ou 32, comprenant en outre un agent de diagnostic ou thérapeutique conjugué à ladite protéine de fusion ou fragment de celle-ci.

35. Protéine de fusion d'anticorps ou fragment de celle-ci de la revendication 32, ledit deuxième MAb étant un anticorps associé à un carcinome ; en particulier, ledit anticorps associé à un carcinome étant choisi dans le groupe constitué de CEA, EGP-1, EGP-2, MUC1, MUC2, MUC3, MUC4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, BrE3, Le-Y, A3, KS-1, VEGF, d'autres anticorps antiangiogenèse, des anticorps antinécrose, et des anticorps anti-produit d'oncogène ; ou ledit anticorps associé à un carcinome se lie à un antigène sur un cancer choisi dans le groupe constitué de cancers gastro-intestinaux et de cancers de l'ovaire.

36. Quantité thérapeutiquement efficace d'un anticorps ou fragment selon l'une quelconque des revendications 1 à 8 et 28 à 29, formulée dans un véhicule pharmaceutiquement acceptable pour utilisation dans un procédé de traitement d'une malignité chez un sujet, comprenant l'étape d'administration de ladite formulation audit sujet.

37. Quantité thérapeutiquement efficace d'un immunoconjugué ou un fragment de liaison d'antigène de celui-ci de l'une quelconque des revendications 9, 17 à 21 et 30, formulée dans un véhicule pharmaceutiquement acceptable pour utilisation dans un procédé de traitement d'une malignité chez un sujet, comprenant l'étape d'administration de ladite formulation audit sujet.

38. Quantité efficace sur le plan diagnostique d'un anticorps ou un fragment de liaison d'antigène de celui-ci selon l'une quelconque des revendications 1 à 8 et 28 à 29, formulé dans un véhicule pharmaceutiquement acceptable pour utilisation dans un procédé de diagnostic/détection d'une malignité chez un sujet, comprenant l'étape d'administration de ladite formulation audit sujet.

39. Quantité efficace sur le plan diagnostique d'un immunoconjugué ou un fragment de celui-ci selon l'une quelconque des revendications 9 à 16, et 30, formulé dans un véhicule pharmaceutiquement acceptable pour utilisation dans un procédé de diagnostic/traitement d'une malignité chez un sujet, comprenant l'étape d'administration de ladite formulation audit sujet.

40. Quantité efficace sur le plan thérapeutique ou diagnostique d'une protéine de fusion ou un fragment de celle-ci de l'une quelconque des revendications 31 à 35, formulée dans un véhicule pharmaceutiquement acceptable pour utilisation dans un procédé de traitement ou de diagnostic/détection d'une malignité chez un sujet, comprenant l'étape d'administration de ladite formulation audit sujet.

41. Anticorps ou fragment de celui-ci selon l'une quelconque des revendications 28 à 29 pour utilisation dans un procédé de traitement ou de diagnostic/détection d'une malignité chez un sujet, comprenant

(i) l'administration à un sujet nécessitant celle-ci de l'anticorps ou de fragments de celui-ci de l'une quelconque des revendications 28 à 29 ;
(ii) l'attente d'un temps suffisant pour qu'une quantité de la protéine non liée soit éliminée de la circulation

sanguine du sujet ; et
(iii) l'administration audit sujet d'une molécule porteuse comprenant un agent de diagnostic, un agent thérapeutique, ou une combinaison de ceux-ci, qui se lie à un site de liaison dudit anticorps.

42. Séquence d'ADN comprenant un acide nucléique codant pour un MAb anti-CSAp ou un fragment de celui-ci choisi dans le groupe constitué de

(a) un MAb anti-CSAp ou un fragment de celui-ci de l'une quelconque des revendications 1 à 30 ;
(b) une protéine de fusion d'anticorps ou un fragment de celle-ci comprenant au moins deux desdits MAb ou fragments de ceux-ci de l'une quelconque des revendications 1 à 30 ;
(c) une protéine de fusion d'anticorps ou un fragment de celle-ci comprenant au moins un premier MAb anti-CSAp ou un fragment de celui-ci comprenant ledit MAb ou un fragment de celui-ci de l'une quelconque des revendications 1 à 30 et au moins un deuxième MAb ou un fragment de celui-ci, autre que le MAb ou un fragment de celui-ci de l'une quelconque des revendications 1 à 30 ; et
(d) une protéine de fusion d'anticorps ou fragment de celui-ci comprenant au moins un premier MAb ou un fragment de celui-ci comprenant ledit MAb ou un fragment de celui-ci de l'une quelconque des revendications 1 à 30 et au moins un deuxième MAb ou fragment de celui-ci, autre que le MAb ou un fragment de celui-ci de l'une quelconque des revendications 1 à 30, ledit deuxième MAb étant choisi dans le groupe constitué d'un anticorps contre pour CEA, EGP-1, EGP-2, MUC-1, MUC-2, MUC-3, MUC-4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, BrE3, Le-Y, A3, KS-1, CD40, et VEGF, et une combinaison de ceux-ci.

43. Vecteur d'expression comprenant la séquence d'ADN de la revendication 42.

44. Cellule hôte comprenant la séquence d'ADN de la revendication 42.

45. Immunoconjugué selon la revendication 9 pour utilisation dans un procédé de transfert d'un agent de diagnostic/détection ou thérapeutique, ou une combinaison de ceux-ci, vers une cible comprenant (i) la fourniture d'une composition qui comprend ledit immunoconjugué et (ii) l'administration à un sujet nécessitant celle-ci de ladite composition.

46. Immunoconjugué pour utilisation selon la revendication 45, ledit agent de diagnostic/détection comprenant au moins un agent de diagnostic photoactif ; en particulier, l'agent de diagnostic photoactif comprenant un chromogène ou un colorant.

47. Immunoconjugué pour utilisation selon la revendication 45, ledit agent de diagnostic étant un radionucléide avec une énergie comprise entre 20 et 2 000 keV ; en particulier ledit radionucléide étant un isotope émetteur gamma, bêta ou de positons ; plus particulièrement ledit radionucléide étant choisi dans le groupe constitué de 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, I-124, 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, 1-131, Yb-169, Hg-197, et TI-201.

48. Immunoconjugué pour utilisation selon la revendication 45, ledit agent de diagnostic étant un agent de contraste.

49. Immunoconjugué pour utilisation selon la revendication 48, ledit agent de contraste étant un ion paramagnétique ; en particulier ledit ion paramagnétique étant un métal comprenant le manganèse, le fer ou le gadolinium.

50. Immunoconjugué pour utilisation selon la revendication 48, ledit agent de contraste étant un agent accentuant les ultrasons, de préférence ledit agent accentuant les ultrasons étant un liposome qui est conjugué à l'anticorps humanisé ou un fragment de celui-ci, plus particulièrement, ledit liposome étant rempli de gaz.

51. Immunoconjugué pour utilisation selon la revendication 48, ledit agent de contraste étant un composé radio-opaque utilisé en radiographie ou en tomodensitométrie ; en particulier, ledit composé radio-opaque étant choisi dans le groupe constitué de composés d'iode, de composés de baryum, de composés de gallium et de composés de thallium ; plus particulièrement, ledit composé radio-opaque étant choisi dans le groupe constitué des baryum, diatrizoate, huile éthiodisée, citrate de gallium, acide iocarmique, acide iocétamique, iodamide, iodipamide, acide iodoxamique, iogulamide, iohexol, iopamidol, acide iopanoïque, acide ioprocémique, acide ioséfamique, acide iosérique, iosulamide méglumine, acide iosémétique, iotasul, acide iotétrique, acide iothalamique, acide iotroxique, acide ioxaglique, acide ioxotrizoïque, ipodate, méglumine, métrizamide, métrizoate, propyliodone, et chlorure thalleux.

52. Immunoconjugué pour utilisation selon la revendication 45, ledit agent thérapeutique étant choisi dans le groupe constitué d'un radionucléide, d'un immunomodulateur, d'une hormone, d'un antagoniste d'hormone, d'une enzyme, d'un inhibiteur d'enzyme, d'un agent thérapeutique photoactif, d'un agent cytotoxique, et d'une combinaison de ceux-ci ; en particulier ledit agent thérapeutique photoactif étant un chromogène ou un colorant.

53. Immunoconjugué pour utilisation selon la revendication 52, ledit agent cytotoxique étant un médicament ou une toxine ; en particulier ledit médicament étant choisi dans le groupe constitué d'agents antimitotiques, d'alkylation, antimétabolites, antiangiogenèse, apoptotiques, anthracyclines, alcaloïdes, inhibiteurs de COX-2 et antibiotiques et des combinaisons de ceux-ci ; ou ledit médicament étant choisi dans le groupe constitué de moutardes azotées, dérivés d'éthylénimine, alkylsulfonates, nitroso-urées, triazènes, analogues d'acide folique, anthracyclines, taxanes, inhibiteurs de COX-2, analogues de pyrimidine, analogues de purine, antibiotiques, enzymes, inhibiteurs d'enzyme, épipodophyllotoxines, complexes de coordination de platine, alcaloïdes de pervenche, urées substituées, dérivés de méthylhydrazine, suppresseurs corticosurrénaux, hormones, antagonistes d'hormone, inhibiteurs d'enzyme, endostatine, taxols, camptothécines, doxorubicines et leurs analogues, et une combinaison de ceux-ci ; ou ladite toxine étant choisie dans le groupe constitué de toxines végétales, microbiennes, et animales ; en particulier ladite toxine étant choisie dans le groupe constitué de la ricine, l'abrine, la toxine alpha, la saporine, une ribonucléase (RNase), une DNase I, l'entérotoxine staphylococcique A, la protéine antivirale de phytolaque, la gélonine, la toxine diphtérique, l'exotoxine de *Pseudomonas,* et l'endotoxine de *Pseudomonas.*

54. Immunoconjugué pour utilisation selon la revendication 52, ledit immunomodulateur étant choisi dans le groupe constitué d'une cytokine, un facteur de croissance de cellule souche, une lymphotoxine, un facteur hématopoïétique, un facteur stimulant les colonies (CSF), un interféron (IFN), un facteur de croissance de cellule souche, l'érythropoïétine, la thrombopoïétine, un anticorps et une combinaison de ceux-ci ; en particulier, ledit anticorps est un anticorps anti-CD40 ou un fragment de celui-ci en particulier ladite lymphotoxine étant un facteur de nécrose tumorale (TNF), ledit facteur hématopoïétique étant une interleukine (IL), ledit facteur stimulant les colonies étant le facteur stimulant les colonies de granulocytes (G-CSF) ou le facteur stimulant les colonies de granulocytes macrophages (GM-CSF), ledit interféron étant les interférons α, β ou γ, ledit facteur de croissance de cellule souche étant appelé « facteur S1 » ; en particulier ladite cytokine étant choisie dans le groupe constitué d'IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, interféron γ, TNF-α et une combinaison de ceux-ci.

55. Immunoconjugué pour utilisation selon la revendication 52, ledit radionucléide étant choisi dans le groupe constitué d'un émetteur Auger, un émetteur β et un émetteur α ; en particulier ledit radionucléide étant un émetteur Auger et ayant une énergie inférieure à 1000 keV, ou ledit radionucléide étant un émetteur β et ayant une énergie comprise entre 20 et 5000 keV ; ou ledit radionucléide étant un émetteur α et ayant une énergie comprise entre 2000 et 10 000 keV.

56. Immunoconjugué pour utilisation selon la revendication 52, ledit radionucléide étant choisi dans le groupe constitué de P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, 1-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, et Bi-213, Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, 1-125, Ho-161, Os-189m, Ir-192, Dy-152, At-2II, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213, Fm-255 et des combinaisons de ceux-ci.

57. Immunoconjugué pour utilisation selon la revendication 52, ledit radionucléide ayant une énergie comprise entre 2000 et 10 000 keV.

58. Anticorps ou fragment de celui-ci selon la revendication 28 ou 29 pour utilisation dans un procédé de transfert d'un agent de diagnostic/détection, un agent thérapeutique, ou une combinaison de ceux-ci, vers une cible comprenant : (i) l'administration à un sujet desdits anticorps ou fragments ; (ii) l'attente d'un temps suffisant pour qu'une quantité de la protéine non liée soit éliminée de la circulation sanguine du sujet ; et (iii) l'administration audit sujet d'une molécule porteuse comprenant un agent de diagnostic/détection, un agent thérapeutique, ou une combinaison de ceux-ci, qui se lie à un site de liaison dudit anticorps.

59. Anticorps ou fragment pour utilisation selon la revendication 58, ladite molécule porteuse se liant à plus d'un site de liaison de la protéine de liaison.

60. Anticorps ou fragment pour utilisation selon la revendication 58, ledit agent de diagnostic/détection ou ledit agent thérapeutique étant choisis dans le groupe comprenant des isotopes, des colorants, des chromogènes, des agents

de contraste, des médicaments, des toxines, des cytokines, des enzymes, des inhibiteurs d'enzyme, des hormones, des antagonistes d'hormone, des facteurs de croissance, des radionucléides, et des métaux.

61. Quantité thérapeutiquement efficace d'un anticorps ou fragment de celui-ci ou protéine de fusion d'anticorps ou fragment de celle-ci comprenant au moins deux MAb ou fragments de ceux-ci pour utilisation dans un procédé de traitement d'une malignité chez un sujet comprenant l'administration de ladite quantité audit sujet, au moins un MAb anti-CSAp ou un fragment de celui-ci ou des protéines de fusion ou des fragments de ceux-ci sont l'une quelconque des revendications 1 à 35 formulés dans un excipient pharmaceutiquement adapté.

62. Quantité thérapeutiquement efficace d'un anticorps ou un fragment de celui-ci comprenant au moins deux MAb ou des fragments de ceux-ci pour utilisation dans un procédé de traitement d'une malignité chez un sujet comprenant l'administration de ladite quantité audit sujet, lesdits MAb étant sélectionnés parmi l'une quelconque des revendications 1 à 35, et formulés dans un excipient pharmaceutiquement adapté.

63. Composition pour utilisation selon la revendication 62, comprenant en outre un deuxième Mab ou un fragment de celui-ci n'étant pas dans l'une quelconque des revendications 1 à 35 ; en particulier ledit deuxième Mab ou fragment de celui-ci étant un Mab nu ou un fragment de celui-ci, ou ledit deuxième MAb ou fragment de celui-ci étant choisi dans le groupe constitué d'anticorps contre BrE3, Le-Y, EGP-1, EGP-2, MUC-1, MUC-2, MUC-3, MUC-4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, A3, KS-1, CEA, VEGF, produits oncogènes, et un anticorps contre CD40 ou un autre immunomodulateur ; en particulier ledit deuxième MAb étant immunoconjugué avec un agent thérapeutique ou de diagnostic/détection.

64. Composition pour utilisation selon la revendication 61, ledit anticorps anti-CSAp étant administré par voie parentérale ; en particulier ledit anticorps anti-CSAp étant administré à une dose de 20 à 2000 milligrammes de protéine par dose ; en particulier ladite dose étant administrée de façon répétée.

65. Composition pour utilisation selon la revendication 61, les régions constantes et de charnière dudit anticorps anti-CSAp humanisé comprenant des régions constantes et de charnière d'un IgG1 humain.

66. Composition pour utilisation selon la revendication 61, ledit anticorps anti-CSAp ou fragment de celui-ci étant administré avant, conjointement avec, ou après un deuxième anticorps conjugué réactif avec un deuxième marqueur de tumeur exprimé par ladite malignité étant administré audit sujet ; ou ledit anticorps anti-CSAp ou fragment de celui-ci étant administré avant, simultanément, ou après au moins un agent thérapeutique ou de diagnostic/détection, en particulier ledit agent thérapeutique ou de diagnostic/détection étant conjugué avec un anticorps qui cible un marqueur de tumeur qui est exprimé par ladite malignité.

67. Composition pour utilisation selon la revendication 61, un premier site de liaison de l'anticorps anti- CSAp ou du fragment de celui-ci étant présent dans une protéine de fusion multispécifique multivalente ou conjugué chimique et un deuxième site de liaison est réactif avec une substance de marqueur de tumeur autre que CSAp.

68. Quantité efficace sur le plan diagnostique d'un conjugué de diagnostic/détection comprenant un MAb anti-CSAp ou fragment de celui-ci ou une protéine de fusion ou un fragment de celui-ci de l'une quelconque des revendications 1 à 35, ledit MAb anti-CSAp ou fragment de celui-ci ou protéine de fusion ou fragment de celui-ci étant lié à au moins un agent de diagnostic/détection, formulé dans un excipient pharmaceutiquement adapté, pour utilisation dans un procédé de diagnostic ou de détection d'une malignité chez un sujet.

69. Quantité thérapeutiquement efficace d'une composition comprenant un MAb anti-CSAp nu ou un fragment de celui-ci ou une protéine de fusion d'anticorps nue ou un fragment de celle-ci de l'une quelconque des revendications 1 à 35 pour utilisation dans un procédé de traitement d'une cellule cancéreuse chez un sujet comprenant (i) l'administration de ladite quantité audit sujet, (ii) la formulation dudit MAb anti-CSAp nu ou fragment de celui-ci ou protéine de fusion d'anticorps ou fragment de celle-ci dans un excipient pharmaceutiquement adapté.

70. Composition pour utilisation selon la revendication 69, ladite composition comprenant en outre un deuxième anticorps nu ou un fragment de celui-ci n'étant pas selon les revendications 1 à 9, 28 à 29, et 31 à 35, en particulier ledit deuxième anticorps ou fragment de celui-ci étant choisi dans le groupe constitué d'anticorps contre CEA, EGP-1, EGP-2, MUC-1, MUC-2, MUC-3, MUC-4, PAM-4, KC4, TAG-72, EGFR, HER2/neu, BrE3, Le-Y, A3, KS-1, CD40, VEGF et d'autres facteurs d'angiogenèse, et des produits oncogènes ; ou ladite composition comprenant en outre un deuxième anticorps nu ou un fragment de celui-ci de l'une quelconque des revendications 1 à 9, 28 à 29, et 31

à 35 ; ou ladite composition comprenant en outre un deuxième anticorps ou un fragment de celui-ci n'étant pas dans l'une quelconque des revendications 1 à 35.

71. Composition pour utilisation selon la revendication 69, ledit anticorps anti-CSA nu étant administré par voie parentérale ; en particulier ledit anticorps anti-CSAp nu étant administré à une dose de 20 à 2000 milligrammes de protéine par dose ; plus particulièrement ladite dose étant administrée de façon répétée.

72. Composition pour utilisation selon la revendication 69, les régions constantes et de charnière dudit anticorps anti-CSAp nu humanisé comprenant des régions constantes et de charnière d'un IgG1 humain.

73. Composition pour utilisation selon la revendication 69, ledit anticorps anti-CSAp nu étant administré avant, conjointement avec, ou après qu'un deuxième anticorps nu réactif avec un deuxième marqueur tumoral exprimé par ladite malignité soit administré audit sujet ; ou ledit anticorps anti-CSAp nu étant administré avant, simultanément à ou après un agent thérapeutique ou de diagnostic/détection.

74. Composition pour utilisation selon les revendications 69 à 73, ledit anticorps anti-CSAp nu étant l'anticorps nu qui se lie à la mucine d'antigène p spécifique au côlon (CSAp).

75. Procédé de diagnostic ou de détection d'une malignité chez un sujet comprenant la conduite d'un essai diagnostique *in vitro* sur un spécimen dudit sujet avec une composition comprenant un MAb anti-CSAp nu ou un fragment de celui-ci ou une protéine de fusion d'anticorps ou fragment de celui-ci nue de l'une quelconque des revendications 1 à 35.

76. Procédé de la revendication 75, ladite malignité étant un carcinome ; en particulier (i) ledit carcinome étant un cancer gastro-intestinal, plus particulièrement ledit carcinome étant le cancer colorectal ou pancréatique ; ou (ii) ledit carcinome étant un cancer ovarien.

77. Procédé de la revendication 75, ledit essai de diagnostic *in vitro* est choisi dans le groupe constitué d'immunoessais, RT-PCR et immunohistochimie ; en particulier (i) ledit essai de diagnostic est RT-PCR ou des immunoessais ; plus particulièrement ledit spécimen est un fluide corporel ou un tissu ou une population de cellules ; ou (ii) ledit essai de diagnostic étant l'immunohistochimie ou l'immunocytochimie.

78. Procédé de la revendication 77, ledit spécimen étant une aliquote de cellules ou un tissu.

79. Procédé de l'une quelconque des revendications 35 à 41 et 45 à 78, ledit sujet étant un mammifère ; en particulier ledit sujet étant un humain ; un animal domestique ; ou ledit sujet étant choisi dans le groupe constitué d'un cheval, un chien, et un chat.

80. Anticorps bispécifique ou fragment d'anticorps ayant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable, ledit bras qui se lie spécifiquement à un tissu ciblé étant l'anticorps tel que défini dans l'une quelconque des revendications 1 à 8 pour utilisation dans un procédé de traitement ou d'identification de tissus malades chez un sujet comprenant :

(a) l'administration dudit anticorps ou fragment d'anticorps audit sujet ;
(b) facultativement, l'administration audit sujet d'une composition de clairance, et en laissant ladite composition éliminer les anticorps ou fragments d'anticorps non localisés de la circulation ;
(c) l'administration audit sujet d'un premier conjugué ciblable qui comprend une partie porteuse qui comprend ou porte au moins un épitope reconnaissable par ledit au moins un autre bras dudit anticorps bispécifique ou fragment d'anticorps, et un ou plusieurs agents thérapeutiques ou diagnostiques conjugués ; et
(d) lorsque ledit agent thérapeutique est une enzyme, l'administration en outre audit sujet de

(1) un promédicament, ladite enzyme étant capable de convertir ledit promédicament en médicament au site cible ; ou
(2) un médicament qui peut être détoxifié dans ledit sujet pour former un intermédiaire de toxicité plus faible, ladite enzyme étant capable de reconvertir ledit intermédiaire détoxifié en une forme toxique, et, par conséquent d'augmenter la toxicité dudit médicament au site cible, ou
(3) un promédicament qui est activé chez ledit sujet par des processus naturels et est soumis à une détoxification par conversion en intermédiaire de toxicité plus faible, ladite enzyme étant capable de re-

convertir ledit intermédiaire détoxifié en une forme toxique, et, par conséquent, d'augmenter la toxicité dudit médicament au site cible, ou
(4) un deuxième conjugué ciblable qui comprend une partie porteuse qui comprend ou porte au moins un épitope reconnaissable par ledit au moins un autre bras dudit anticorps bispécifique ou fragment d'anticorps, et un promédicament, ladite enzyme étant capable de convertir ledit promédicament en médicament au site cible.

81. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 80, ledit conjugué ciblable comprenant au moins deux haptènes HSG.

82. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 80, comprenant en outre, lorsque ledit premier conjugué ciblable comprend un promédicament, l'administration d'un deuxième conjugué ciblable qui comprend une partie porteuse qui comprend ou porte au moins un épitope reconnaissable par ledit au moins un autre bras dudit anticorps bispécifique ou fragment d'anticorps, et une enzyme capable de convertir ledit promédicament en médicament ou de reconvertir un intermédiaire détoxifié dudit médicament en une forme toxique.

83. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 80, ledit agent de diagnostic/détection étant un radionucléide.

84. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 80, ledit agent de diagnostic/détection étant un radionucléide ayant une énergie comprise entre 20 et 2 000 keV.

85. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 83, ledit agent de diagnostic/détection émettant de 25 à 600 keV de particules gamma et/ou positons ; ou ledit radionucléide est un isotope émetteur gamma, bêta ou de positons ; en particulier ledit radionucléide étant choisi dans le groupe constitué de 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, 1-124, Cr-51, Co-57, Co-58, Fe-59, Cu-67, Ga-67, Se-75, Ru-97, Tc-99m, In-111, In-114m, 1-123, I-125, I-131, Yb-169, Hg-197, et Tl-201.

86. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 80, ledit agent de diagnostic/détection comprenant au moins un agent de diagnostic photoactif ; en particulier, l'agent de diagnostic photoactif comprenant un chromogène ou un colorant.

87. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 80, ledit agent de diagnostic/détection étant un composé radio-opaque ; en particulier, ledit composé radio-opaque étant choisi dans le groupe constitué de composés d'iode, de composés de baryum, de composés de gallium et de composés de thallium ; en particulier, ledit composé radio-opaque étant choisi dans le groupe constitué des baryum, diatrizoate, huile éthiodisée, citrate de gallium, acide iocarmique, acide iocétamique, iodamide, iodipamide, acide iodoxamique, iogulamide, iohexol, iopamidol, acide iopanoïque, acide ioprocémique, acide ioséfamique, acide iosérique, iosulamide méglumine, acide iosémétique, iotasul, acide iotétrique, acide iothalamique, acide iotroxique, acide ioxaglique, acide ioxotrizoïque, ipodate, méglumine, métrizamide, métrizoate, propyliodone, et chlorure thalleux.

88. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 80, ledit agent de diagnostic/détection étant un agent de contraste.

89. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 88, ledit agent de contraste étant un ion paramagnétique ; en particulier ledit ion paramagnétique comprenant un métal choisi dans le groupe constitué du manganèse, du fer et du gadolinium.

90. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 88, ledit agent de contraste étant un agent accentuant les ultrasons ; en particulier, ledit agent accentuant les ultrasons étant un liposome qui est conjugué à l'anticorps humanisé ou un fragment de celui-ci ; plus particulièrement, ledit liposome étant rempli de gaz.

91. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 80, ledit agent thérapeutique étant choisi dans le groupe constitué d'un radionucléide, d'atomes de bore, de gadolinium ou d'uranium, d'un immunomodulateur, d'une cytokine, d'une hormone, d'un antagoniste d'hormone, d'une enzyme, d'un inhibiteur d'enzyme, d'un agent thérapeutique photoactif, d'un agent cytotoxique, d'un inhibiteur d'angiogenèse, et d'une combinaison de ceux-ci.

92. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 91, ledit agent cytotoxique étant un médicament, un promédicament ou une toxine ; en particulier ledit médicament étant choisi dans le groupe constitué des glucuronide d'épirubicine, CPT-11, glucuronide d'étoposide, glucuronide de daunomicine et glucuronide de doxorubicine ; en particulier ladite toxine étant choisie dans le groupe constitué d'une toxine de plante, d'une toxine animale et d'une toxine microbienne ; ou ladite toxine est choisie dans le groupe constitué de la ricine, l'abrine, une ribonucléase (RNase), une DNase I, l'entérotoxine staphylococcique A, la protéine antivirale de phytolaque, la gélonine, la toxine diphtérique, l'exotoxine de *Pseudomonas*, et l'endotoxine de *Pseudomonas* ; en particulier ledit médicament étant choisi dans le groupe constitué d'agents antimitotiques, d'alkylation, antimétabolites, inhibiteurs d'angiogenèse, apoptotiques, alcaloïdes, inhibiteurs de COX-2 et antibiotiques et des combinaisons de ceux-ci ; ou ledit médicament étant choisi dans le groupe constitué de moutardes azotées, dérivés d'éthylénimine, alkylsulfonates, nitroso-urées, triazènes, analogues d'acide folique, anthracyclines, taxanes, inhibiteurs de COX-2, analogues de pyrimidine, analogues de purine, antibiotiques, enzymes, épipodophyllotoxines, complexes de coordination de platine, alcaloïdes de pervenche, urées substituées, dérivés de méthylhydrazine, suppresseurs corticosurrénaux, hormones, antagonistes d'hormone, endostatine, taxols, camptothécines, doxorubicines et leurs analogues, et une combinaison de ceux-ci

93. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 91, ledit immunomodulateur étant choisi dans le groupe constitué d'une cytokine, un facteur de croissance de cellule souche, une lymphotoxine, un facteur hématopoïétique, un facteur stimulant les colonies (CSF), un interféron (IFN), un facteur de croissance de cellule souche, l'érythropoïétine, la thrombopoïétine, un anticorps agoniste ou antagoniste d'un immunomodulateur, et une combinaison de ceux-ci ; en particulier, ladite lymphotoxine étant un facteur de nécrose tumorale (TNF), ledit facteur hématopoïétique étant une interleukine (IL), ledit facteur stimulant les colonies étant le facteur stimulant les colonies de granulocytes (G-CSF) ou le facteur stimulant les colonies de granulocytes macrophages (GM-CSF), ledit interféron étant les interférons α, β ou γ, ledit facteur de croissance de cellule souche étant appelé « facteur S1 ».

94. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 93, ladite cytokine étant choisie dans le groupe constitué d'IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, interféron γ, TNF-α et une combinaison de ceux-ci.

95. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 91, ledit radionucléide étant choisi dans le groupe constitué d'un émetteur Auger, un émetteur β et un émetteur α ; ou ledit radionucléide étant choisi dans le groupe constitué de P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, 1-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, et Bi-213, Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, I-125, Ho-161, Os-189m, Ir-192, Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213, Fm-255 et des combinaisons de ceux-ci.

96. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 91, ledit atome de bore étant B-10, et/ou ledit atome de gadolinium étant Gd-157, et/ou ledit atome d'uranium étant U-235.

97. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 95, ledit radionucléide ayant une énergie comprise entre 20 et 10 000 keV ; ou ledit radionucléide étant un émetteur Auger et ayant une énergie inférieure à 1000 keV ; ou ledit radionucléide étant un émetteur β et ayant une énergie comprise entre 20 et 5000 keV ; ou ledit radionucléide étant un émetteur et ayant une énergie comprise entre 2000 et 10 000 keV.

98. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 80, ledit conjugué ciblable comprenant un ou plusieurs isotopes radioactifs utiles pour tuer un tissu malade ; en particulier ledit conjugué ciblable comprend des atomes 10B, et ledit procédé comprenant en outre l'étape d'irradiation desdits atomes de bore localisés audit tissu malade, de manière à effectuer un BNCT dudit tissu malade ; ou le conjugué ciblable comprenant un ou plusieurs agents pour thérapie photodynamique, en particulier ledit agent pour thérapie photodynamique étant un photosensibilisateur, plus particulièrement ledit photosensibilisateur étant choisi dans le groupe constitué du cycle A monoacide de benzoporphyrine (BPD-MA), de l'étiopurpurine d'étain (SnET2), de la phtalocyanone d'aluminium sulfonée (AlSPc) et de la texaphyrine de lutétium (Lutex).

99. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 80, ledit tissu ciblé étant une tumeur ; en particulier ladite tumeur produisant ou étant associée à l'antigène p spécifique au côlon (CSAp).

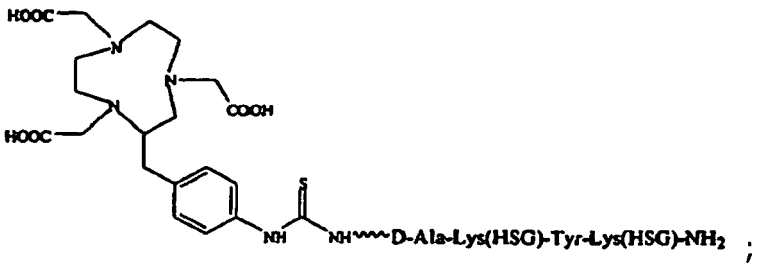
100. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 80, ledit anticorps ou fragment de celui-ci qui se lie à la mucine d'antigène p spécifique au côlon (CSAp) comprenant le Fv de la MAb de l'une quelconque

des revendications 1 à 8.

101. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 80, ledit anticorps bispécifique étant une protéine de fusion ; en particulier la protéine de fusion étant trivalente, et incorporant le Fv d'un anticorps réactif avec CSAp.

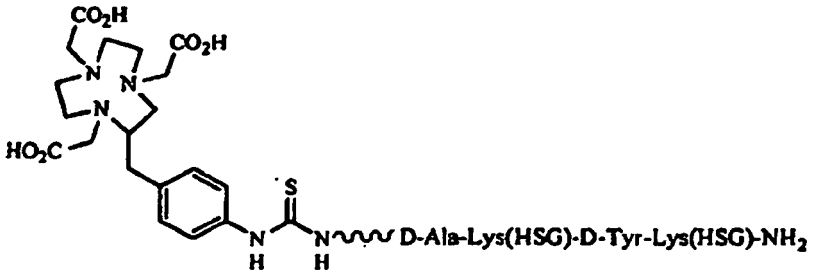
102. Quantité efficace d'un anticorps bispécifique ou un fragment d'anticorps comprenant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable, ledit bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 et un conjugué ciblable choisi dans le groupe constitué de

(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)



;

et
(v)



pour utilisation dans un procédé pour détecter ou traiter des tumeurs exprimant CSAp chez un mammifère, comprenant l'administration dudit anticorps ou fragment d'anticorps et l'administration dudit conjugué.

103. Substances pour utilisation selon la revendication 102, comprenant en outre l'administration audit sujet d'une composition de clairance, et en laissant ladite composition éliminer les anticorps ou fragments d'anticorps non localisés de la circulation.

104. Trousse utile pour traiter ou identifier des tissus malades chez un sujet comprenant :

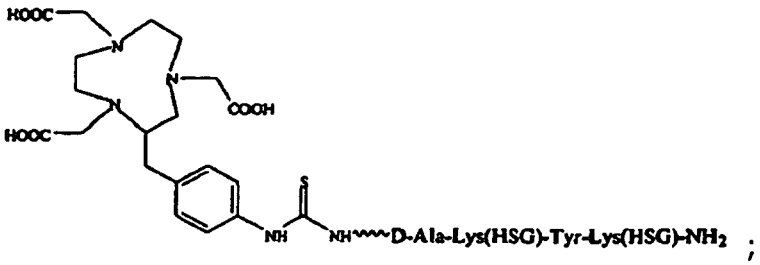
(a) anticorps bispécifique ou fragment d'anticorps ayant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable, ledit bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 ;
(b) un premier conjugué ciblable qui comprend une partie porteuse qui comprend ou porte au moins un épitope reconnaissable par ledit au moins un autre bras dudit anticorps bispécifique ou fragment d'anticorps, et un ou plusieurs agents thérapeutiques ou diagnostiques conjugués ; et
(c) facultativement, une composition de clairance utile pour éliminer les anticorps et fragments d'anticorps non localisés ; et

(d) facultativement, lorsque ledit agent thérapeutique conjugué audit premier conjugué ciblable est une enzyme,

(1) un promédicament, ladite enzyme étant capable de convertir ledit promédicament en médicament au site cible ; ou
(2) un médicament qui peut être détoxifié dans ledit sujet pour former un intermédiaire de toxicité plus faible, ladite enzyme étant capable de reconvertir ledit intermédiaire détoxifié en une forme toxique, et, par conséquent d'augmenter la toxicité dudit médicament au site cible, ou
(3) un promédicament qui est activé chez ledit sujet par des processus naturels et est sujet à une détoxification par conversion en intermédiaire de toxicité plus faible, ladite enzyme étant capable de reconvertir ledit intermédiaire détoxifié en une forme toxique, et, par conséquent, d'augmenter la toxicité dudit médicament au site cible, ou
(4) un deuxième conjugué ciblable qui comprend une partie porteuse qui comprend ou porte au moins un épitope reconnaissable par ledit au moins un autre bras dudit anticorps bispécifique ou fragment d'anticorps, et un promédicament, ladite enzyme étant capable de convertir ledit promédicament en médicament au site cible.

105. Trousse de la revendication 104, ledit conjugué ciblable étant choisi dans le groupe constitué de :

(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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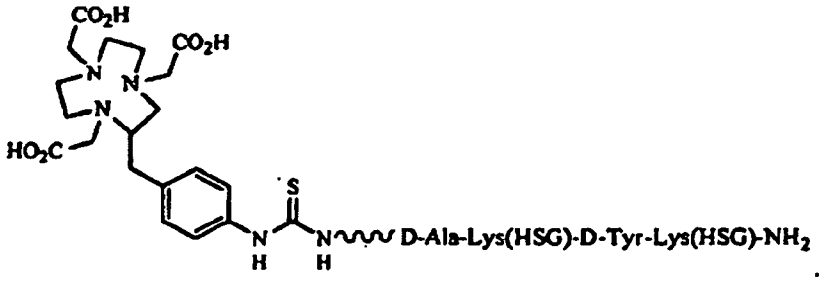

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106. Procédé *in vitro* de criblage d'un conjugué ciblable comprenant :

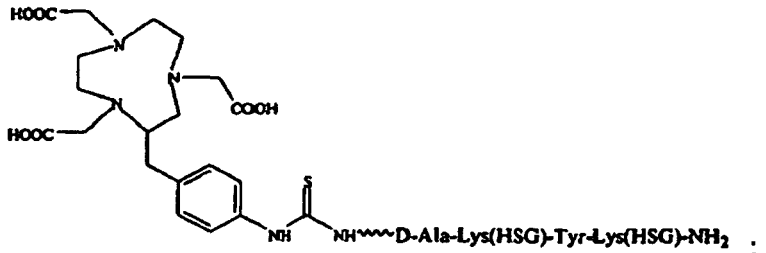
(a) la mise en contact de ladite construction ciblable avec un anticorps bispécifique ou un fragment d'anticorps ayant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement audit conjugué ciblable pour obtenir un mélange, ledit bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 ; et
(b) facultativement l'incubation dudit mélange ; et
(c) l'analyse dudit mélange.

107. Quantité efficace d'un anticorps bispécifique ou un fragment d'anticorps comprenant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable, ledit

bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 ; pour utilisation dans un procédé pour l'imagerie de tissu malin ou de cellules malignes dans un mammifère comprenant :

(a) l'administration de la quantité efficace de l'anticorps bispécifique ou fragment d'anticorps et
(b) l'administration d'un conjugué ciblable choisi dans le groupe constitué de

(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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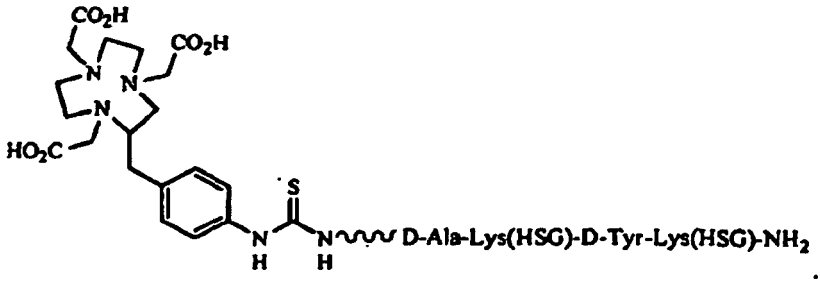

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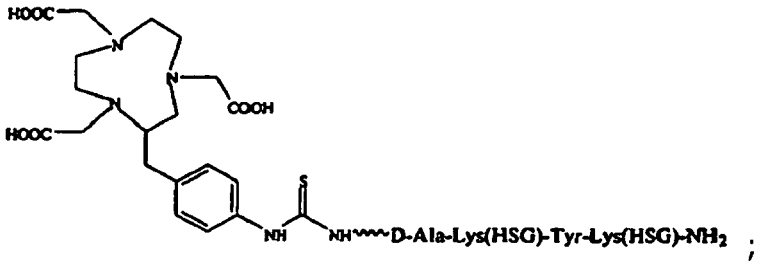
108. Quantité efficace d'un anticorps bispécifique ou un fragment d'anticorps comprenant au moins un bras qui se lie spécifiquement à un tissu ciblé exprimant CSAp et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable, ledit bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou un fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 pour utilisation dans un procédé d'identification / mise en évidence peropératoire de tissus malades exprimant CSAp, chez un sujet, comprenant :

(a) l'administration de la quantité efficace de l'anticorps bispécifique ou fragment d'anticorps et
(b) l'administration d'un conjugué ciblable choisi dans le groupe constitué de

(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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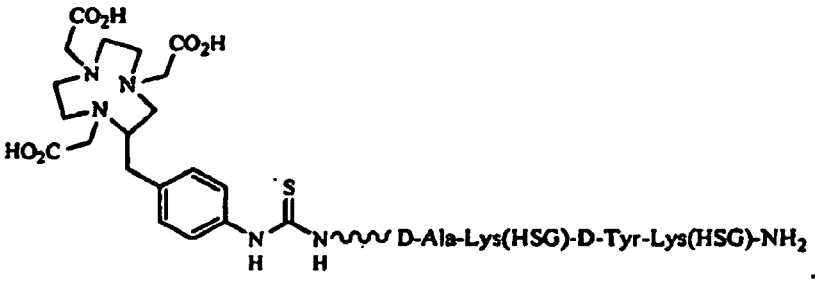
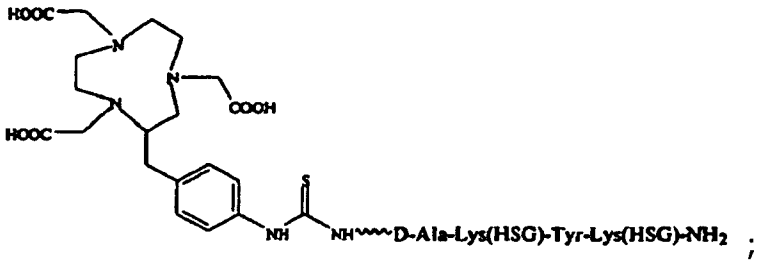


109. Quantité efficace d'un anticorps bispécifique ou un fragment d'anticorps comprenant au moins un bras qui se lie spécifiquement à un tissu ciblé exprimant CSAp et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable ; ledit bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou un fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 pour utilisation dans un procédé pour l'identification endoscopique de tissus malades exprimant CSAp, chez un sujet, comprenant :

(a) l'administration de la quantité efficace de l'anticorps bispécifique ou fragment d'anticorps et
(b) l'administration d'un conjugué ciblable choisi dans le groupe constitué de

(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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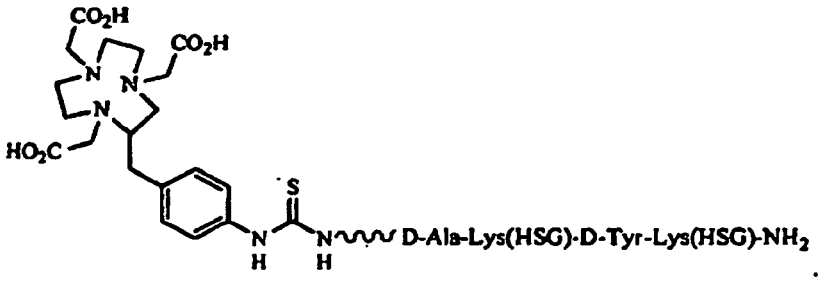
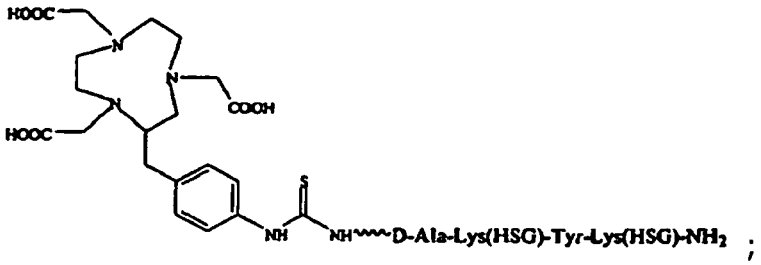


110. Quantité efficace d'un anticorps bispécifique ou un fragment d'anticorps comprenant au moins un bras qui se lie spécifiquement à un tissu ciblé exprimant CSAp et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable, ledit bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 pour utilisation dans un procédé pour l'identification intravasculaire de tissus malades exprimant CSAp, chez un sujet, comprenant :

(a) l'administration de la quantité efficace de l'anticorps bispécifique ou fragment d'anticorps et
(b) l'administration d'un conjugué ciblable choisi dans le groupe constitué de

(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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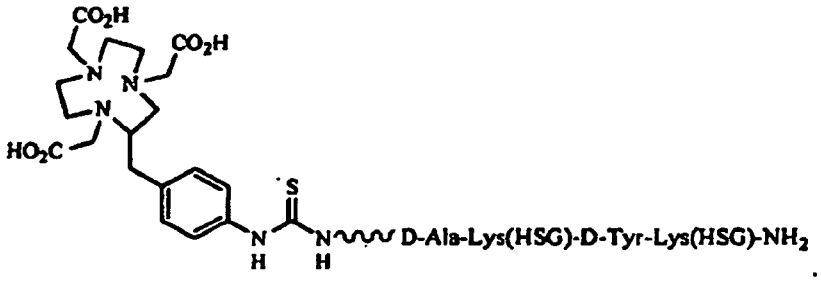


111. Fragment d'anticorps bispécifique $F(ab)_2$ ou $F(ab')_2$, l'anticorps bispécifique ou fragment ayant un premier site de liaison d'anticorps qui se lie spécifiquement à un antigène CSAp comme défini dans l'une quelconque des revendications 1 à 8, et a un deuxième site de liaison d'anticorps qui se lie spécifiquement à un haptène pour utilisation dans un procédé de détection de lésions pendant une procédure endoscopique, laparoscopique, de cathéter intravasculaire, ou chirurgicale, le procédé comprenant :

(a) l'injection à un sujet qui doit subir une telle procédure du fragment d'anticorps bispécifique $F(ab)_2$ ou $F(ab')_2$, et en laissant le fragment d'anticorps s'accumuler aux sites cibles ;
(b) éventuellement éliminer les fragments d'anticorps non ciblés en utilisant un agent de clairance anti-idiotype

galactosylé si le fragment bispécifique n'est pas en grande partie éliminé de la circulation dans un délai d'environ 24 heures après l'injection, et l'injection d'un haptène marqué bivalent, qui est rapidement localisé au site cible et est éliminé par les reins ;
(c) la détection de la présence de l'haptène par détection à courte portée de taux élevés de marqueur accumulé aux sites cibles avec des moyens de détection, dans un délai de 48 heures après la première injection, et la conduite de ladite procédure, ladite détection étant effectuée sans l'utilisation d'un agent de contraste ou d'un agent de soustraction.

112. Procédé de la revendication 111, ledit haptène étant marqué avec un radio-isotope diagnostique, un agent d'accentuation d'image IRM ou un marqueur fluorescent.

113. Quantité efficace d'un immunoconjugué ou fragment de celui-ci tel que défini dans l'une quelconque des revendications précédentes pour utilisation dans un procédé pour détection de lésion à courte portée, pendant une procédure opératoire, intravasculaire, laparoscopique, ou endoscopique, le procédé comprenant :

(a) l'injection à un sujet qui doit subir une telle procédure par voie parentérale de la quantité efficace de l'immunoconjugué ou fragment de celui-ci,
(b) la conduite de la procédure dans un délai de 48 heures après l'injection ;
(c) le balayage de l'intérieur accédé du sujet à courte portée avec un moyen de détection pour détecter la présence dudit anticorps marqué ou fragment de celui-ci ; et
(d) la localisation des sites d'accumulation dudit anticorps marqué ou fragment de celui-ci par détection de taux élevés dudit anticorps marqué ou fragment de celui-ci à de tels sites avec les moyens de détection.

114. Substances pour utilisation selon la revendication 113, ledit immunoconjugué ou fragment de celui-ci comprenant un radioisotope qui émet à une énergie de 20 à 1 000 keV ; en particulier le radioisotope étant choisi dans le groupe constitué des technétium-99m, iode-125, iode-131, iode-123, indium-111, fluor-18, gallium-68 et gallium-67M ; ou l'immunoconjugué ou fragment de celui-ci comprenant un agent non isotopique, en particulier ledit agent non isotopique étant un agent photoactif ; plus particulièrement ledit agent photoactif étant un agent fluorescent.

115. Anticorps bispécifique ou fragment d'anticorps ayant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable comprenant au moins deux haptènes HSG, l'au moins un autre bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou un fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 pour utilisation dans un procédé de traitement ou d'identification de tissus malades chez un sujet, comprenant :

(a) l'administration dudit anticorps ou fragment d'anticorps audit sujet ;
(b) facultativement, l'administration audit sujet d'une composition de clairance, et en laissant ladite composition éliminer les anticorps ou fragments d'anticorps non localisés de la circulation ;
(c) l'administration audit sujet d'un conjugué ciblable qui comprend une partie porteuse qui comprend ou porte au moins deux haptènes HSG et peut comprendre un cation diagnostique ou thérapeutique, et/ou un ou plusieurs agents thérapeutiques ou diagnostiques chélatés ou chimiquement liés, ou des enzymes ; et
(d) lorsque ledit conjugué ciblable comprend une enzyme, en outre l'administration audit sujet de

(1) un promédicament, ladite enzyme étant capable de convertir ledit promédicament en médicament au site cible ; ou
(2) un médicament qui peut être détoxifié dans ledit sujet pour former un intermédiaire de toxicité plus faible, ladite enzyme étant capable de reconvertir ledit intermédiaire détoxifié en une forme toxique, et, par conséquent d'augmenter la toxicité dudit médicament au site cible, ou
(3) un promédicament qui est activé chez ledit sujet par des processus naturels et est sujet à une détoxification par conversion en intermédiaire de toxicité plus faible, ladite enzyme étant capable de reconvertir ledit intermédiaire détoxifié en une forme toxique, et, par conséquent, d'augmenter la toxicité dudit médicament au site cible.

116. Anticorps ou fragment d'anticorps selon la revendication 115, ledit agent de diagnostic émettant de 25 à 600 keV de particules gamma et/ou positons ; ou en particulier, ledit agent de diagnostic étant choisi dans le groupe constitué de 18F, 52Fe, 62Cu, 64Cu, 67Cu, 67Ga, 68Ga, 86Y, 89Zr, 94mTc, 94Tc, 99mTc, 111In, 123I, 124I, 125I, et 131I

117. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 115, ledit agent thérapeutique étant un

médicament, un promédicament ou une toxine ; en particulier ledit promédicament étant choisi dans le groupe constitué des glucuronide d'épirubicine, CPT-11, glucuronide d'étoposide, glucuronide de daunomicine et glucuronide de doxorubicine ; en particulier ladite toxine étant choisie dans le groupe constitué de la ricine, l'abrine, une ribonucléase, une DNase I, l'entérotoxine staphylococcique A, la protéine antivirale de phytolaque, la gélonine, la toxine diphtérique, l'exotoxine de *Pseudomonas,* et l'endotoxine de *Pseudomonas.*

118. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 115 comprenant en outre un nucléide thérapeutique ; en particulier ledit nucléide thérapeutique étant choisi dans le groupe constitué de 32P, 33P, 47Sc, 64Cu, 67Cu, 67Ga, 90Y, 111Ag, 111In, 125I, 131I, 142Pr, 153Sm, 161Tb, 166Dy, 166Ho, 177Lu, 186Re, 188Re, 189Re, 212Pb, 212Bi, 213Bi, 211At, 223Ra et 225Ac.

119. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 115, ledit conjugué ciblable comprenant un ou plusieurs isotopes radioactifs utiles pour tuer un tissu malade ; ou ledit conjugué ciblable comprenant des atomes 10B, et ledit procédé comprend en outre l'étape d'irradiation desdits atomes de bore localisés audit tissu malade, de manière à effectuer un BNCT dudit tissu malade.

120. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 115, ledit conjugué ciblable comprenant une ou plusieurs toxines ; en particulier ladite toxine étant choisie dans le groupe constitué de la ricine, l'abrine, une ribonucléase, une DNase I, l'entérotoxine staphylococcique A, la protéine antivirale de phytolaque, la gélonine, la toxine diphtérique, l'exotoxine de *Pseudomonas,* et l'endotoxine de *Pseudomonas.*

121. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 115, ledit conjugué ciblable comprenant un ou plusieurs médicaments ; ou ledit conjugué ciblable comprenant un ou plusieurs promédicaments ; en particulier ledit promédicament étant choisi dans le groupe constitué des glucuronide d'épirubicine, CPT-11, glucuronide d'étoposide, glucuronide de daunomicine et glucuronide de doxorubicine.

122. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 115, ledit conjugué ciblable comprenant un ou plusieurs agents de diagnostic utiles pour détecter un tissu malade, en particulier l'agent de diagnostic étant choisi dans le groupe constitué de 118F, 52Fe, 62Cu, 64Cu, 67Cu, 67Ga, 68Ga, 86Y, 89Zr, 94mTc, 94Tc, 99mTc, 111In, 123I, 124I, 125I, et 131I ; en particulier ledit isotope radioactif étant utilisé pour effectuer la tomographie par émission de positons (PET).

123. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 115, ledit conjugué ciblable comprenant un ou plusieurs agents d'accentuation d'image pour utilisation en imagerie par résonance magnétique (IRM), en particulier ledit agent d'accentuation étant choisi dans le groupe constitué de Mn, Fe et Gd ; ou le conjugué ciblable comprenant un ou plusieurs agents pour thérapie photodynamique, en particulier ledit agent pour thérapie photodynamique étant un photosensibilisateur, plus particulièrement ledit photosensibilisateur étant choisi dans le groupe constitué du cycle A monoacide de benzoporphyrine (BPD-MA), de l'étiopurpurine d'étain (SnET2), de la phtalocyanone d'aluminium sulfonée (AlSPc) et de la texaphyrine de lutétium (Lutex).

124. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 115, (i) ledit au moins un bras qui se lie spécifiquement à un tissu ciblé étant un anticorps monoclonal ou un fragment d'un anticorps monoclonal ; (ii) ledit au moins un autre bras qui se lie spécifiquement à un conjugué ciblable étant un anticorps monoclonal ou un fragment d'un anticorps monoclonal ; (iii) ledit au moins un bras qui se lie spécifiquement à un tissu ciblé étant un anticorps humain, chimérique ou humanisé ou un fragment d'un anticorps humain, chimérique ou humanisé ; ou (iv) ledit au moins un autre bras qui se lie spécifiquement à un conjugué ciblable étant un anticorps humain, chimérique ou humanisé ou un fragment d'un anticorps humain, chimérique ou humanisé.

125. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 115, ledit anticorps bispécifique ou fragment d'anticorps comprenant en outre un nucléide thérapeutique, en particulier ledit radionucléide thérapeutique étant choisi dans le groupe constitué de 32P, 33P, 47Sc, 64Cu, 67Cu, 67Ga, 90Y, 111Ag, 111In, 125I, 131I, 142Pr, 153Sm, 161Tb, 166Dy, 166Ho, 177Lu, 186Re, 188Re, 189Re, 212Pb, 212Bi, 213Bi, 211At, 223Ra et 225Ac.

126. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 115, ledit conjugué ciblable comprenant la doxorubicine, SN-38, l'étoposide, le méthotrexate, la 6-mercaptopurine ou l'étoposide phosphate.

127. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 115, ledit tissu ciblé étant une tumeur ; en particulier ladite tumeur produisant ou étant associée à l'antigène p spécifique au côlon (CSAp) ; plus particulièrement

l'anticorps bispécifique comprenant le Fv de MAb Mu9 et le Fv de MAb 679 ; encore plus particulièrement Mu9 et/ou 679 étant chimérisés ou humanisés, ou Mu9 et/ou 679 étant Mu9 et 679 humains, ou l'anticorps bispécifique comprenant un ou plusieurs des CDR de Mu9 ; ou l'anticorps bispécifique comprenant un ou plusieurs des CDR de 679.

128. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 127, l'anticorps bispécifique étant une protéine de fusion.

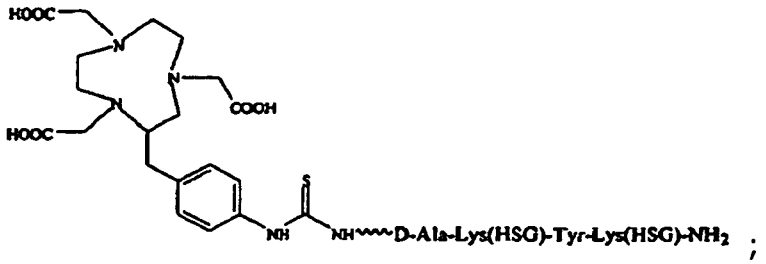
129. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 127, la tumeur produisant l'antigène carcinoembryonnaire (CEA) ; en particulier l'anticorps bispécifique comprenant le Fv de MAb MN14 et le Fv de MAb 679 ; plus particulièrement MN14, et/ou 679 étant chimérisés ou humanisés ; ou MN14, et/ou 679 étant MN14 et 679 humains ; ou l'anticorps bispécifique comprenant un ou plusieurs des CDR de MN14 ; ou l'anticorps bispécifique comprenant un ou plusieurs des CDR de 679 ; ou l'anticorps bispécifique étant une protéine de fusion, en particulier la protéine de fusion étant trivalente, et incorporant le Fv d'un anticorps réactif avec CSAp.

130. Anticorps ou fragment d'anticorps pour utilisation selon la revendication 129, l'anticorps bispécifique incorporant un anticorps anti-CEA de classe III et le Fv de 679.

131. Quantité efficace d'un anticorps bispécifique ou un fragment d'anticorps comprenant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable pour utilisation dans un procédé pour détecter ou traiter des cellules ou des tissus cibles dans un mammifère, comprenant :

l'administration dudit anticorps ou fragment d'anticorps audit mammifère ;
ledit au moins un bras étant capable de se lier à un fragment de liaison complémentaire sur les cellules ou tissus cibles ou sur une molécule produite par ou associée à celui-ci, un tel fragment étant l'antigène CSAp et ledit bras qui se lie spécifiquement à un tissu ciblé est l'anticorps ou un fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 ; et
l'administration d'un conjugué ciblable choisi dans le groupe constitué de

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2 ;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 ;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2 ;
(d)



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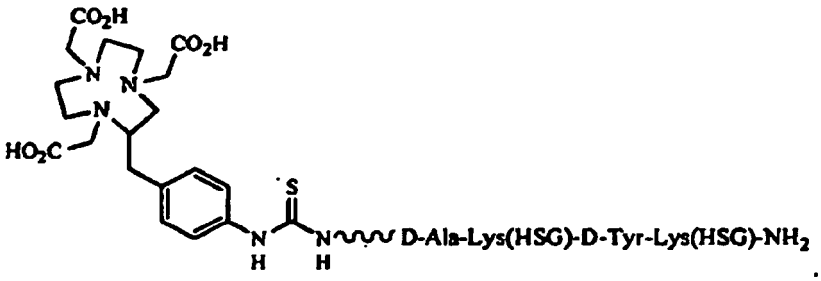

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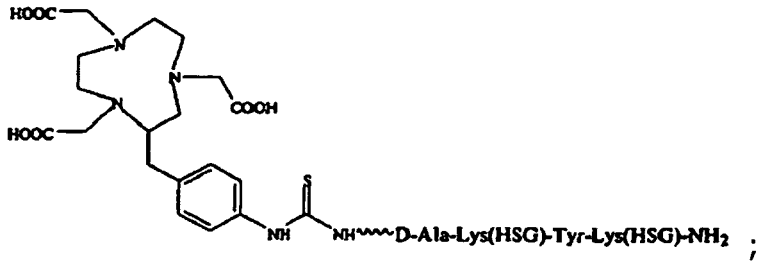
132. Anticorps bispécifique ou fragment d'anticorps ayant au moins un bras qui se lie spécifiquement à un tissu ciblé et

au moins un autre bras qui se lie spécifiquement à un conjugué ciblable pour utilisation dans un procédé de traitement ou d'identification de tissus malades chez un sujet, comprenant :

l'administration audit anticorps ou fragment d'anticorps audit sujet ;
facultativement, l'administration audit sujet d'une composition de clairance, et en laissant ladite composition éliminer les anticorps ou fragments d'anticorps non localisés de la circulation ; et
l'administration d'un conjugué ciblable choisi dans le groupe constitué de :

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2 ;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 ;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2 ;
(d)



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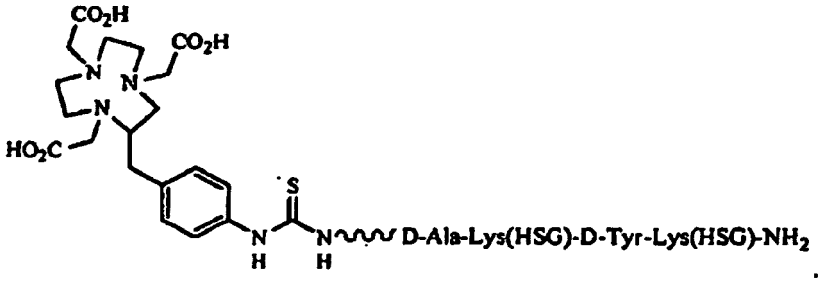

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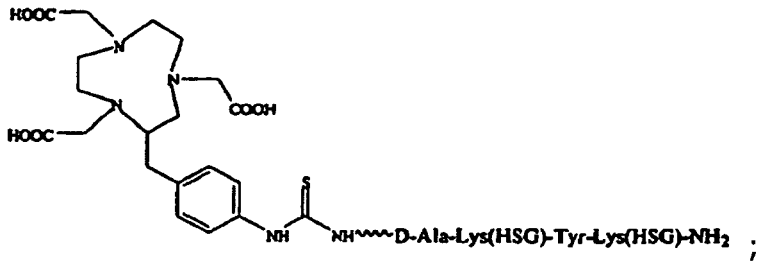
l'anticorps ou fragment d'anticorps étant un anticorps ou fragment d'anticorps selon l'une quelconque des revendications 1 à 8.

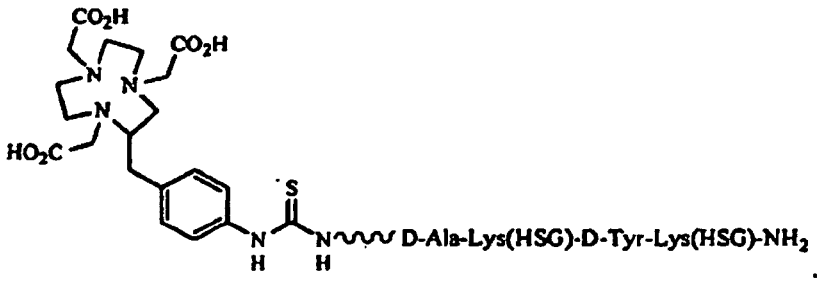
133. Trousse utile pour traiter ou identifier des tissus malades chez un sujet comprenant :

(a) un anticorps bispécifique ou un fragment d'anticorps ayant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable, le bras qui se lie spécifiquement au tissu ciblé étant un anticorps ou un fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8, et le conjugué étant choisi dans le groupe constitué de

(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)



et
(v)



(b) un conjugué ciblable qui comprend une partie porteuse qui comprend ou porte au moins un épitope reconnaissable par ledit au moins un autre bras dudit anticorps bispécifique ou fragment d'anticorps, et un ou plusieurs agents thérapeutiques ou diagnostiques conjugués, ou enzymes ; et
(c) éventuellement, une composition de clairance utile pour éliminer les anticorps et fragments d'anticorps non localisés ; et
(d) facultativement, lorsque ledit conjugué ciblable comprend une enzyme,

(1) un promédicament, ladite enzyme étant capable de convertir ledit promédicament en médicament au site cible ; ou
(2) un médicament qui peut être détoxifié dans ledit sujet pour former un intermédiaire de toxicité plus faible, ladite enzyme étant capable de reconvertir ledit intermédiaire détoxifié en une forme toxique, et, par conséquent d'augmenter la toxicité dudit médicament au site cible, ou
(3) un promédicament qui est activé chez ledit sujet par des processus naturels et est sujet à une détoxification par conversion en intermédiaire de toxicité plus faible, ladite enzyme étant capable de reconvertir ledit intermédiaire détoxifié en une forme toxique, et, par conséquent, d'augmenter la toxicité dudit médicament au site cible.

134. Procédé de criblage *in vitro* pour criblage pour un conjugué ciblable comprenant :

la mise en contact de ladite construction ciblable avec un anticorps bispécifique ou un fragment d'anticorps ayant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement audit conjugué ciblable pour obtenir un mélange ;
ledit au moins un bras étant capable de se lier à un fragment de liaison complémentaire sur les cellules cibles, tissus ou sur une molécule produite par ou associée à ceux-ci, le fragment de liaison étant l'antigène CSAp et ledit au moins un bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou un fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 ; et
facultativement l'incubation dudit mélange ; et
l'analyse dudit mélange.

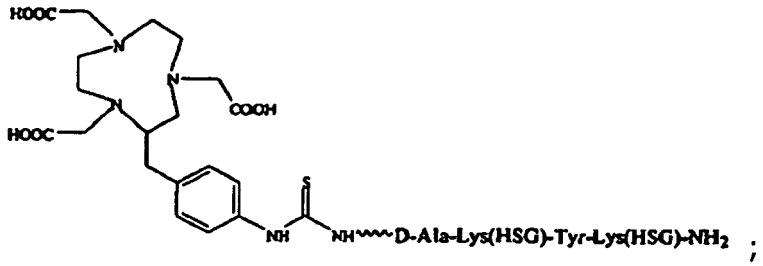
135. Procédé de la revendication 134, ladite analyse comprenant un procédé analytique choisi dans le groupe constitué de FABMS, RMN à haut champ ou CLHP d'exclusion.

136. Quantité efficace d'un anticorps bispécifique ou un fragment d'anticorps comprenant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable ;

ledit au moins un bras étant capable de se lier à un fragment de liaison complémentaire sur les cellules, tissus cibles ou sur une molécule produite par ou associée à ceux-ci, le fragment étant l'antigène CSAp et ledit au moins un bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou un fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 pour utilisation dans un procédé pour l'imagerie d'un tissu normal chez un mammifère, comprenant :

l'administration de la quantité efficace de l'anticorps bispécifique ou fragment d'anticorps ; et
l'administration d'un conjugué ciblable choisi dans le groupe constitué de

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2 ;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 ;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2 ;
(d)



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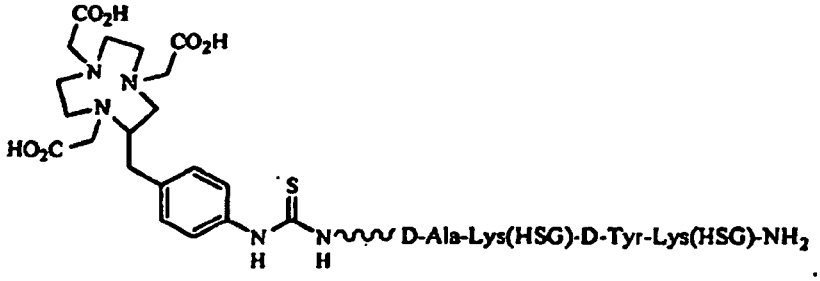

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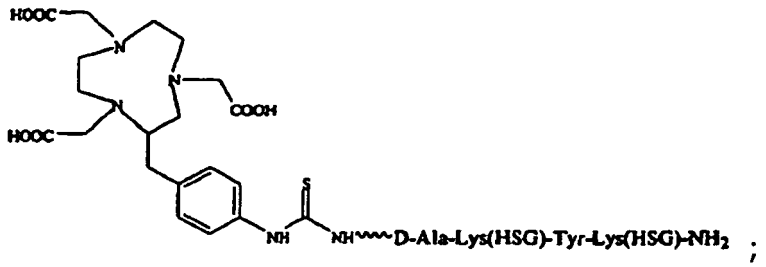
137. Substance pour utilisation selon la revendication 136, ledit tissu normal étant un tissu d'ovaire, de thymus, de parathyroïde ou de rate.

138. Quantité efficace d'un anticorps bispécifique ou un fragment d'anticorps comprenant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable ;
ledit au moins un bras étant capable de se lier à un fragment de liaison complémentaire sur les cellules, tissus cibles ou sur une molécule produite par ou associée à ceux-ci, le fragment étant l'antigène CSAp et ledit au moins un bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou un fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 pour utilisation dans un procédé d'identification peropératoire de tissus malades, chez un sujet, comprenant :

l'administration de la quantité efficace de l'anticorps bispécifique ou fragment d'anticorps et
l'administration d'un conjugué ciblable choisi dans le groupe constitué de

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2 ;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 ;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2 ;
(d)



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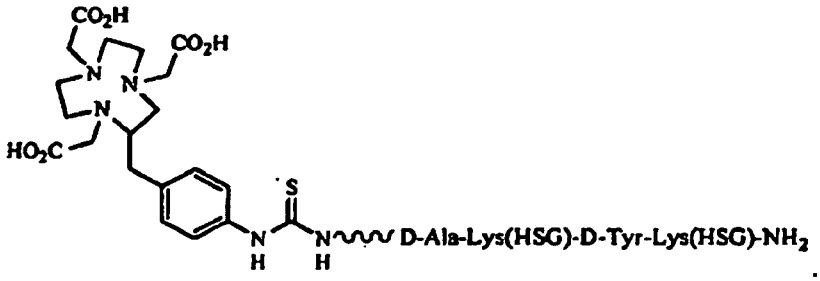
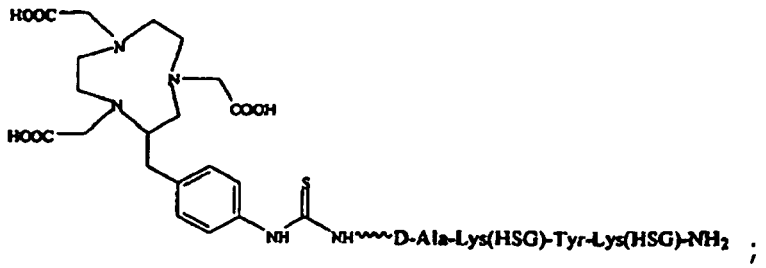


139. Quantité efficace d'un anticorps bispécifique ou un fragment d'anticorps comprenant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable ; ledit au moins un bras étant capable de se lier à un fragment de liaison complémentaire sur les cellules, tissus cibles ou sur une molécule produite par ou associée à ceux-ci, le fragment étant l'antigène CSAp et ledit au moins un bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou un fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 pour utilisation dans un procédé pour l'identification endoscopique de tissus malades, chez un sujet, comprenant :

l'administration de la quantité efficace de l'anticorps bispécifique ou fragment d'anticorps ; et
l'administration d'un conjugué ciblable choisi dans le groupe constitué de

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2 ;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 ;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2 ;
(d)



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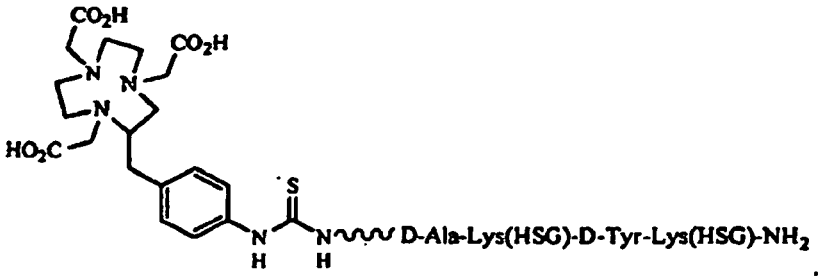
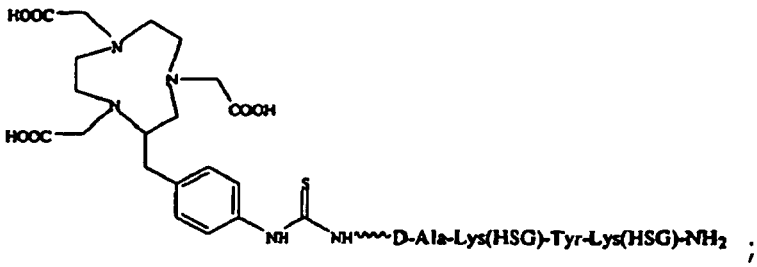


140. Quantité efficace d'un anticorps bispécifique ou un fragment d'anticorps comprenant au moins un bras qui se lie spécifiquement à un tissu ciblé et au moins un autre bras qui se lie spécifiquement à un conjugué ciblable ; ledit au moins un bras étant capable de se lier à un fragment de liaison complémentaire sur les cellules, tissus cibles ou sur une molécule produite par ou associée à ceux-ci, le fragment étant l'antigène CSAp et ledit au moins un bras qui se lie spécifiquement à un tissu ciblé étant un anticorps ou un fragment de celui-ci comme défini dans l'une quelconque des revendications 1 à 8 pour utilisation dans un procédé pour l'identification intravasculaire de tissus malades, chez un sujet, comprenant :

l'administration de la quantité efficace de l'anticorps bispécifique ou fragment d'anticorps ; et
l'administration d'un conjugué ciblable choisi dans le groupe constitué de

(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2 ;
(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 ;
(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2 ;
(d)



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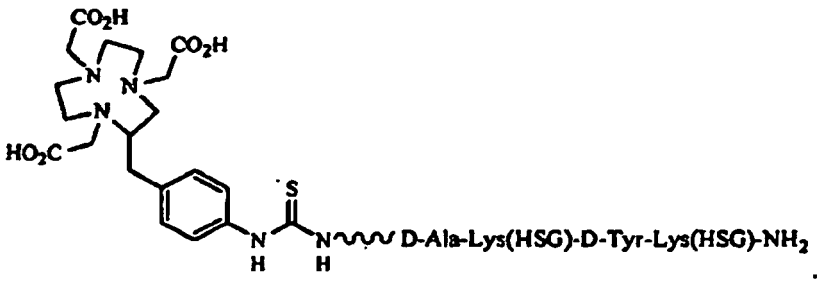

141. Anticorps bispécifique ou fragment d'anticorps, quantité efficace de l'anticorps bispécifique ou fragment d'anticorps, trousse ou procédé de l'une quelconque des revendications 131, 132, 133, 134, 136, 138, 139 ou 140 ledit conjugué ciblable comprenant en outre un agent de diagnostic choisi dans le groupe constitué de ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, ^{94m}Tc ^{94}Tc, ^{99m}Tc ^{111}In ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154\text{-}158}Gd$ et ^{175}Lu ; en particulier ledit conjugué ciblable comprenant en outre un nucléide thérapeutique choisi dans le groupe constitué de ^{32}P, ^{33}P, ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, $I^{66}Ho$, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{211}At, ^{223}Ra et ^{225}Ac.

```
GACATTGTGATGTCACAATCTCCATCCTCCCTGGCTGTGTCACCAGGAGAGAAGGTCACTATGACCTGCAAATCCAGTCAGAGTCTGTTC   90
 D  I  V  M  S  Q  S  P  S  S  L  A  V  S  P  G  E  K  V  T  M  T  C  K  S  S  Q  S  L  F   30

AACAGTAGAACCCGAAAGAACTACTTGGGTTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTTCTGATCTACTGGGCATCTACTCGG  180
 N  S  R  T  R  K  N  Y  L  G  W  Y  Q  Q  K  P  G  Q  S  P  K  L  L  I  Y  W  A  S  T  R   60
 CDR1                                                                          CDR2

GAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAACAGTGTGCAGTCTGAAGACCTGGCA  270
 E  S  G  V  P  D  R  F  T  G  S  G  S  G  T  D  F  T  L  T  I  N  S  V  Q  S  E  D  L  A   90

GTTTATTACTGCACTCAAGTTTATTATCTGTGCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAACGG                      339
 V  Y  Y  C  T  Q  V  Y  Y  L  C  T  F  G  A  G  T  K  L  E  L  K  R                      113
             CDR3
```

FIG. 1

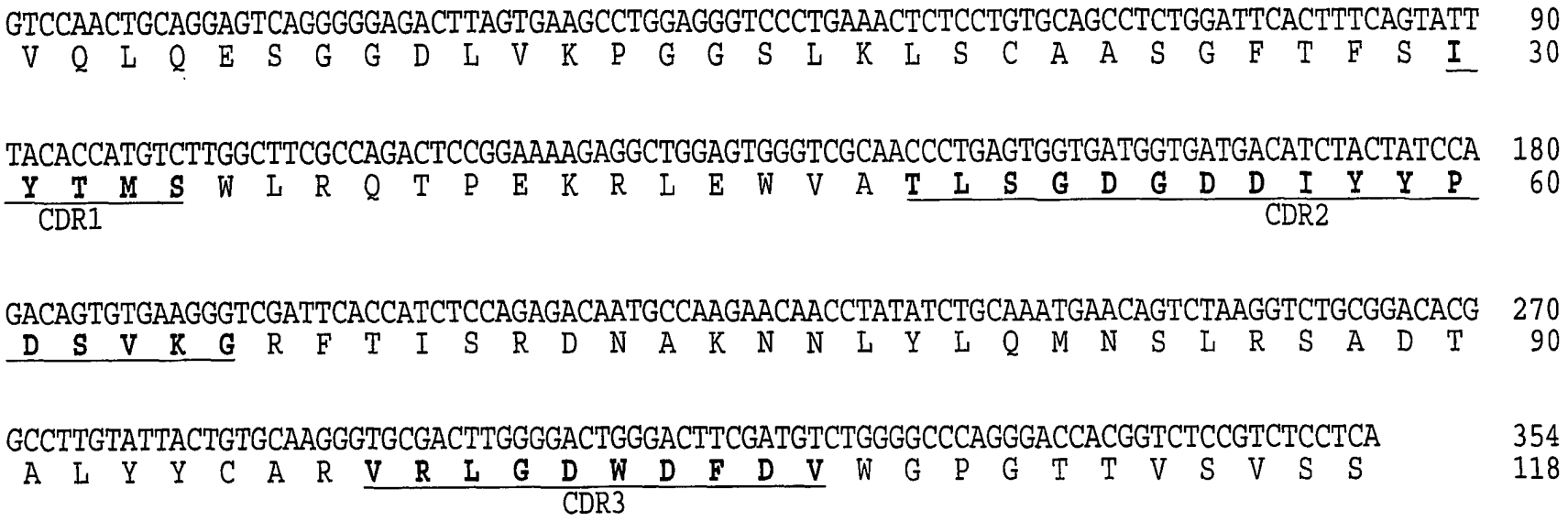
GTCCAACTGCAGGAGTCAGGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTATT 90
V Q L Q E S G G D L V K P G G S L K L S C A A S G F T F S I 30
TACACCATGTCTTGGCTTCGCCAGACTCCGGAAAAGAGGCTGGAGTGGGTCGCAACCCTGAGTGGTGATGGTGATGACATCTACTATCCA 180
Y T M S W L R Q T P E K R L E W V A T L S G D G D D I Y Y P 60
CDR1
CDR2
GACAGTGTGAAGGGTCGATTCACCATCTCCAGAGACAATGCCAAGAACAACCTATATCTGCAAATGAACAGTCTAAGGTCTGCGGACACG 270
D S V K G R F T I S R D N A K N N L Y L Q M N S L R S A D T 90
GCCTTGTATTACTGTGCAAGGGTGCGACTTGGGGACTGGGACTTCGATGTCTGGGGCCCAGGGACCACGGTCTCCGTCTCCTCA 354
A L Y Y C A R V R L G D W D F D V W G P G T T V S V S S 118
CDR3

FIG. 2

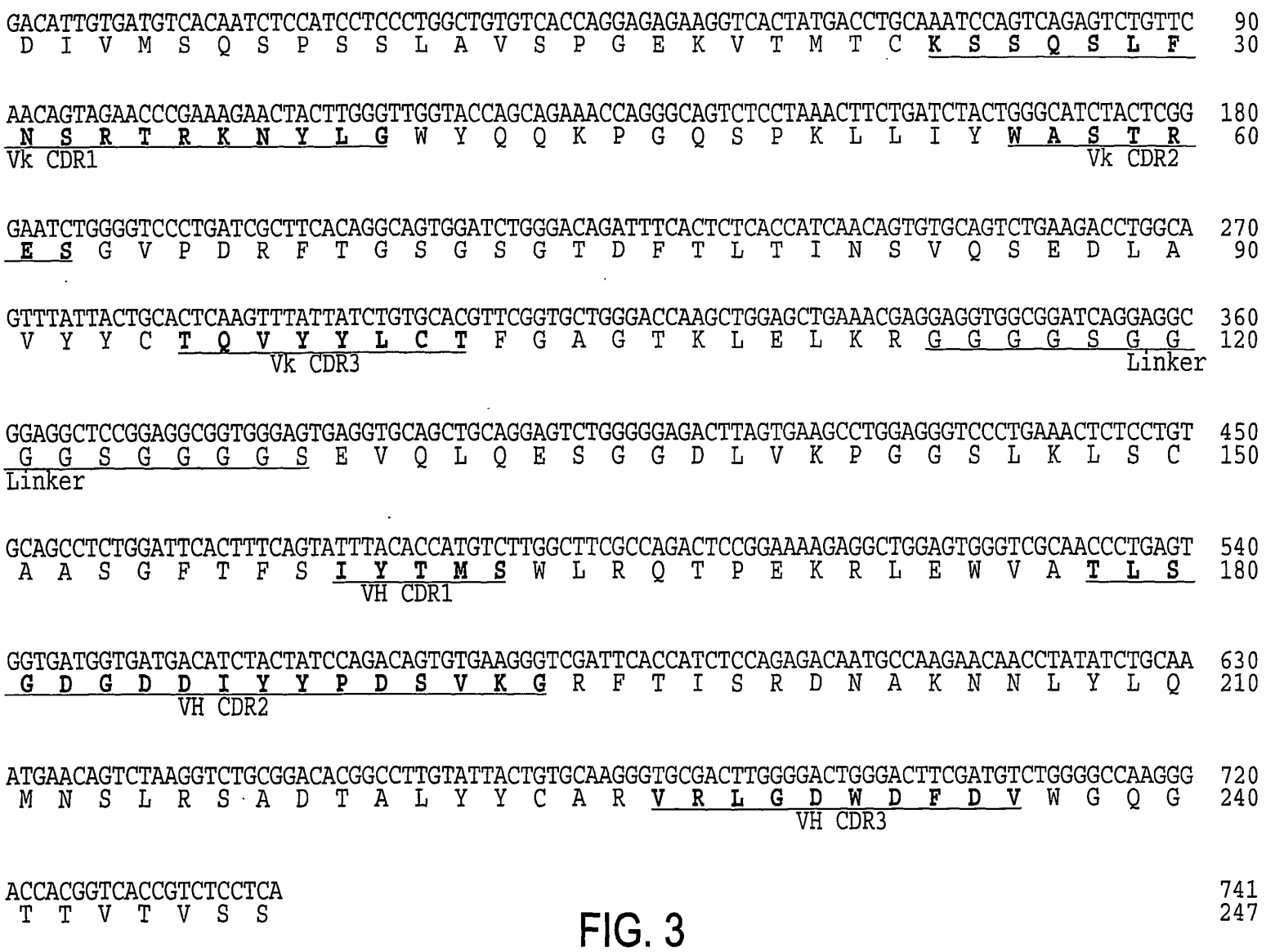
GACATTGTGATGTCACAATCTCCATCCTCCCTGGCTGTGTCACCAGGAGAGAAGGTCACTATGACCTGCAAATCCAGTCAGAGTCTGTTC 90
D I V M S Q S P S S L A V S P G E K V T M T C K S S Q S L F 30
AACAGTAGAACCCGAAAGAACTACTTGGGTTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTTCTGATCTACTGGGCATCTACTCGG 180
N S R T R K N Y L G W Y Q Q K P G Q S P K L L I Y W A S T R 60
Vk CDR1
Vk CDR2
GAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAACAGTGTGCAGTCTGAAGACCTGGCA 270
E S G V P D R F T G S G S G T D F T L T I N S V Q S E D L A 90
GTTTATTACTGCACTCAAGTTTATTATCTGTGCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAACGAGGAGGTGGCGGATCAGGAGGC 360
V Y Y C T Q V Y Y L C T F G A G T K L E L K R G G G G S G G 120
Vk CDR3
Linker
GGAGGCTCCGGAGGCGGTGGGAGTGAGGTGCAGCTGCAGGAGTCTGGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGAAACTCTCCTGT 450
G G S G G G G S E V Q L Q E S G G D L V K P G G S L K L S C 150
Linker
GCAGCCTCTGGATTCACTTTCAGTATTTACACCATGTCTTGGCTTCGCCAGACTCCGGAAAAGAGGCTGGAGTGGGTCGCAACCCTGAGT 540
A A S G F T F S I Y T M S W L R Q T P E K R L E W V A T L S 180
VH CDR1
GGTGATGGTGATGACATCTACTATCCAGACAGTGTGAAGGGTCGATTCACCATCTCCAGAGACAATGCCAAGAACAACCTATATCTGCAA 630
G D G D D I Y Y P D S V K G R F T I S R D N A K N N L Y L Q 210
VH CDR2
ATGAACAGTCTAAGGTCTGCGGACACGGCCTTGTATTACTGTGCAAGGGTGCGACTTGGGGACTGGGACTTCGATGTCTGGGGCCAAGGG 720
M N S L R S A D T A L Y Y C A R V R L G D W D F D V W G Q G 240
VH CDR3
ACCACGGTCACCGTCTCCTCA 741
T T V T V S S 247

FIG. 3

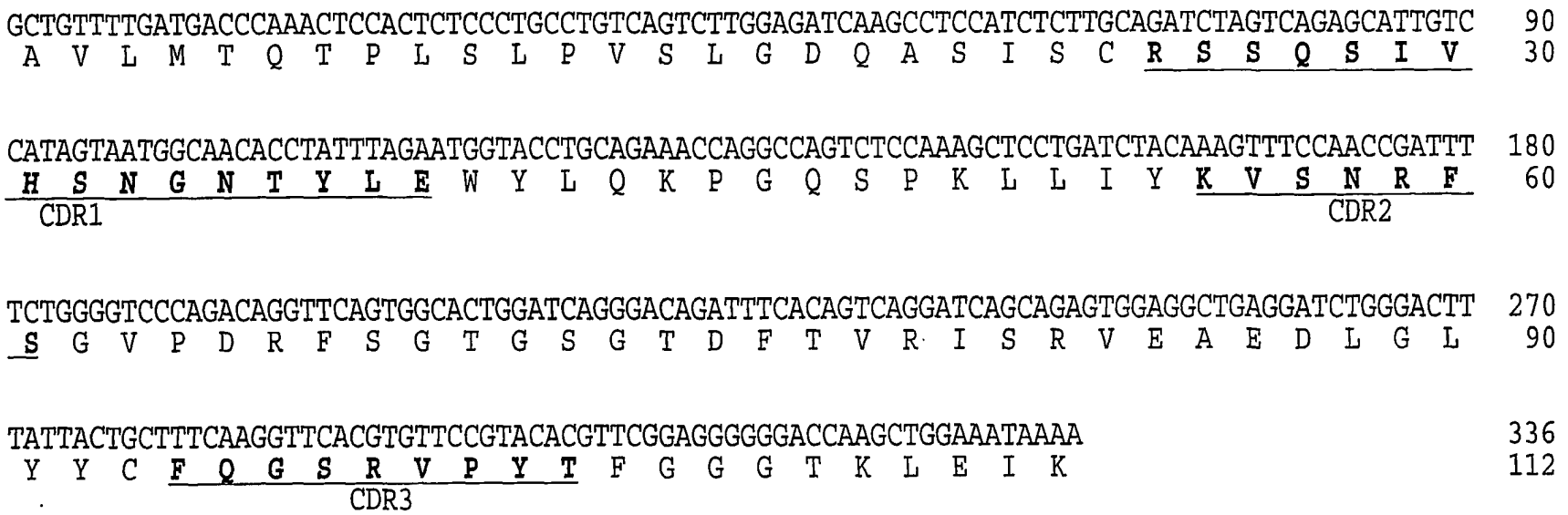
GCTGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTGTC 90
A V L M T Q T P L S L P V S L G D Q A S I S C R S S Q S I V 30
CATAGTAATGGCAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTT 180
H S N G N T Y L E W Y L Q K P G Q S P K L L I Y K V S N R F 60
CDR1
CDR2
TCTGGGGTCCCAGACAGGTTCAGTGGCACTGGATCAGGGACAGATTTCACAGTCAGGATCAGCAGAGTGGAGGCTGAGGATCTGGGACTT 270
S G V P D R F S G T G S G T D F T V R I S R V E A E D L G L 90
TATTACTGCTTTCAAGGTTCACGTGTTCCGTACACGTTCGGAGGGGGGACCAAGCTGGAAATAAAA 336
Y Y C F Q G S R V P Y T F G G G T K L E I K 112
CDR3

FIG. 4

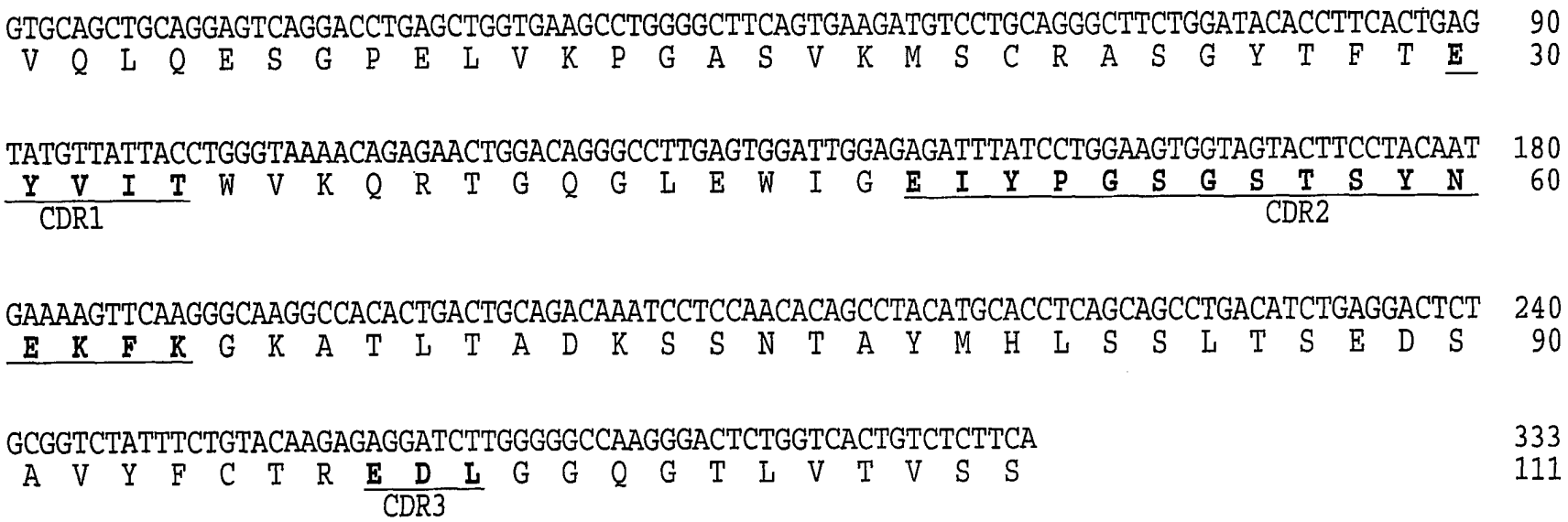
GTGCAGCTGCAGGAGTCAGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAGATGTCCTGCAGGGCTTCTGGATACACCTTCACTGAG 90
V Q L Q E S G P E L V K P G A S V K M S C R A S G Y T F T E 30
TATGTTATTACCTGGGTAAAACAGAGAACTGGACAGGGCCTTGAGTGGATTGGAGAGATTTATCCTGGAAGTGGTAGTACTTCCTACAAT 180
Y V I T W V K Q R T G Q G L E W I G E I Y P G S G S T S Y N 60
CDR1
CDR2
GAAAAGTTCAAGGGCAAGGCCACACTGACTGCAGACAAATCCTCCAACACAGCCTACATGCACCTCAGCAGCCTGACATCTGAGGACTCT 240
E K F K G K A T L T A D K S S N T A Y M H L S S L T S E D S 90
GCGGTCTATTTCTGTACAAGAGAGGATCTTGGGGGCCAAGGGACTCTGGTCACTGTCTCTTCA 333
A V Y F C T R E D L G G Q G T L V T V S S 111
CDR3

FIG. 5

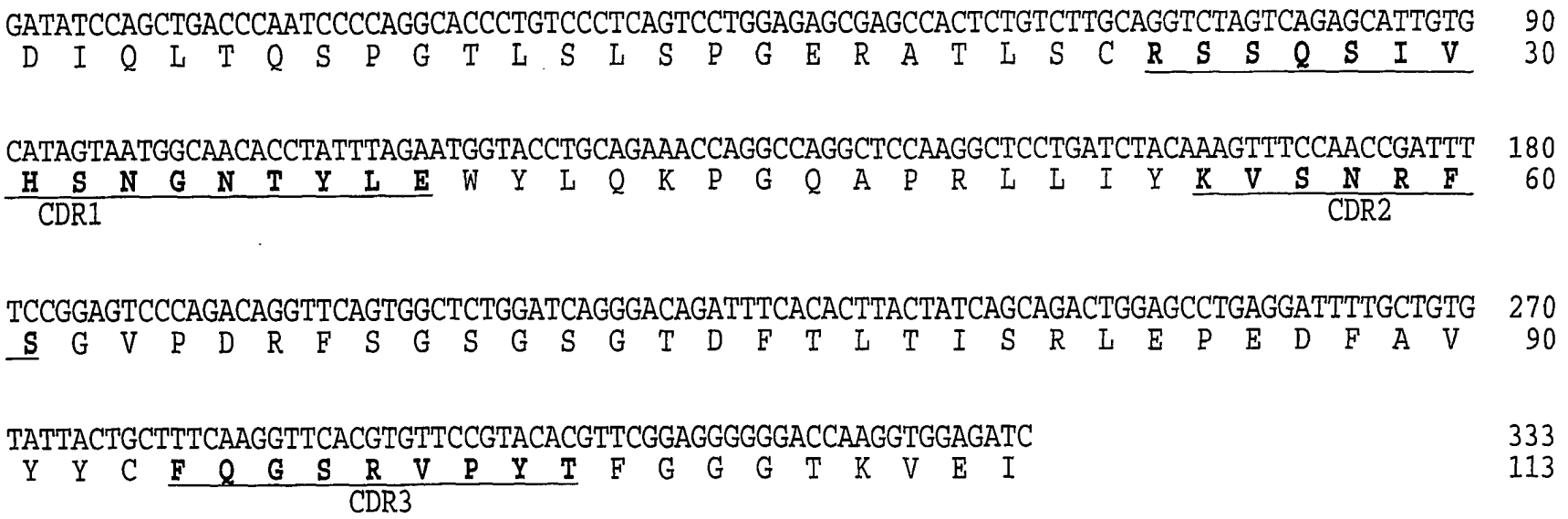
GATATCCAGCTGACCCAATCCCCAGGCACCCTGTCCCTCAGTCCTGGAGAGCGAGCCACTCTGTCTTGCAGGTCTAGTCAGAGCATTGTG 90
D I Q L T Q S P G T L S L S P G E R A T L S C R S S Q S I V 30
CATAGTAATGGCAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGGCTCCAAGGCTCCTGATCTACAAAGTTTCCAACCGATTT 180
H S N G N T Y L E W Y L Q K P G Q A P R L L I Y K V S N R F 60
CDR1
CDR2
TCCGGAGTCCCAGACAGGTTCAGTGGCTCTGGATCAGGGACAGATTTCACACTTACTATCAGCAGACTGGAGCCTGAGGATTTTGCTGTG 270
S G V P D R F S G S G S G T D F T L T I S R L E P E D F A V 90
TATTACTGCTTTCAAGGTTCACGTGTTCCGTACACGTTCGGAGGGGGGACCAAGGTGGAGATC 333
Y Y C F Q G S R V P Y T F G G G T K V E I 113
CDR3

FIG. 6

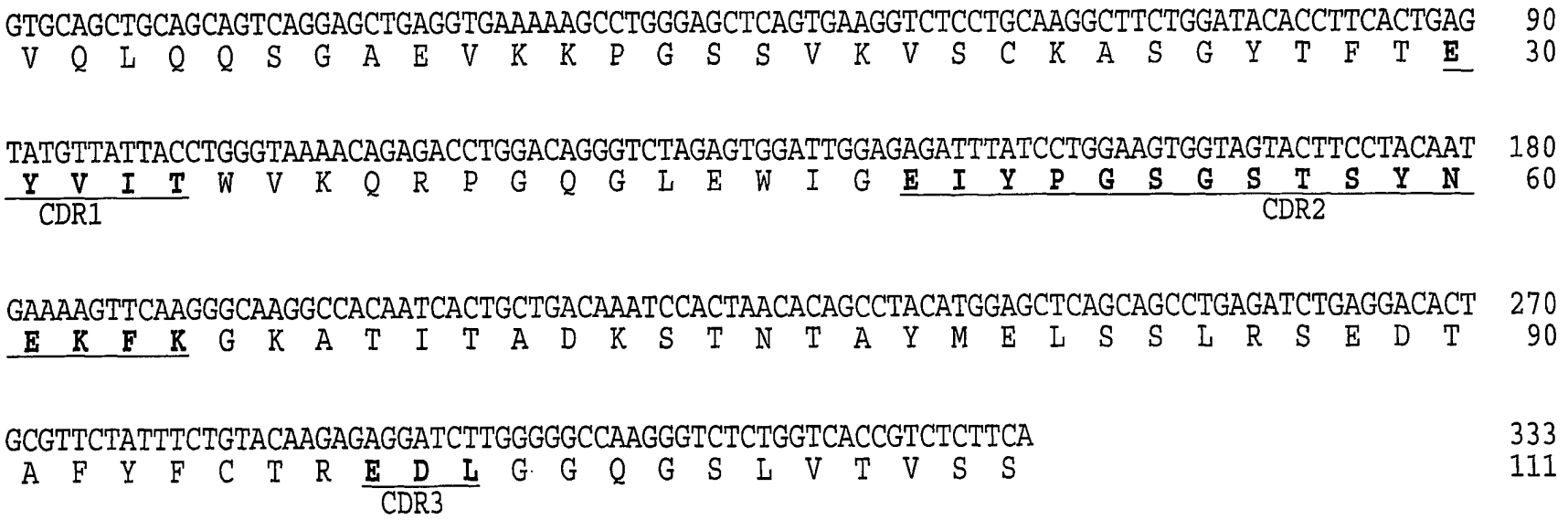
GTGCAGCTGCAGCAGTCAGGAGCTGAGGTGAAAAAGCCTGGGAGCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACTGAG 90
V Q L Q Q S G A E V K K P G S S V K V S C K A S G Y T F T E 30
TATGTTATTACCTGGGTAAAACAGAGACCTGGACAGGGTCTAGAGTGGATTGGAGAGATTTATCCTGGAAGTGGTAGTACTTCCTACAAT 180
Y V I T W V K Q R P G Q G L E W I G E I Y P G S G S T S Y N 60
CDR1
CDR2
GAAAAGTTCAAGGGCAAGGCCACAATCACTGCTGACAAATCCACTAACACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGACACT 270
E K F K G K A T I T A D K S T N T A Y M E L S S L R S E D T 90
GCGTTCTATTTCTGTACAAGAGAGGATCTTGGGGGCCAAGGGTCTCTGGTCACCGTCTCTTCA 333
A F Y F C T R E D L G G Q G S L V T V S S 111
CDR3

FIG. 7

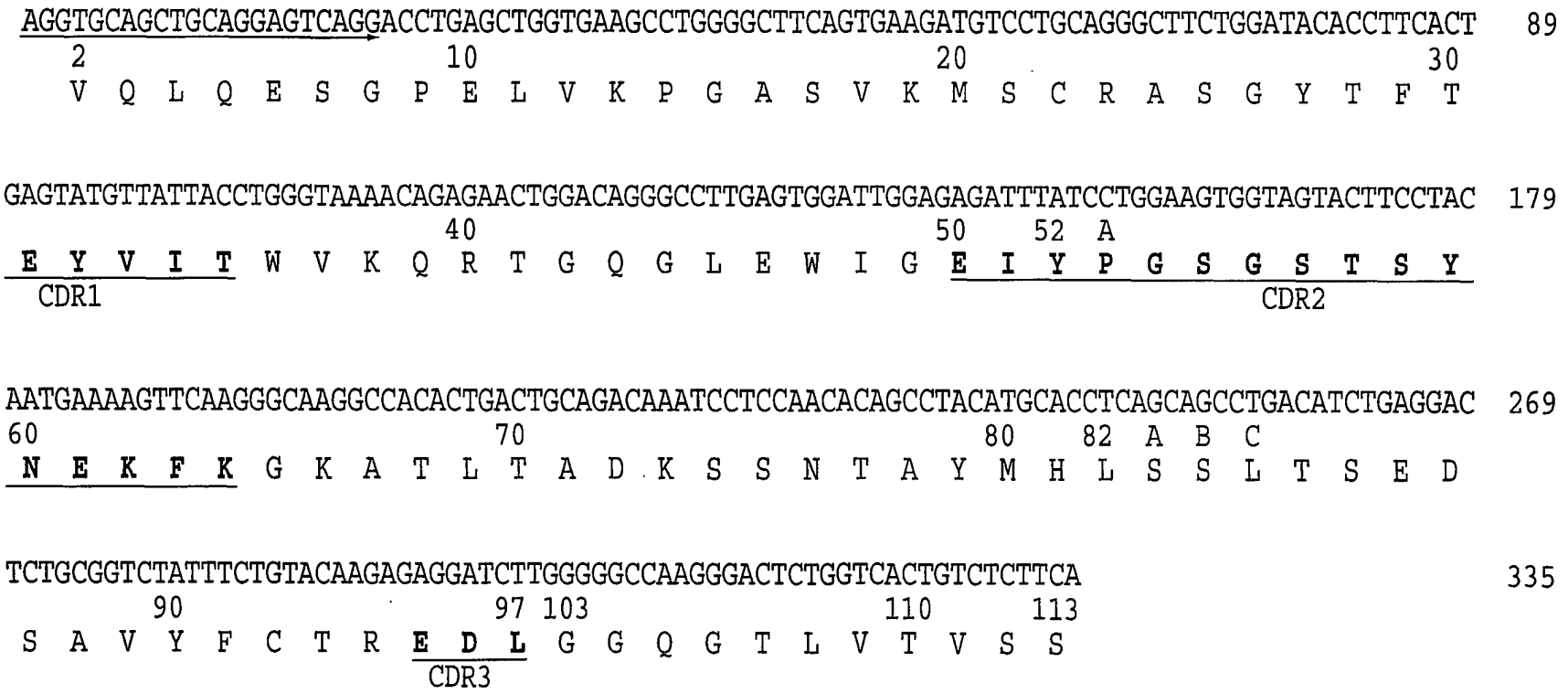
AGGTGCAGCTGCAGGAGTCAGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAGATGTCCTGCAGGGCTTCTGGATACACCTTCACT 89
2 10 20 30
V Q L Q E S G P E L V K P G A S V K M S C R A S G Y T F T
GAGTATGTTATTACCTGGGTAAAACAGAGAACTGGACAGGGCCTTGAGTGGATTGGAGAGATTTATCCTGGAAGTGGTAGTACTTCCTAC 179
40 50 52 A
E Y V I T W V K Q R T G Q G L E W I G E I Y P G S G S T S Y
CDR1
CDR2
AATGAAAAGTTCAAGGGCAAGGCCACACTGACTGCAGACAAATCCTCCAACACAGCCTACATGCACCTCAGCAGCCTGACATCTGAGGAC 269
60 70 80 82 A B C
N E K F K G K A T L T A D K S S N T A Y M H L S S L T S E D
TCTGCGGTCTATTTCTGTACAAGAGAGGATCTTGGGGGCCAAGGGACTCTGGTCACTGTCTCTTCA 335
90 97 103 110 113
S A V Y F C T R E D L G G Q G T L V T V S S
CDR3

FIG. 8

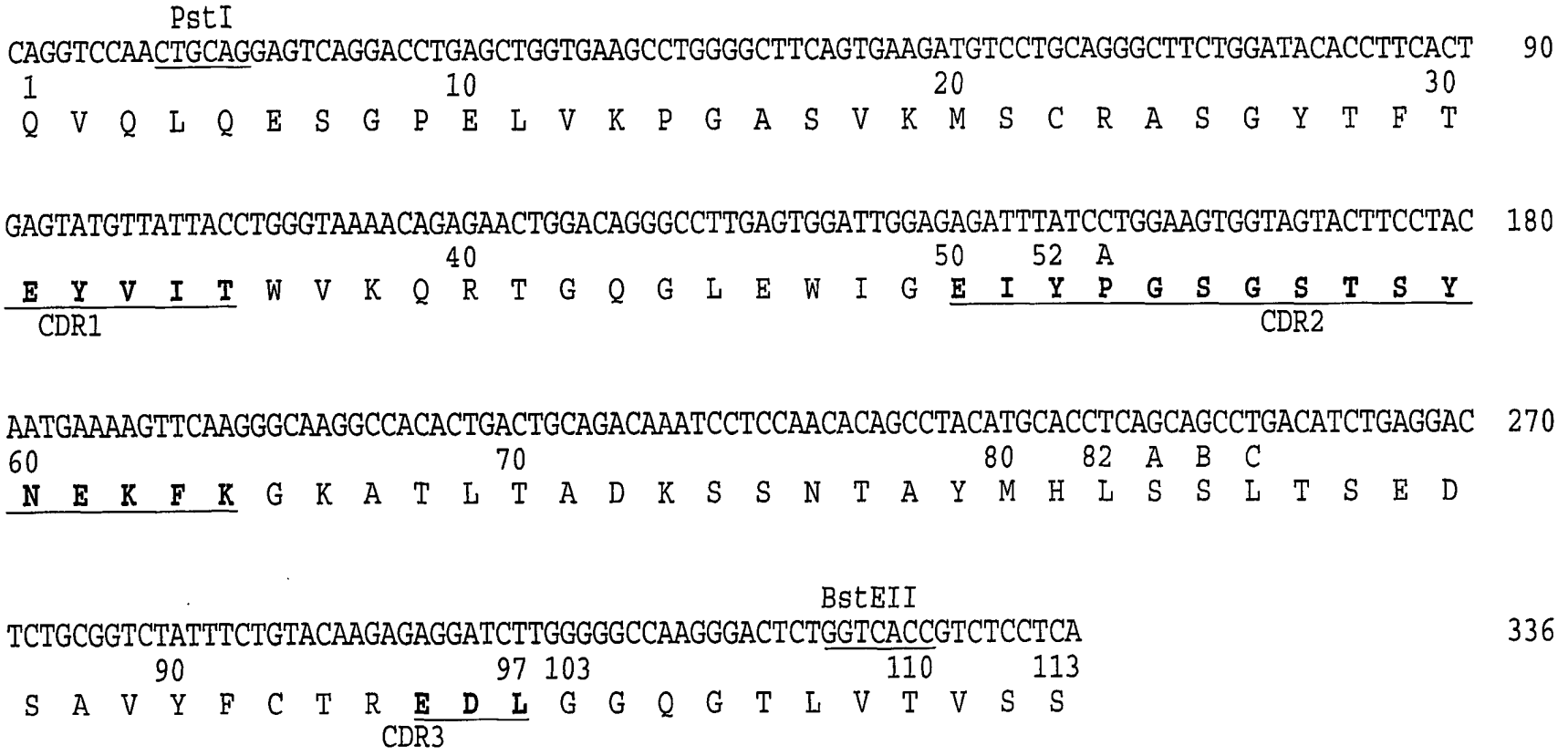
PstI
CAGGTCCAACTGCAGGAGTCAGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAGATGTCCTGCAGGGCTTCTGGATACACCTTCACT 90
1 10 20 30
Q V Q L Q E S G P E L V K P G A S V K M S C R A S G Y T F T
GAGTATGTTATTACCTGGGTAAAACAGAGAACTGGACAGGGCCTTGAGTGGATTGGAGAGATTTATCCTGGAAGTGGTAGTACTTCCTAC 180
40 50 52 A
E Y V I T W V K Q R T G Q G L E W I G E I Y P G S G S T S Y
CDR1
CDR2
AATGAAAAGTTCAAGGGCAAGGCCACACTGACTGCAGACAAATCCTCCAACACAGCCTACATGCACCTCAGCAGCCTGACATCTGAGGAC 270
60 70 80 82 A B C
N E K F K G K A T L T A D K S S N T A Y M H L S S L T S E D
BstEII
TCTGCGGTCTATTTCTGTACAAGAGAGGATCTTGGGGGCCAAGGGACTCTGGTCACCGTCTCCTCA 336
90 97 103 110 113
S A V Y F C T R E D L G G Q G T L V T V S S
CDR3

FIG. 9A

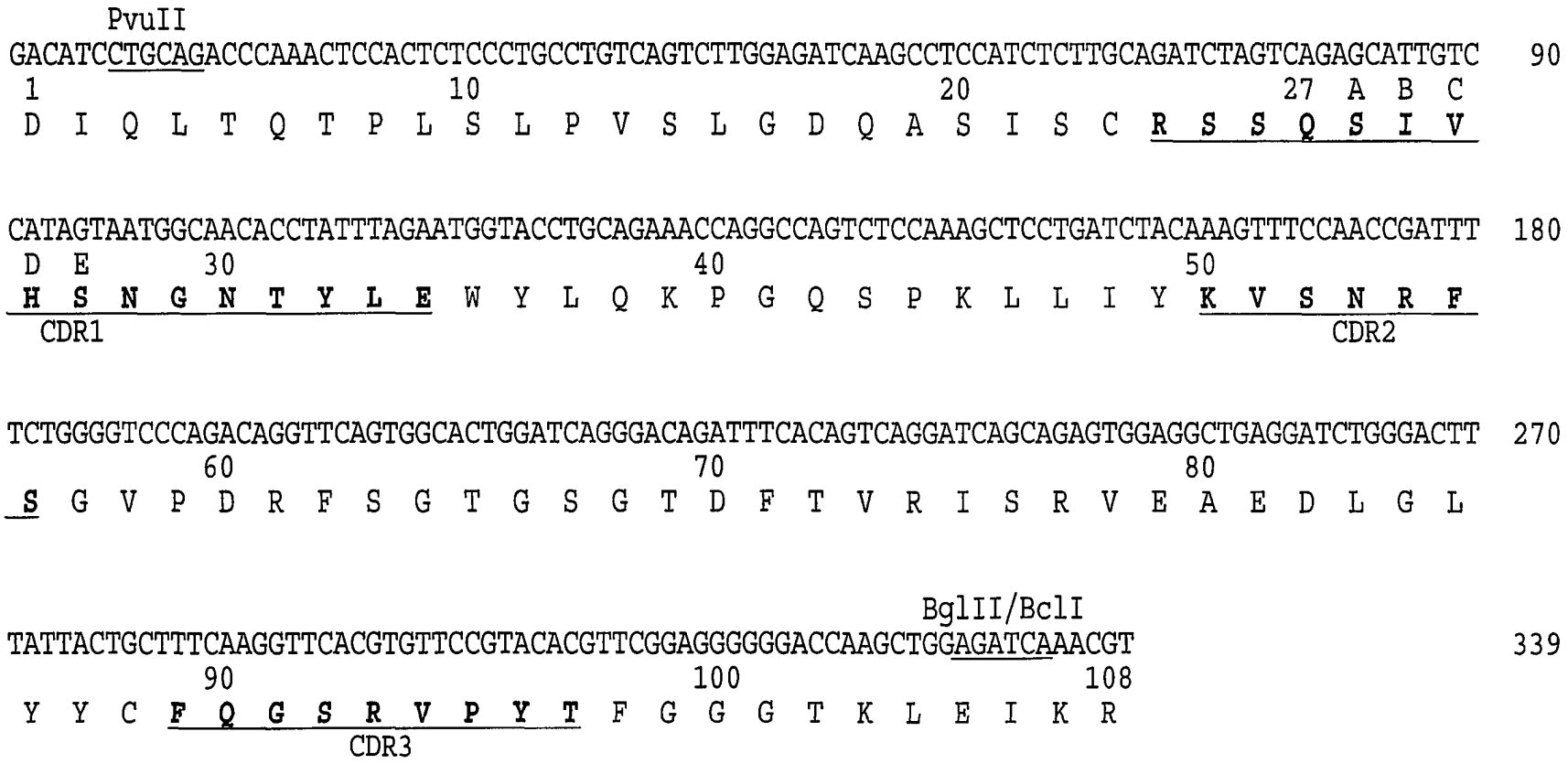
PvuII
GACATCCTGCAGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTGTC 90
1 10 20 27 A B C
D I Q L T Q T P L S L P V S L G D Q A S I S C R S S Q S I V
CATAGTAATGGCAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTT 180
D E 30 40 50
H S N G N T Y L E W Y L Q K P G Q S P K L L I Y K V S N R F
CDR1 CDR2
TCTGGGGTCCCAGACAGGTTCAGTGGCACTGGATCAGGGACAGATTTCACAGTCAGGATCAGCAGAGTGGAGGCTGAGGATCTGGGACTT 270
60 70 80
S G V P D R F S G T G S G T D F T V R I S R V E A E D L G L
BglII/BclI
TATTACTGCTTTCAAGGTTCACGTGTTCCGTACACGTTCGGAGGGGGGACCAAGCTGGAGATCAAACGT 339
90 100 108
Y Y C F Q G S R V P Y T F G G G T K L E I K R
CDR3

FIG. 9B

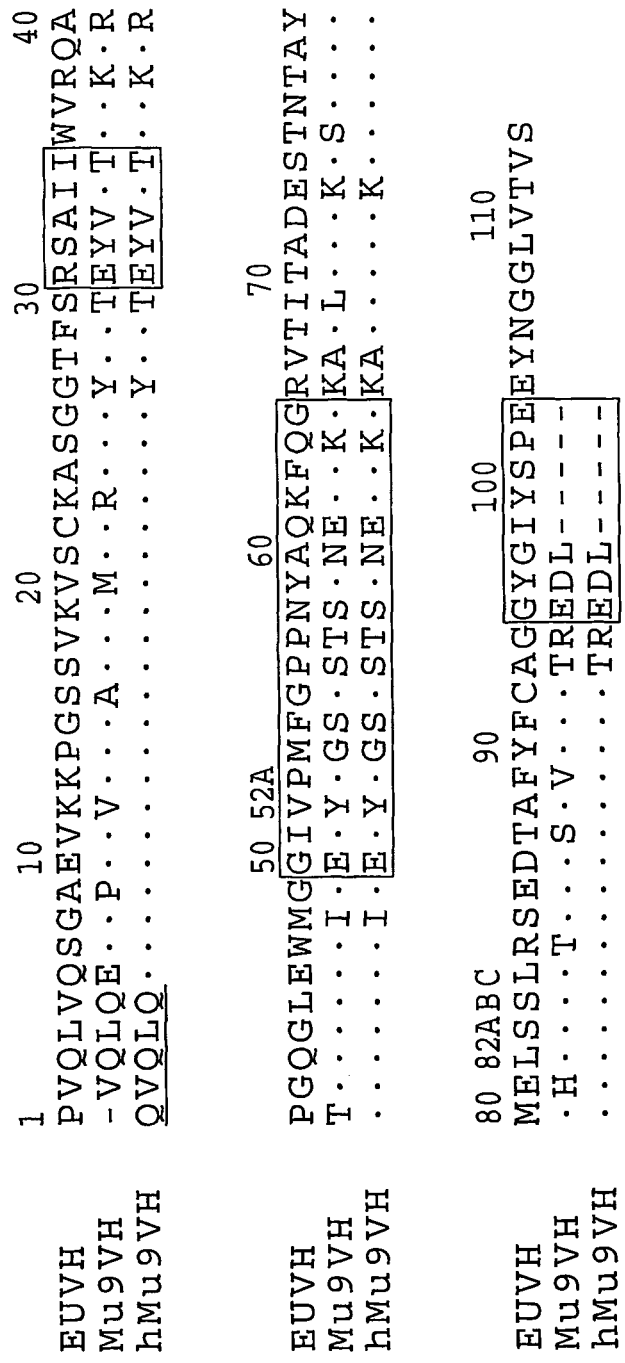
1 10 20 30 40
EUVH PVQLVQSGAEVKKPGSSVKVSCKASGGTFSRSAIIWVRQA
Mu9VH -VQLQE··P··V···A···M··R···Y··TEYV·T··K·R
hMu9VH QVQLQ·····Y··TEYV·T··K·R
50 52A 60 70
EUVH PGQGLEWMGGIVPMFGPPNYAQKFQGRVTITADESTNTAY
Mu9VH T·····I·E·Y·GS·STS·NE··K·KA·L···K·S····
hMu9VH ·····I·E·Y·GS·STS·NE··K·KA·····K·····
80 82ABC 90 100 110
EUVH MELSSLRSEDTAFYFCAGGYGIYSPEEYNGGLVTVS
Mu9VH ·H····T···S·V···TREDL-----
hMu9VH ·····TREDL-----
103 110 113
NEWMVH WGQGSLVTVSS
Mu9VH G···T··TVSS
hMu9VH G·····TVSS

FIG. 10A

```
                1                   10                  20          27A B C D E   30
WOLVk   EIVLTQSPGTLSLSPGERATLSCRASQS-----VSSGYLGW
Mu9Vk   AVLM··T·LS·PV·L·DQ·SI··  ·S···IVHSNGNT··E·
hMu9Vk  DIQL·····················  ·S···IVHSNGNT··E·

                40                  50                  60                  70
WOLVk   YQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTI
        ·L·····S·K····KV·N·FS·V·····T·····VR·
hMu9Vk  ·L·····S·K····KV·N·FS·V·····T·····VR·
hMu9Vk  ·L·····S·K····KV·N·FS·V·····T·····VR·

                80                  90                  100         108
WOLVk   SRLEPEDFAVYYCQQYGSLGRTFGQGTKVEIKR
        ··V·A··LGL····F·GSRVPY···G···LEIK-
Mu9Vk   ··V·A··LGL····F·GSRVPY···G···LEIK-
hMu9Vk  ··V·A··LGL····F·GSRVPY···G···EIKR
```

FIG. 10B

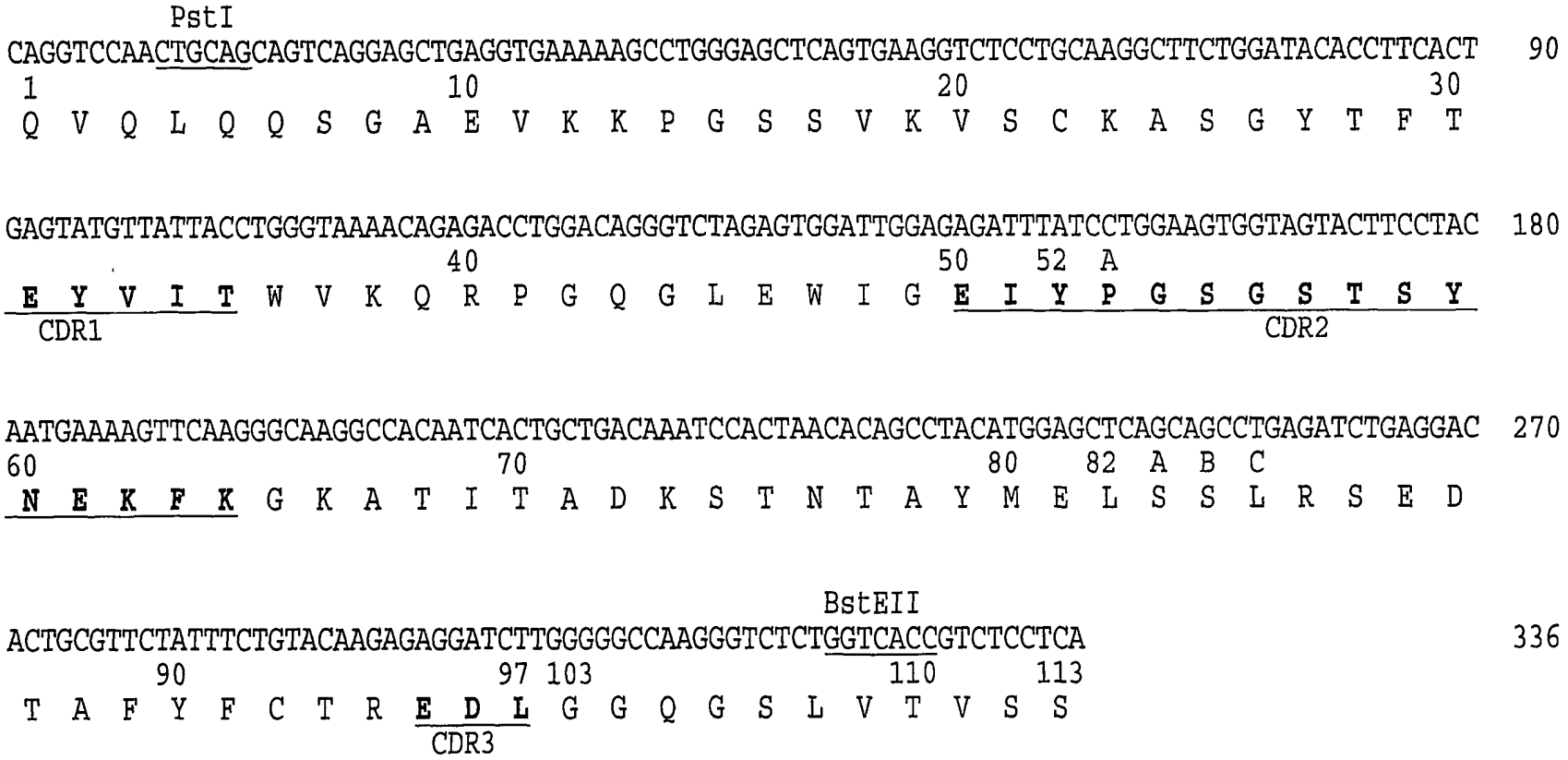
PstI
CAGGTCCAACTGCAGCAGTCAGGAGCTGAGGTGAAAAAGCCTGGGAGCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACT 90
1 10 20 30
Q V Q L Q Q S G A E V K K P G S S V K V S C K A S G Y T F T
GAGTATGTTATTACCTGGGTAAAACAGAGACCTGGACAGGGTCTAGAGTGGATTGGAGAGATTTATCCTGGAAGTGGTAGTACTTCCTAC 180
40 50 52 A
E Y V I T W V K Q R P G Q G L E W I G E I Y P G S G S T S Y
CDR1 CDR2
AATGAAAAGTTCAAGGGCAAGGCCACAATCACTGCTGACAAATCCACTAACACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGAC 270
60 70 80 82 A B C
N E K F K G K A T I T A D K S T N T A Y M E L S S L R S E D
BstEII
ACTGCGTTCTATTTCTGTACAAGAGAGGATCTTGGGGGCCAAGGGTCTCTGGTCACCGTCTCCTCA 336
90 97 103 110 113
T A F Y F C T R E D L G G Q G S L V T V S S
CDR3

FIG. 11A


PvuII
GACATCCAGCTGACCCAATCCCCAGGCACCCTGTCCCTCAGTCCTGGAGAGCGAGCCACTCTGTCTTGCAGGTCTAGTCAGAGCATTGTG 90
1 10 20 27 A B C
D I Q L T Q S P G T L S L S P G E R A T L S C R S S Q S I V
CATAGTAATGGCAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGGCTCCAAGGCTCCTGATCTACAAAGTTTCCAACCGATTT 180
D E 30 40 50
H S N G N T Y L E W Y L Q K P G Q A P R L L I Y K V S N R F
CDR1
CDR2
TCCGGAGTCCCAGACAGGTTCAGTGGCTCTGGATCAGGGACAGATTTCACACTTACTATCAGCAGACTGGAGCCTGAGGATTTTGCTGTG 270
60 70 80
S G V P D R F S G S G S G T D F T L T I S R L E P E D F A V
BglII/BclI
TATTACTGCTTTCAAGGTTCACGTGTTCCGTACACGTTCGGAGGGGGGACCAAGGTGGAGATCAAACGT 336
90 100 108
Y Y C F Q G S R V P Y T F G G G T K V E I K R
CDR3

FIG. 11B

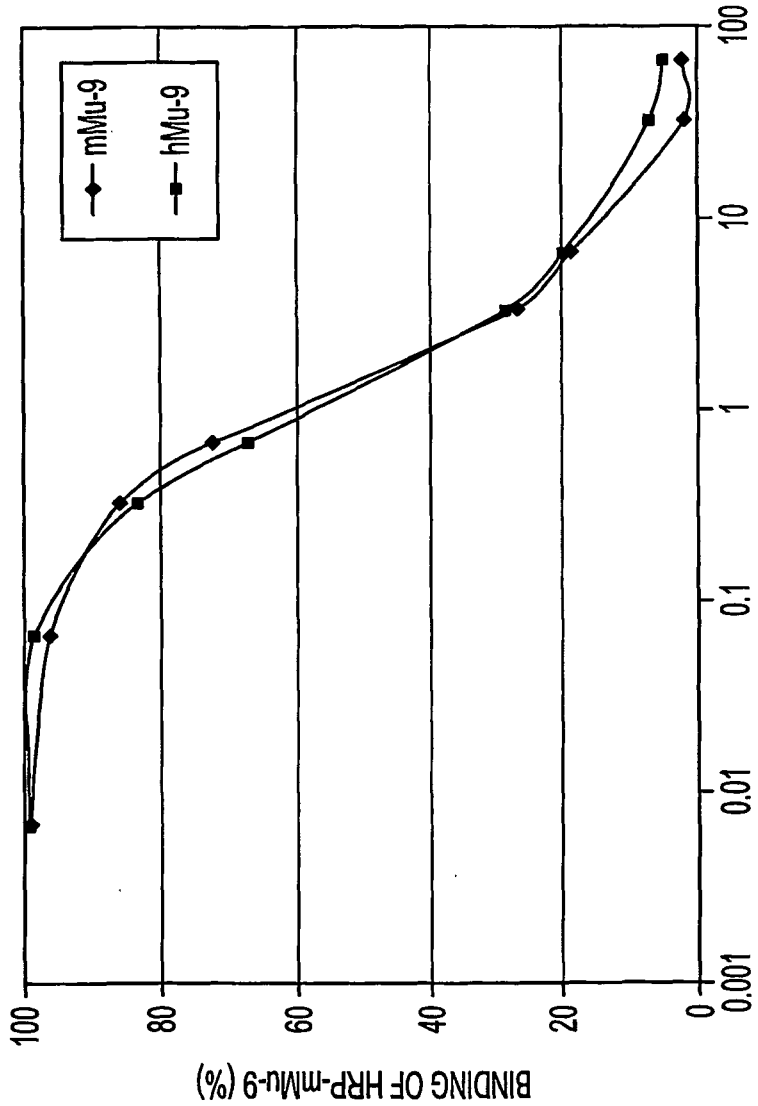
mMu-9
hMu-9
BINDING OF HRP-mMu-9 (%)
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
COMPETING Ab (nM)

FIG. 12

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 09337756 B **[0001]**
- US 4863713 A, Goodwin **[0006]**
- US 5256395 A, Barbet **[0006]**
- US 5274076 A **[0006]**
- US 4923922 A, Baldwin **[0010]**
- US 4916213 A **[0010]**
- US 4918163 A, Young **[0010]**
- US 5204095 A **[0010]**
- US 5196337 A, Irie **[0010]**
- US 5134075 A, Hellstrom **[0010]**
- US 5171665 A **[0010]**
- US 6342221 B, Thorpe **[0010] [0117] [0288]**
- US 5965132 A, Epstein **[0010] [0117]**
- US 6004554 A **[0010] [0117] [0288]**
- US 6071491 A **[0010] [0090]**
- US 6017514 A **[0010] [0090] [0288]**
- US 5882626 A **[0010] [0090] [0288]**
- US 5019368 A **[0010] [0090] [0288]**
- US 5716595 A, Goldenberg **[0057] [0097]**
- US 6096289 A **[0057] [0097] [0180] [0216]**
- US 2001006618 A **[0057]**
- US 5567610 A **[0076]**
- US 5229275 A **[0076]**
- US 5332567 A **[0089]**
- US 5057313 A, Shih **[0091] [0194]**
- US 5443953 A, Hansen **[0091] [0195]**
- US 5156840 A, McKearn **[0091]**
- US 4975278 A **[0094]**
- US 5270196 A **[0094]**
- US 5660829 A **[0094]**
- US 5587161 A **[0094]**
- US 5405990 A **[0094]**
- US 5561119 A **[0094]**
- US 5646298 A **[0094]**
- US 5338678 A **[0094]**
- US 5545548 A **[0094]**
- US 6331175 B **[0097]**
- US 348818 A **[0097]**
- US 4824659 A, Hawthorne **[0100]**
- US 4150149 A **[0106]**
- US 4361544 A **[0106] [0216]**
- US 4444744 A **[0106] [0107] [0216]**
- US 4361644 A **[0107]**
- WO 0029584 A1 **[0114]**
- US 5827690 A **[0115] [0150] [0156]**
- US 5633425 A **[0116]**
- US 03074566 A **[0116]**
- US 4704692 A **[0144]**
- US 4946778 A **[0144]**
- US 4036945 A, Goldenberg **[0146]**
- US 4331647 A **[0146] [0216]**
- US 60342103 B **[0153]**
- US 2003148409 A **[0153]**
- US 20030342103 A **[0153]**
- US 5874540 A **[0160]**
- US 4818709 A **[0160] [0216]**
- US 6077499 A **[0174] [0211]**
- US 09921290 B **[0176]**
- US 342104 P, G.L.Griffiths and W.J. McBride **[0179]**
- US 09382186 B **[0190]**
- US 6254868 B, Leung **[0195]**
- US 5753206 A **[0205] [0211]**
- US 6126916 A **[0211]**
- US 6010680 A **[0211]**
- US 5776095 A **[0211]**
- US 5776094 A **[0211]**
- US 5776093 A **[0211]**
- US 5772981 A **[0211]**
- US 5746996 A **[0211]**
- US 5697902 A **[0211]**
- US 5328679 A **[0211]**
- US 5128119 A **[0211]**
- US 5101827 A **[0211]**
- US 4735210 A **[0211]**
- US 4348376 A **[0216]**
- US 5851527 A **[0216] [0221]**
- US 09205243 B **[0226]**
- US 09911610 B **[0228]**
- US 5262524 A **[0228]**
- US 5091542 A **[0228]**
- US 5892020 A **[0228]**
- US 6025165 A **[0228]**
- US 5837242 A **[0228]**
- WO 0034317 A, Carr **[0288]**

Non-patent literature cited in the description

- **GOODWIN et al.** *J. Nucl. Med.,* 1992, vol. 33, 1366-1372 **[0006]**
- **KRANENBORG et al.** 55. *Cancer Res,* 1995, 5864s-5867s **[0006]**
- *Cancer,* 1997, vol. 80, 2390-2397 **[0006]**

- **BODEN et al.** *Bioconjugate Chem.,* 1995, vol. 6, 373-379 **[0006]**
- **SCHUHMACHER et al.** *Cancer Res.,* 1995, vol. 55, 115-123 **[0006]**
- **GOLDENBERG.** *Semin. Nucl. Med.,* 1989, vol. 19, 332 **[0010]**
- Radioimmunotherapy. **GOLDENBERG, DM.** Nuclear Medicine Annual. Lippincott, William & Wilkins, 2001, 167-206 **[0010]**
- **THORPE.** *TIBTECH,* 1993, vol. 11, 42 **[0010]**
- **GOLDENBERG.** *Scientific American, Science & Medicine,* 1994, vol. 1, 64 **[0010]**
- **VAUGHAN et al.** *Brit. J. Radiol.,* 1987, vol. 60, 567 **[0010]**
- **GOLD et al.** *Cancer Res.,* 1990, vol. 50, 6405 **[0011]**
- **BLUMENTHAL et al.** *Int. J. Cancer,* 1989, vol. 22, 292 **[0011]**
- **SHARKEY et al.** *Cancer,* 1994, vol. 73, 864 **[0011]**
- **PANT et al.** *Cancer,* 1982, vol. 50, 919 **[0011]**
- **KRISHNAN et al.** *Cancer,* 1997, vol. 80, 2667-2674 **[0072]**
- **GREEN et al.** *Nature Genet.,* 1994, vol. 7, 13 **[0075]**
- **LONBERG et al.** *Nature,* 1994, vol. 368, 856 **[0075]**
- **TAYLOR et al.** *Int. Immun.,* 1994, vol. 6, 579 **[0075]**
- **MCCAFFERTY et al.** *Nature,* 1990, vol. 348, 552-553 **[0075]**
- **JOHNSON ; CHISWELL.** *Current Opinion in Structural Biology,* 1993, vol. 3, 5564-571 **[0075]**
- Remington's Pharmaceutical Sciences. Mack Publishing Co, 1990 **[0091]**
- **SHIH et al.** *Int. J. Cancer,* 1988, vol. 41, 832 **[0091] [0194]**
- **KOHLER ; MILSTEIN.** *Nature,* 1975, vol. 256, 495 **[0103]**
- CURRENT PROTOCOLS IN IMMUNOLOGY. John Wiley & Sons, 1991, vol. 1, 2.5.1-2.6.7 **[0103]**
- Purification of Immunoglobulin G (IgG. **BAINES et al.** METHODS IN MOLECULAR BIOLOGY. The Humana Press, Inc, 1992, vol. 10, 79-104 **[0104]**
- Immunodiagnosis of Cancer. **HERBERMAN.** The Clinical Biochemistry of Cancer. American Association of Clinical Chemists, 1979, 347 **[0106]**
- **YU et al.** *Nat. Immunol.,* 2000, vol. 1, 252-256 **[0108]**
- **TOMIZUKA et al.** *Nature Genetics,* 1997, vol. 16, 133 **[0111]**
- **BARBAS et al.** *METHODS: A Companion to Methods in Enzymology,* 1991, vol. 2, 119 **[0112]**
- **WINTER et al.** *Ann. Rev. Immunol.,* 1994, vol. 12, 433 **[0112]**
- **ORLANDI et al.** *Proc. Nat'l Acad. Sci. USA,* 1989, vol. 86, 3833 **[0113]**
- **LEUNG et al.** *Hybridoma,* 1994, vol. 13, 469 **[0113] [0265] [0281]**
- **JONES et al.** *Nature,* 1986, vol. 321, 522 **[0113]**
- **RIECHMANN et al.** *Nature,* 1988, vol. 332, 323 **[0113]**
- **VERHOEYEN et al.** *Science,* 1988, vol. 239, 1534 **[0113] [0114] [0136]**
- **CARTER et al.** *Proc. Nat'l Acad. Sci. USA,* 1992, vol. 89, 4285 **[0113]**
- **SANDHU.** *Crit. Rev. Biotech.,* 1992, vol. 12, 437 **[0113]**
- **SINGER et al.** *J. Immun.,* 1993, vol. 150, 2844 **[0113]**
- **TEMPEST et al.** *Biotechnology,* 1991, vol. 9, 266 **[0114]**
- **COLMAN, A.** *Biochem. Soc. Symp.,* 1998, vol. 63, 141-147 **[0115] [0150] [0156]**
- **MENDEZ et al.** *Nature Genetics,* 1997, vol. 15, 146-156 **[0116]**
- **COUTO, J ; CHRISTIAN, R ; PETERSON, J ; CERIANI, R.** *Cancer Res.,* 1995, vol. 55 (23), 5973s-5977s **[0116]**
- **SAMBROOK et al.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Press, 1989 **[0123]**
- **LEUNG et al.** *Hybridoma,* 1994, vol. 13, 469-476 **[0125]**
- **GILLES et al.** *J. Immunol. Methods,* 1989, vol. 125, 191 **[0126] [0131]**
- **LOSMAN et al.** *Clin. Cancer Res.,* 1999, vol. 5, 3101 **[0126] [0131]**
- **LOSMAN et al.** *Cancer,* 1997, vol. 80, 2660 **[0126] [0131]**
- **BARNES et al.** *Cytotechnology,* 2000, vol. 32, 109-123 **[0126] [0131]**
- **WERNER et al.** *Arzneim.-Forsch./Drug Res.,* 1998, vol. 48 (8), 870-880 **[0126] [0131]**
- **ORLANDI et al.** *Proc. Natl. Acad. Sci., U.S.A.,* 1989, vol. 86, 3833 **[0127]**
- **LEUNG et al.** *BioTechniques,* 1993, vol. 15, 286 **[0127]**
- **SANGER et al.** *Proc. Natl. Acad. Sci. USA,* 1977, vol. 74, 5463 **[0128]**
- **CO et al.** *J. Immunol.,* 1992, vol. 148, 1149 **[0132]**
- **TEMPEST et al.** *Bio/Technology,* 1991, vol. 9, 266 **[0136]**
- CURRENT PROTOCOLS IN MOLECULAR BIOLOGY. John Wiley & Sons, 1987 **[0138]**
- **HUSE.** ANTIBODY ENGINEERING: A PRACTICAL GUIDE. W. H. Freeman & Co, 1992, 103-120 **[0139]**
- **UHLMANN.** *Gene,* 1988, vol. 71, 29 **[0140]**
- **WOSNICK et al.** *Gene,* 1988, vol. 60, 115 **[0140]**
- **GREENWOOD et al.** *Biochem. J.,* 1963, vol. 89, 123 **[0142]**
- **HUSE et al.** *Science,* 1989, vol. 246, 1274-1281 **[0143]**
- **R. RAAG ; M. WHITLOW.** Single Chain Fvs. *FASEB,* 1995, vol. 9, 73-80 **[0144]**
- **R.E. BIRD ; B.W. WALKER.** Single Chain Antibody Variable Regions. *TIBTECH,* 1991, vol. 9, 132-137 **[0144]**
- **VAUGHN et al.** *Nat. Biotechnol.,* 1996, vol. 14, 309-314 **[0145]**
- **RIDDER et al.** *Biotechnology,* 1995, vol. 13, 255-260 **[0145]**
- **JACKSON et al.** *Br. J. Cancer,* 1998, vol. 78, 181-188 **[0145]**

- **OSBOURN et al.** *Immunotechnology,* 1996, vol. 2, 181-196 **[0145]**
- **NISONOFF et al.** *Arch Biochem. Biophys.,* 1960, vol. 89, 230 **[0146]**
- **PORTER.** *Biochem. J.,* 1959, vol. 73, 119 **[0146]**
- **EDELMAN et al.** METHODS IN ENZYMOLOGY. Academic Press, 1967, vol. 1, 422 **[0146]**
- **LARRICK et al.** *Methods: A Companion to Methods in Enzymology,* 1991, vol. 2, 106 **[0147]**
- Genetic Manipulation of Monoclonal Antibodies. **COURTENAY-LUCK et al.** MONOCLONAL ANTIBODIES: PRODUCTION, ENGINEERING AND CLINICAL APPLICATION. Cambridge University Press, 1995, 166-179 **[0147]**
- Genetic Manipulation and Expression of Antibodies. **WARD et al.** MONOCLONAL ANTIBODIES: PRINCIPLES AND APPLICATIONS. Wiley-Liss, Inc, 1995, 137-185 **[0147]**
- **FITZGERALD et al.** *Protein Eng.,* 1997, vol. 10 (10), 1221-1225 **[0152]**
- **COLOMA et al.** *Nature Biotech.,* 1997, vol. 15, 159-163 **[0152]**
- **MACK et al.** *Proc. Natl. Acad. Sci.,* 1995, vol. 92, 7021-7025 **[0153]**
- **ZHENPING et al.** *Biotechnology,* 1996, vol. 14, 192-196 **[0155]**
- **YANG et al.** *Appl. Environ. Microbiol.,* 1998, vol. 64, 2869-2874 **[0155]**
- **FIEDLER et al.** *Biotech.,* 1995, vol. 13, 1090-1093 **[0157]**
- **FIEDLER et al.** *Immunotechnology,* 1997, vol. 3, 205-216 **[0157]**
- **MAHIOUZ et al.** *J. Immunol. Methods,* 1998, vol. 212, 149-160 **[0159]**
- **MILLER, L.K.** *Ann. Rev. Microbiol.,* 1988, vol. 42, 177 **[0159]**
- **BEI et al.** *J. Immunol. Methods,* 1995, vol. 186, 245 **[0159]**
- REMINGTON'S PHARMACEUTICAL SCIENCES. Mack Publishing Co, 1995 **[0172]**
- GOODMAN AND GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS. MacMillan Publishing Co, 1985 **[0172]**
- PHOTODYNAMIC THERAPY OF TUMORS AND OTHER DISEASES. Libreria Progetto, 1985 **[0173]**
- **VAN DEN BERGH.** Chem. Britain. 1986, vol. 22, 430 **[0173]**
- **MEW et al.** *J. Immunol.,* 1983, vol. 130, 1473 **[0173] [0219]**
- *Cancer Res.,* 1985, vol. 45, 4380 **[0173] [0219]**
- **OSEROFF et al.** *Proc. Natl. Acad. Sci. USA,* 1986, vol. 83, 8744 **[0173] [0219]**
- *Photochem. Photobiol.,* 1987, vol. 46, 83 **[0173] [0219]**
- **HASAN et al.** *Prog. Clin. Biol. Res.,* 1989, vol. 288, 471 **[0173] [0219]**
- **TATSUTA et al.** *Lasers Surg. Med.,* 1989, vol. 9, 422 **[0173] [0219]**
- **PELEGRIN et al.** *Cancer,* 1991, vol. 67, 2529 **[0173] [0219]**
- **PASTAN et al.** *Cell,* 1986, vol. 47, 641 **[0174]**
- **GOLDENBERG.** *CA - A Cancer Journal for Clinicians,* 1994, vol. 44, 43 **[0174]**
- **YU et al.** Int. J. Cancer. 1994, vol. 56, 244 **[0193]**
- **WONG.** CHEMISTRY OF PROTEIN CONJUGATION AND CROSS-LINKING. CRC Press, 1991 **[0193]**
- Modification of Antibodies by Chemical Methods. **UPESLACIS et al.** MONOCLONAL ANTIBODIES: PRINCIPLES AND APPLICATIONS. Wiley-Liss, Inc, 1995, 187-230 **[0193]**
- Production and Characterization of Synthetic Peptide-Derived Antibodies. **PRICE et al.** MONOCLONAL ANTIBODIES: PRODUCTION, ENGINEERING AND CLINICAL APPLICATION. Cambridge University Press, 1995, 60-84 **[0193]**
- **SHIH et al.** *Int. J. Cancer,* 1990, vol. 46, 1101 **[0194]**
- **LEUNG et al.** *J. Immunol.,* 1995, vol. 154, 5919 **[0195]**
- **GREENE ; WUTS.** Protective Groups in Organic Synthesis. John Wiley and Sons, 1999 **[0200]**
- **KARACAY et al.** *Bioconjugate Chem.,* 2000, vol. 11, 842-854 **[0209]**
- Photodynamic Therapy of Tumors and Other Diseases. Libreria Progetto, 1985 **[0219]**
- **VAN DEN BERGH.** *Chem. Britain,* 1986, vol. 22, 430 **[0219]**
- **ARCAMONE.** *Cancer Res.,* 1985, vol. 45, 5995 **[0222]**
- **POTTER et al.** *Cancer Res.,* 1998, vol. 58, 2646-2651 **[0223]**
- **POTTER et al.** *Cancer Res.,* 1998, vol. 58, 3627-3632 **[0223]**
- **HANDE et al.** *Cancer Res.,* 1988, vol. 48, 1829-1834 **[0224]**
- **WANG et al.** *Cancer Res.,* 1982, vol. 52, 4484-4491 **[0224]**
- **BAKINA et al.** *J. Med Chem.,* 1997, vol. 40, 4013-4018 **[0224]**
- **LANDSDORP et al.** *Euro. J. Immunol.,* 1986, vol. 16, 679-83 **[0228]**
- **KROTT et al.** *Protein Engineering,* 1997, vol. 10 (4), 423-433 **[0228]**
- **ANSEL et al.** PHARMACEUTICAL DOSAGE FORMS AND DRUG DELIVERY SYSTEMS. Lea & Febiger, 1990 **[0231] [0233]**
- REMINGTON'S PHARMACEUTICAL SCIENCES. Mack Publishing Company, 1990 **[0231] [0233]**
- **SHERWOOD et al.** *Bio/Technology,* 1992, vol. 10, 1446 **[0233]**
- **SALTZMAN et al.** *Biophys. J.,* 1989, vol. 55, 163 **[0233]**
- **SAMBROOK et al.** MOLECULAR CLONING: A LABORATORY MANUAL. Cold Spring Harbor Laboratory, 1989 **[0237]**

- **AUSUBEL et al.** CURRENT PROTOCOLS IN MOLECULAR BIOLOGY. John Wiley & Sons, 1997 **[0237]**
- **KARACAY.** *Bioconjugate Chem.,* 2000, vol. 11, 842-854 **[0242] [0256]**
- **DANGLES.** *J. Org. Chem.,* 1987, vol. 52, 4984-4993 **[0246]**
- **ORLANDI et al.** *Proc. Natl. Acad. Sci. USA,* 1989, vol. 86, 3833 **[0265]**
- **QU et al.** *Clin. Cancer Rec.,* 1999, vol. 5, 3095s-3100s **[0277]**
- **BAMIAS, A. ; EPENETOS,A.A.** Two-step strategies for the diagnosis and treatment of cancer with bioconjugates. *Antibody, Immunoconjugates, Radiopharm.,* 1992, vol. 5, 385-395 **[0288]**
- **BARBET, J. ; PELTIER, P. ; BARDET, S. ; VUILLEZ, JP. ; BACHELOT, I. ; DENET, S. ; OLIVIER, P. ; LECIA, F. ; CORCUFF, B. ; HUGLO, D.** Radioimmunodetection of medullary thyroid carcinoma using indium-111 bivalent hapten and anti-CEA x anti-DTPA-indium bispecifc antibody. *J.Nucl.Med.,* 1998, vol. 39, 1172-1178 **[0288]**
- **BOS, ES. ; KUIJPERS, WHA. ; MEESTERS-WINTERS, M. ; PHAM, DT. ; DEHAAN, AS. ; VAN DOORMALEN,AM. ; KASPERSON,F.M. ; VANBOECKEL, CAA ; GOUEGEON-BERTRAND, F.** In vitro evaluation of DNA-DNA hybridization as a two-step approach in radioimmunotherapy of cancer. *Cancer Res.,* 1994, vol. 54, 3479-3486 **[0288]**
- **DI CARLO, A. ; MARIANO, A. ; D'ALESSANDRO, V. ; BELLI, G. ; ROMANO, G. ; MACCHIA, V.** Evaluation of epidermal growth factor receptor, carcinoembryonic antigen and Lewis carbohydrate antigens in human colorectal and liver neoplasias. *Oncol. Rep.,* 2001, vol. 8, 387-392 **[0288]**
- **GAUTHEROT, E. ; BOUHOU, J. ; LEDOUSSAL, J-M. ; MANETTI, C. ; MARTIN, M. ; ROUVIER, E. ; BARBET, J.** Therapy for colon carcinoma xenografts with bi-specific antibody-targeted, iodine-131-labeled bivalent hapten. *Cancer,* 1997, vol. 80, 2618-2623 **[0288]**
- **GAUTHEROT, E. ; BOUHOU, J. ; LOUCIF, E. ; MANETTI, C. ; MARTIN, M. ; LEDOUSSAL, J.M. ; ROUVIER, E. ; BARBET, J.** Radioimmunotherapy of LS174T colon carcinoma in nude mice using an iodine-131-labeled bivalent hapten combined with an anti-CEA x anti-indium-DTPA bi-specific antibody. *J.Nucl.Med.,* 1997, vol. 38, 7 **[0288]**
- **GOODWIN, D.A. ; MEARES, CF. ; MCCALL, MJ. ; MCTIGUE,M. ; CHAOVAPONG, W.** Pre-targeted immunoscintigraphy of murine tumors with indium-111-labeled bifunctional haptens. *J.Nucl.Med.,* 1988, vol. 29, 226-234 **[0288]**
- **GREENWOOD, F.C. ; HUNTER, W.M.** The preparation of I-131 labeled human growth hormone of high specific radioactivity. *Biochem.,* 1963, vol. 89, 114-123 **[0288]**
- **HAWKINS, G.A. ; MCCABE, R.P. ; KIM, C.-H. ; SUBRAMANIAN, R. ; BREDEHORST, R. ; MCCULLERS, G.A. ; VOGEL,C.-W. ; HANNA, M.G.JR. ; POMATA, N.** Delivery of radionuclides to pretargeted monoclonal antibodies using dihydrofolate reductase and methotrexate in an affinity system. *Cancer Res.,* 1993, vol. 53, 2368-2373 **[0288]**
- **INFUSA, H. ; ADACHI, T. ; KIYOKAWA, T. ; NAKATANI, Y. et al.** Ley glycolipid-recognizing monoclonal antibody inhibits procoagulant activity and metastasis of uman adenocarcinoma. *Int. J. Oncol.,* 2001, vol. 19, 941-946 **[0288]**
- **KODA, K. ; GLASSY, M.C. ; MCKNIGHT, M.E. ; YASUTOMI, J. ; SAITO, N. ; DAN, M. ; NAKAJIMA, N.** Immunotherapy for recurrent colorectal cancers with human monoclonal antibody SK-1. *Anticancer Res.,* 2001, vol. 21, 621-627 **[0288]**
- **KRANENBORG, M.H. ; BOERMAN, O.C. ; OOSTERWIJK-WAKKA, J. ; WEIJERT, M. ; CORSTENS, F. ; OOSTERWIJK, E.** Development and characterization of anti-renal cell carcinoma x antichelate bi-specific monoclonal antibodies for two-phase targeting of renal cell carcinoma. *Cancer Res,* 1995, vol. 55, 5864s-5867s **[0288]**
- **LOSMAN M.J. ; QU Z. ; KRISHNAN I.S. ; WANG J. ; HANSEN H.J. ; GOLDENBERG D.M. ; LEUNG S.O.** *Clin. Cancer Res.,* 1999, vol. 5 (10), 3101s-3105s **[0288]**
- **MAETA, M. SAITO ; H. OKA, S. ; TSUJITANI, S. ; IKEGUCHI, M. ; KAIBARA, N.** Mutated p53 in tumors, mutant p53 and p53-specific antibodies in the circulation in patients with gastric cancer. *J. Exp. Clin. Cancer Res.,* 2000, vol. 19, 489-95 **[0288]**
- **PENEFSKY, H.S.** A centrifuged column procedure for the measurement of ligand binding by beef heart F1. Part G. *Methods Enzymol,* 1979, vol. 56, 527-530 **[0288]**
- **RITTER, G. ; COHEN, L.S. ; WILLIAMS, C., JR. ; RICHARDS, E.C. ; OLD, L.J. ; WELT S.** Serological analysis of human anti-human antibody responses in colon cancer patients treated with repeated doses of humanized monoclonal antibody A33. *Cancer Res.,* 2001, vol. 61, 6851-6859 **[0288]**
- **SCHUHMACHER, J. ; KLIVENYI, G. ; MATYS,R. ; STADLER, M. ; REGIERT, T. ; HAUSER,H. ; DOLL, J. ; MAIER-BORST, W. ; ZOLLER, M.** Multistep tumor targeting in nude mice using bi-specific antibodies and a gallium chelate suitable for immunocintigraphy with positron emission tomography. *Cancer Res.,* 1995, vol. 55, 115-123 **[0288]**
- **SCHWARTZBERG, L.S.** Clinical experience with edrecolomab: a monoclonal antibody therapy for colorectal carcinoma. *Crit. Rev. Oncol. Hematol.,* 2001, vol. 40, 17-24 **[0288]**

- **SHARKEY, RM. ; KARACAY, GRIFFITHS, GL. ; BEHR, TM. ; BLUMENTHAL,RD. ; MATTES,MJ. ; HANSEN, HJ. ; GOLDENBERG.** Development of a streptavidin-anti-carcinoembryonic ntigen antibody, radiolabeled biotin pretargeting method for radioimmunotherapy of colorectal cancer. Studies in a human colon cancer xenograft model. *Bioconjugate Chem,* 1997, vol. 8, 595-604 **[0288]**
- **STAIB, L. ; BIREBENT, B. ; SOMASUNDARAM, R. ; PUREV, E. ; BRAUMULLER, H. et al.** Immunogenicity of recombinant GA733-2E antigen (CO17-1A, EGP, Dsi-4, KSA, Ep-CAM) in gastro-intestinal carcinoma patients. *Int. J. Cancer,* 2001, vol. 92, 79-87 **[0288]**
- **STICKNEY, DR. ; ANDERSON, LD. ; SLATER, JB. ; AHLEM, CN. ; KIRK, GA. ; SCHWEIGHARDT, SA ; FRINCKE, JM.** Bifunctional antibody: a binary radiopharmaceutical delivery system for imaging colorectal carcinoma. *Cancer Res.,* 1991, vol. 51, 6650-6655 **[0288]**
- **TODRYK, S.M. ; TURR, A.L. ; GREEN, M.H. ; SMALLWOOD, J.A. ; HALANEK, N. ; DALGLEISH, A.G. ; GLENNIE, M.J.** CD40 ligation for immunotherapy of solid tumours. *J. Immunol Methods,* 2001, vol. 248, 139-147 **[0288]**
- **TORDSSON, J. ; LAVASANI, S. ; OHLSSON, L. ; KARLSTROM, P. ; SVEDBERG, H. ; ABRAHMSEN, L. ; BRODIN, T.** A3—a novel colon and pancreatic cancer reactive antibody from a primate phage library seleted using intact tumour cells. *Int. J. Cancer,* 2000, vol. 87, 559-568 **[0288]**
- **TURNER, J.G. ; RAKHMILEVICH, A.L. ; BURDELYA, L. ; NEAL., Z. ; IMBODEN, M. ; SONDEL, P.M. ; YU, H.** Anti-CD40 antibody induces antitumor and antimetastatic effects: the role of NK cells. *J. Immunol.,* 2001, vol. 166, 89-94 **[0288]**
- *Res.,* 1991, vol. 51, 6650-6655 **[0288]**

专利名称(译)	生产和使用与双特异性抗体一起使用的新型肽基试剂		
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当前申请(专利权)人(译)	IMMUNOMEDICS INC.		
[标]发明人	HANSEN HANS J GRIFFITHS GARY L LEUNG SHUI ON MCBRIDE WILLIAM J QU ZHENGXING GOLDENBERG DAVID M		
发明人	HANSEN, HANS, J. GRIFFITHS, GARY, L. LEUNG, SHUI-ON MCBRIDE, WILLIAM, J. QU, ZHENGXING GOLDENBERG, DAVID, M.		
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CPC分类号	C07K16/468 A61K47/6863 A61K47/6899 A61K51/1048 A61K51/1063 A61K2039/505 B82Y5/00 C07K16/18 C07K16/30 C07K16/3007 C07K16/3046 C07K16/44 C07K2317/12 C07K2317/13 C07K2317/24 C07K2317/31 C07K2317/55 C07K2317/622 C07K2319/00		
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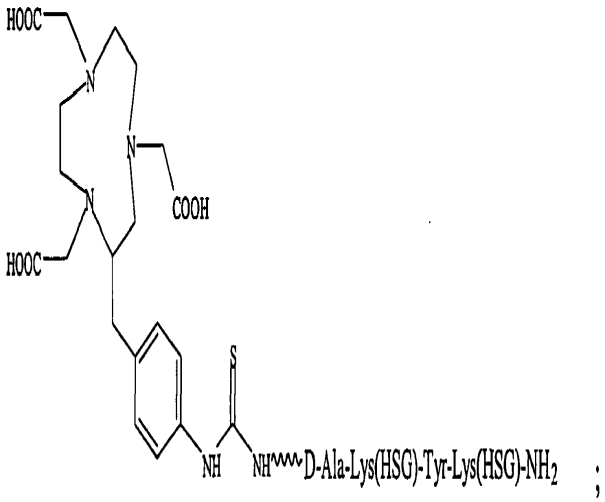
摘要(译)

本公开涉及双特异性抗体或抗体片段，其具有至少一个对靶组织具有反应性的臂和至少一个对接头部分具有反应性的臂。接头部分包括已制备抗体的半抗原。抗原接头与一种或多种治疗剂或诊断剂或酶缀合。本公开内容提供了用于产生双特异性抗体或抗体片段的构建体和方法，以及使用它们的方法。


HOOC
N
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D-Ala-Lys(HSG)-Tyr-Lys(HSG)-NH2
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