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(54) **ANTIBODIES TO MUC16 AND METHODS OF USE THEREOF**

(57) The disclosure provides antibodies, and antigen-binding fragments thereof, that specifically bind to a polypeptide, or antigenic portion thereof, wherein the polypeptide is selected from a) MUC16 ectodomain polypeptide, b) MUC16 cytoplasmic domain polypeptide, and c) MUC16 extracellular domain polypeptide that contains a cysteine loop polypeptide. The disclosure's antibodies and compositions containing them are useful in diagnostic and therapeutic applications for diseases in which MUC16 is overexpressed, such as cancer.

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Description

[0001] This application claims priority to co-pending U.S. provisional Application Serial No. 61/317,964, filed on March 26, 2010, which is herein incorporated by reference in its entirety for all purposes.

[0002] This invention was made with government support under PO1-CA52477-16 awarded by the United States Public Health Service (US PHS). The government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The invention relates to antibodies, and antigen-binding fragments thereof, that specifically bind to a polypeptide, or antigenic portion thereof, wherein the polypeptide is selected from a) MUC16 ectodomain polypeptide, b) MUC16 cytoplasmic domain polypeptide, and c) MUC16 extracellular domain polypeptide that contains a cysteine loop polypeptide. The invention's antibodies and compositions containing them are useful in diagnostic and therapeutic applications for diseases in which MUC16 is overexpressed, such as cancer.

BACKGROUND OF THE INVENTION

[0004] Cell surface markers and shed antigens are used in the diagnosis of several cancers. For example, the CA125 antigen, recognized by the OC125 antibody, is a tissue-specific, circulating antigen expressed in ovarian cancer. The CA125 antigen is encoded by the MUC16 gene, cloned by Lloyd and Yin. The full-length gene describes a complex tethered mucin protein present primarily in a variety of gynecologic tissues, especially neoplasms. OC125 and other related antibodies react with glycosylation-dependent antigens present exclusively in the cleaved portion of the molecule.

[0005] A serum assay can detect elevated levels of the circulating CA125 antigen in many epithelial ovarian cancer patients, and this antigen, derived using the ovarian cell line OVCA433, is recognized by the OC125 antibody (1-2). The detection of circulating CA125 in serum has proven to be a useful tool for the management of ovarian cancer patients and clinical trials (3-4). However, CA125 is neither sufficiently sensitive nor specific for general cancer screening (5-6). A variety of CA125 linked antibodies including VK8 and M11 have subsequently been defined as present on ovarian cancer cells (7-9). Although these antibodies have been used to develop serum assays and various other studies in ovarian cancer, they have significant shortcomings for clinical use in screening or tissue delivery. These antibodies are not useful as screening tools, nor can they detect the proximal residual MUC16 protein fragment after cleavage. This has limited their diagnostic and therapeutic applications.

[0006] For example, OC125, M11, and most other antibodies prepared against ovarian cancer cell extracts are directed at complex, glycosylation-dependent antigens. These antigens are exclusively present in the shed portion of MUC16 and cannot be employed to follow the biology of the proximal portion of MUC16 and may not accurately reflect tissue distribution since the glycosylation patterns can vary substantially among tissues. Because the vast majority of MUC16-reactive antibodies, including OC125, react with the glycosylation-dependent antigen present exclusively in the cleaved portion of the molecule, the true distribution of MUC16 expression is not known (21). There is currently no antibody available to track the fate of the remaining MUC16 protein fragment after cleavage and CA125 release.

[0007] Thus, there remains a need for the identification of antibodies that are directed against sequences in the peptide backbone of MUC16, and that are useful for diagnosis and treatment of cancers in which MUC16 is expressed and/or overexpressed.

SUMMARY OF THE INVENTION

[0008] The invention provides an antibody, or an antigen-binding fragment thereof, that specifically binds to a polypeptide, or antigenic portion thereof, wherein the polypeptide is selected from the group of a) MUC16 ectodomain polypeptide, b) MUC16 cytoplasmic domain polypeptide, and c) MUC16 extracellular domain polypeptide that contains a cysteine loop polypeptide CQVSTFRSVPNRHHTGVDSLC (SEQ ID NO:19). In one embodiment, the antibody internalizes into a cell. While not intending to limit the invention to a particular sequence of MUC 16 ectodomain, in one embodiment, the MUC16 ectodomain polypeptide comprises a polypeptide selected from the group of Polypeptide 1 NFSPLARRVDRVAIYEE (SEQ ID NO:01) and Polypeptide 2 TLDRSSVLVDGYSPNRNE (SEQ ID NO:02). In another embodiment, the antibody lacks specific binding to a glycosylated MUC16 extracellular domain. In yet a further embodiment, the antibody specifically binds to the Polypeptide 2 (SEQ ID NO:02) of the MUC16 ectodomain polypeptide, and wherein the antibody comprises a variable heavy (V_H) chain encoded by SEQ ID NO:06, and a variable light (V_L) chain encoded by SEQ ID NO:07. In yet another alternative embodiment, the antibody specifically binds to the Polypeptide 2 (SEQ ID NO:02) of the MUC16 ectodomain polypeptide, and wherein the antibody comprises a variable heavy (V_H) chain encoded by SEQ ID NO:04, and a variable light (V_L) chain encoded by SEQ ID NO:05. In a further embodiment, the antibody specifically binds to the Polypeptide 1 (SEQ ID NO:01) of the MUC16 ectodomain polypeptide, and wherein the antibody comprises

a variable heavy (V_H) chain encoded by SEQ ID NO:08, and a variable light (V_L) chain encoded by at least one of SEQ ID NO:09 and SEQ ID NO:10. In one embodiment, the MUC16 cytoplasmic domain polypeptide comprises VTTRR RKKEGEYNVQ QQ (SEQ ID NO:18). More preferably, but without limitation, the MUC16 cytoplasmic domain polypeptide comprises Polypeptide 3 CGVLVTTRRRKKEGEYNVQQQ (SEQ ID NO:03). In an alternative embodiment, the MUC16 extracellular domain polypeptide that contains a cysteine loop polypeptide comprises CQVSTFRSVPNRHHTGVDSLC (SEQ ID NO:19). More preferably, but without limitation, the MUC16 extracellular domain polypeptide comprises Polypeptide 4 KSYF SDCQVSTFRS VPNRHHTGVD SLCNFSPL (SEQ ID NO:15). In yet another alternative embodiment, the antibody specifically binds to the Polypeptide 4 (SEQ ID NO:15) of the MUC16 extracellular domain polypeptide, and wherein the antibody comprises a variable heavy (V_H) chain encoded by SEQ ID NO:11, and a variable light (V_L) chain encoded by SEQ ID NO:12. In a further alternative embodiment, the antibody is selected from the group of a chimeric antibody, a monoclonal antibody, a recombinant antibody, an antigen-binding fragment of a recombinant antibody, a humanized antibody, and an antibody displayed upon the surface of a phage. In another embodiment, the antigen-binding fragment is selected from the group of a Fab fragment, a F(ab')2 fragment, and a Fv fragment. In an alternative embodiment, the antibody, or antigen-binding fragment thereof, is covalently linked to a cytotoxic agent or a prodrug of a cytotoxic agent. In a preferred embodiment, the antibody is a monoclonal antibody produced by a hybridoma cell line.

[0009] The invention also provides an isolated monoclonal antibody, or an antigen-binding fragment thereof, produced by a hybridoma cell line, wherein the antibody specifically binds to a polypeptide, or antigenic portion thereof, wherein the polypeptide is selected from the group of a) MUC16 ectodomain polypeptide, b) MUC16 cytoplasmic domain polypeptide, and c) MUC16 extracellular domain polypeptide that contains a cysteine loop polypeptide CQVSTFRSVPNRHHTGVDSLC (SEQ ID NO:19). In one embodiment, the MUC16 ectodomain polypeptide comprises Polypeptide 1 (SEQ ID NO:01) and the antibody is selected from the group of 9B11.20.16, 10A2, 2F4, 23D3, 30B1, and 31B2. In an alternative embodiment, the MUC16 ectodomain polypeptide comprises Polypeptide 2 (SEQ ID NO:02), and wherein the antibody is selected from the group of 4H11.2.5, 13H1, 29G9, 9C9.21.5.13, 28F8, 23G12, 9C7.6, 11B6, 25G4, 5C2.17, 4C7, 26B2, 4A5.37, 4A2, 25H3, and 28F7.18.10. In yet a further embodiment, the MUC16 cytoplasmic domain polypeptide comprises Polypeptide 3 CGVLVTTRRRKKEGEYNVQQQ (SEQ ID NO:03), and wherein the antibody is selected from the group of 31A3.5.1, 19D1, 10F6, 22E10, 22F1, 3H8, 22F11, 4D7, 24G12, 19G4, 9A5, 4C2, 31C8, 27G4, and 6H2. In another alternative embodiment, the MUC16 extracellular domain polypeptide comprises Polypeptide 4 KSYF SDCQVSTFRS VPNRHHTGVD SLCNFSPL (SEQ ID NO:15), and wherein the antibody is selected from the group of 24B3 and 9C7.

[0010] The invention additionally provides a composition comprising (a) any one or more of the antibodies, or antigen-binding fragments thereof, that are described herein, and (b) a pharmaceutically acceptable carrier.

[0011] Also provided by the invention is a hybridoma cell line that produces a monoclonal antibody that specifically binds to a polypeptide, or antigenic portion thereof, selected from the group of a) MUC16 ectodomain polypeptide, b) MUC16 cytoplasmic domain polypeptide, and c) MUC16 extracellular domain polypeptide that contains a cysteine loop polypeptide CQVSTFRSVPNRHHTGVDSLC (SEQ ID NO:19).

[0012] The invention additionally provides a method for detecting a disease that comprises overexpression of MUC16 in a subject, comprising a) providing i) a sample from a subject, and ii) any one or more of the antibodies, or antigen-binding fragments thereof, that are described herein, b) contacting the sample with the antibody under conditions for specific binding of the antibody with its antigen, and c) detecting an increased level of binding of the antibody to the sample compared to a control sample lacking the disease, thereby detecting the disease in the subject. In one embodiment, the disease is cancer. In a preferred embodiment, the cancer is selected from the group of ovarian cancer and breast cancer. While not intending to limit the method of detection, in one embodiment, detecting binding of the antibody to the sample is immunohistochemical, enzyme-linked immunosorbent assay (ELISA), fluorescence-activated cell sorting (FACS), Western blot, immunoprecipitation, and/or radiographic imaging.

[0013] Also provided herein is a method for treating a disease that comprises overexpression of MUC16, comprising administering to a subject having the disease a therapeutically effective amount of any one or more of the antibodies, or antigen-binding fragments thereof, that are described herein. In one embodiment, the disease is cancer, as exemplified by ovarian cancer and breast cancer.

[0014] The invention also provides an isolated antibody, or an antigen-binding fragment thereof, that specifically binds to a MUC16 polypeptide or to an antigenic portion thereof, wherein the MUC16 polypeptide is selected from the group of a) TLDRKSVFVDGYSQNRDD (SEQ ID NO:21), b) KSYFSDCQVLAFRSVSNNNNHTGVDSLCNFSPL (SEQ ID NO:22), c) SLYSNCRLASLRPKKNGTATGVNAICSYHQN (SEQ ID NO:23), d) KSYFSDCQVNNFRS, e) TLDRSSVLVDGYSQNRDD, and f) TLDRSSVLVDGYSQNRDD. In one embodiment, the antibody is selected from the group of a monoclonal antibody, a chimeric antibody, a recombinant antibody, an antigen-binding fragment of a recombinant antibody, a humanized antibody, and an antibody displayed upon the surface of a phage. In a preferred embodiment, the antibody is a monoclonal antibody produced by hybridoma cells selected from the group of 12B10-3G10, 10C4-3H5, 10C4-1F2, 10C4-2H8, 10C4-1G7, 17F2-3G5, 17F2-3F6, 17F2-2F9, 17F2-1E11, 12B10-3F7, 12B10-2F6, 12B10-2F10, 25E9-3, 25E9-5, 25E9-1, 25E9-16, 21B8-1H11, 21B8-3G6, 21B8-3H9, 21B8-1G8, 21E1-1E3, 21E1-1G9, 21E1-2G7,

21E1-3G12, 4H1-2E1, 4H1-2E3, 4H1-3E1, 4H1-3H3, 15A8-2E2, 15A8-2E10, 15A8-2E11, 15A8-3D2, 22B5-1F6, 22B5-3G9, 22B5-2G8, and 22B5-3F11. In a particular embodiment, the MUC16 polypeptide is TLDRKSVFVDGYSQNRDD (SEQ ID NO:21), and the antibody comprises a variable heavy (V_H) chain sequence SEQ ID NO:27, and a variable light (V_L) chain sequence SEQ ID NO:29, such as the monoclonal antibody produced by hybridoma cell 12B10-3G10. In an alternative embodiment, the antigen-binding fragment is selected from the group of a Fab fragment, a F(ab')2 fragment, and a Fv fragment. In a more preferred embodiment, the antibody, or antigen-binding fragment thereof, is covalently linked to a cytotoxic agent and/or to a prodrug of a cytotoxic agent. In a further embodiment, the antibody specifically binds to human MUC16 (SEQ ID NO:25). In another embodiment, the antibody internalizes into a cell. In an alternative embodiment, the antibody lacks specific binding to a glycosylated MUC16 extracellular domain.

[0015] The invention also provides a composition comprising (a) any one or more of the invention's antibodies and/or antigen-binding fragments thereof, and (b) a pharmaceutically acceptable carrier.

[0016] The invention further provides a hybridoma cell that produces an antibody, or an antigen-binding fragment thereof, that specifically binds to a MUC16 polypeptide or to an antigenic portion thereof, wherein the MUC16 polypeptide is selected from the group of a) TLDRKSVFVDGYSQNRDD (SEQ ID NO:21), b) KSYFSDCQVLAFRSVSNNNNHTGVDSLCNFSPL (SEQ ID NO:22), c) SLYSNCRLASLRPKKNGTATGVNAICSYHQN (SEQ ID NO:23), d) KSYFSDCQVNNFRS, e) TLDRSSVLVDGYSQNRDD, and f) TLDRSSVLVDGYSQNRDD.

[0017] The invention also provides an isolated nucleotide sequence comprising a polynucleotide that encodes at least one of a variable heavy (V_H) chain sequence and the variable light (V_L) chain sequence of an antibody that specifically binds to a MUC16 polypeptide, wherein the MUC16 polypeptide is selected from the group of a) TLDRKSVFVDGYSQNRDD (SEQ ID NO:21), b) KSYFSDCQVLAFRSVSNNNNHTGVDSLCNFSPL (SEQ ID NO:22), c) SLYSNCRLASLRPKKNGTATGVNAICSYHQN (SEQ ID NO:23), d) KSYFSDCQVNNFRS, e) TLDRSSVLVDGYSQNRDD, and f) TLDRSSVLVDGYSQNRDD. In one embodiment, the MUC16 polypeptide is TLDRKSVFVDGYSQNRDD (SEQ ID NO:21) and the polynucleotide encoding the variable heavy (V_H) chain sequence comprises SEQ ID NO:26, and wherein the polynucleotide encoding the variable light (V_L) chain sequence comprises SEQ ID NO:28.

[0018] The invention also provides a method for producing an antibody that specifically binds to a MUC16 polypeptide or to an antigenic portion thereof, comprising administering to a subject an immunologically effective amount of a MUC16 polypeptide selected from the group of a) TLDRKSVFVDGYSQNRDD (SEQ ID NO:21), b) KSYFSDCQVLAFRSVSNNNNHTGVDSLCNFSPL (SEQ ID NO:22), c) SLYSNCRLASLRPKKNGTATGVNAICSYHQN (SEQ ID NO:23), d) KSYFSDCQVNNFRS, e) TLDRSSVLVDGYSQNRDD, and f) TLDRSSVLVDGYSQNRDD.

[0019] The invention additionally provides a method for identifying a subject as having disease, comprising determining the level, in a sample from the subject, of specific binding of any one or more of the invention's antibodies and/or antigen-binding fragments thereof, with the MUC16 polypeptide or with the antigenic portion thereof, wherein detecting an altered level of the specific binding relative to a control sample identifies the subject as having disease. In one embodiment, the disease is cancer exemplified by ovarian cancer and breast cancer. In another embodiment, the method further comprises detecting an altered level of binding of the antibody to the sample compared to a control sample. Optionally, the detecting is selected from the group of immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), fluorescence-activated cell sorting (FACS), Western blot, immunoprecipitation, and radiographic imaging.

[0020] The invention also provides a method for reducing one or more symptoms of disease comprising administering to a subject in need thereof a therapeutically effective amount of any one or more of the invention's antibodies and/or antigen-binding fragment thereof. In one embodiment, the disease is cancer, exemplified by ovarian cancer and breast cancer. Optionally, the method further comprises detecting a reduction in one or more symptoms of the disease after the administration step.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021]

Figure 1: Three MUC16 carboxy terminus peptides were synthesized at the MSKCC Microchemistry Core Facility. Polypeptide 1 is near the putative cleavage site, Polypeptide 2 is before the transmembrane, and Polypeptide 3 is the internal peptide, which is inside the transmembrane.

Figure 2: Comparison staining of high-grade serous ovarian carcinomas using OC125 (left panel) and 4H11 (right panel)

Figure 3: Immunohistochemical scoring of OC125 and 4H11 on tissue microarrays of high-grade ovarian serous carcinoma. Only membranous and/or cytoplasmic staining was considered positive. Score 0: No staining; Score 1: <5% strong or weak; Score 2: 5-50% strong or weak; Score 3: 51-75% strong or 51-100% weak; Score 4: 76-99% strong; Score 5: 100% strong. Figure 3A: OC125 (Score 0); Figure 3B: OC125 (Score 1); Figure 3C: OC125 (Score 2); Figure 3D: OC125 (Score 3); Figure 3E: OC125 (Score 4); Figure 3F: OC125 (Score 5); Figure 3G: 4H11 (Score 0); Figure 3H: 4H11 (Score 1); Figure 3I: 4H11 (Score 2); Figure 3J: 4H11 (Score 3); Figure 3K: 4H11 (Score 4);

Figure 3L: 4H11 (Score 5).

Figure 4: Western blot analysis. Figure 4A: Western blot analysis of GST-ΔMUC16^{c114} fusion protein with monoclonal antibodies 9C9.21.5.13 and 4H11.2.5. Figure 4B: Western blot analysis of SKOV3-phrGFP-ΔMUC16^{c114} and SKOV3-phrGFP-AMUC16^{c334} protein extract and probed with monoclonal antibodies 9C9.21.5.13 and 4H11.2.5.

Figure 5A: MUC16 carboxy terminus monoclonal antibodies binding affinity on OVCAR3 cells (Panels A-D). Figure 5B: Internalization of radio-labeled 4H11 and OC125 monoclonal antibodies on SKOV3-phrGFP-ΔMUC16^{c334} stable transfected cells.

Figure 6A-D: Comparison staining intensities of OC125 and 4H11 monoclonal antibodies on tissue microarrays containing cancers of the prostate (2A, concordant), lung (2B, discordant), breast (2C, discordant), and pancreas (2D, discordant).

Figure 7: FACS analysis as described in the Material and Methods section was performed with commercial antibodies and MUC16 carboxy terminus monoclonal antibodies on OVCAR3 wt, SKOV3-phrGFP-ΔMUC16^{c114} and SKOV3-phrGFP-ΔMUC16^{c334} stable transfected cell lines.

Figure 8: Nucleotide sequence encoding antibody variable heavy (V_H) chain and antibody variable light (V_L) chain. (A) 4A5 V_H (SEQ ID NO:04), (B) 4A5 V_L (SEQ ID NO:05), (C) 4H11 V_H (SEQ ID NO:06), (D) 4H11 VL (SEQ ID NO:07), (E) 9B11 V_H (SEQ ID NO:08), (F) 9B11 $V_{L.A}$ (SEQ ID NO:09), (G) 9B11 $V_{L.B}$ (SEQ ID NO:10), (H) 24B3 V_H (SEQ ID NO:11), (I) 24B3 V_L (SEQ ID NO:12).

Figure 9: (A) Homo sapiens MUC16 (GenBank NP_078966) (SEQ ID NO:13), (B) Polypeptide 1 (SEQ ID NO:O1), (C) Polypeptide 2 (SEQ ID NO:02), (D) Polypeptide 3 (SEQ ID NO:03), (E) Transmembrane domain (SEQ ID NO:14), (F) Polypeptide 4 (SEQ ID NO:15) containing a cysteine loop polypeptide (SEQ ID NO:19).

Figure 10: Schematic of MUC16 structure.

Figure 11. Design and *in vitro* analysis of MUC-CD targeted CARs. (A) Schematic diagram of the first generation 4H11z and second generation 4H11-28z retroviral vectors. 4H11scFv: MUC16 specific scFv derived from the heavy (V_H) and light (V_L) chain variable regions of the monoclonal antibody 4H11; CD8: CD8 hinge and transmembrane domains; CD28: CD28 transmembrane and cytoplasmic signaling domains; ζ chain: T cell receptor ζ chain cytoplasmic signaling domain; LTR: long terminal repeat; black box: CD8 leader sequence; grey box: $(Gly_4Ser)_3$ linker; arrows indicate start of transcription. (B) FACS analysis of human T cells retrovirally transduced to express either the 4H11z or 19z1 CAR. (C) 4H11z$^+$- but not 19z1$^+$T cells expand on 3T3(MUC-CD/B7.1) AAPC. CAR$^+$were co-cultured on 3T3(MUC-CD/B7.1) AAPC monolayers at 3 x 10^6 CAR$^+$T cells/well of a 6 well plate. Proliferation of CAR$^+$ T cells, normalized to the CAR$^+$ T cell fraction as assessed by FACS for the CAR$^+$ fraction in combination with viable T cell counts obtained on days 2, 4 and 7, as assessed by trypan blue exclusion assays.

Figure 12. *In vitro* comparison of T cells modified to express the first generation 4H11z CAR to T cells modified to express the second generation co-stimulatory 4H11-28z CAR. (A) CAR$^+$ T cells were co-cultured on MUC-CD monolayers with (right panel) or without B7.1 (left panel). 3 x 10^6 CAR$^+$T cells were co-cultured on AAPC monolayers in 6 well tissue culture plates in cytokine-free medium. Total viable T cell counts were assessed on days 2, 4 and 7, by trypan blue exclusion assays. 4H11-28z$^+$ T cells markedly expanded when compared to 4H11z$^+$ T cells upon co-culture with 3T3(MUC-CD) AAPCs, **p=0.0023 (4H11z compared to 4H11-28z). In contrast, both 4H11z$^+$ and 4H11-28z$^+$ T cells expanded similarly on 3T3(MUC-CD/B7.1) AAPCs, p=0.09, (4H11z compared to 4H11-28z). Control 19-28z$^+$ T cells did not proliferate on 3T3(MUC-CD), **p=0.0056 (19-28z compared to 4H11z), **p=0.0011 (19-28z compared to 4H11-28z), or on 3T3(MUC-CD/B7.1), **p=0.0026 (19-28z compared to 4H11z), **p=0.0087 (19-28z compared to 4H11-28z). (B) 4H11-28z$^+$but not 4H11z$^+$ T cells secrete IL-2 upon co-culture with 3T3(MUC-CD) AAPCs. Tissue culture supernatants at day 2 following activation on 3T3(MUC-CD) AAPCs were analyzed for cytokine secretion. 4H11-28z$^+$ T cells, in contrast to 4H11z$^+$ T cells, demonstrated enhanced secretion of IL-2 consistent with T cell co-stimulation mediated through the 4H11-28z CAR. ***p=0.0008 (19z1 or 19-28z compared to 4H11z), **p=0.0026 (19z1 or 19-28z compared to 4H11-28z), **p=0.0046 (4H11z compared to 4H11-28z). Furthermore, both 4H11-28z$^+$ and 4H11z$^+$T cells secreted IFNγ. *p=0.011 (4H11z compared to 4H11-28z). Control 19z1 and 1928z transduced T cells failed to secrete either IL-2 or IFNγ. **p=0.0034 (19z1 compared to 4Hllz), **p=0.036 (19-28z compared to 4H11z), ***p=0.0008 (19-28z compared to 4H11-28z). (C) Expansion of CAR$^+$T cells following 3 cycles of stimulation on 3T3(MUC-CD/B7.1). Human T cells transduced to express either 4H11z or 4H11-28z CARs demonstrated a >2 log expansion over 2 cycles of stimulation on 3T3(MUC-CD/B7.1) AAPCs. Arrows indicate 1st and 2nd cycles of restimulation on AAPCs. (D) FACS analysis of the CAR$^+$ T cell fraction of 4H11-28z$^+$ T cells increased following each weekly cycle of stimulation. (I) FACS following initial transduction, (II) FACS at 7 days following first stimulation on AAPCs, (III) FACS at 7 days following second stimulation on AAPCs. These data are representative of one of three different experiments using three different healthy donor T cell populations, all of which demonstrated similar proliferation and cytokine secretion patterns.

Figure 13. MUC-CD targeted T cells specifically expand and lyse MUC-CD$^+$ tumor cells. (A) Cytotoxicity assay of 4H11z$^+$ and 4H11-28z$^+$T cells targeting OV-CAR(MUC-CD) tumor cells demonstrates efficient cytotoxicity mediated by T cells from healthy donors modified to express the first and second generation MUC-CD targeted CARs. Control

T cells modified to express the first and second generation CD19-targeted 19z1 and 19-28z CARs failed to demonstrate significant lysis of target tumor cells. (B) Healthy donor T cells modified to express the 4H11-28z CAR equally lyse primary patient ascites-derived MUC-CD$^+$ tumor cells when compared to T cells modified to express the control 19-28z CAR. This data represents 1 or 3 experiments targeting primary tumor cells from 3 ovarian carcinoma patients with similar results. (C) Autologous T cells isolated from peripheral blood, when modified with the 4H11-28z CAR, exhibit significant lysis of autologous MUC-CD$^+$ ascites-derived tumor cells when compared to control 19-28z$^+$ autologous T cells. These data represent 1 of 3 experiments utilizing T cells and autologous tumor cells from 3 different ovarian carcinoma patients with similar results. (D) Antigen specific proliferation of MUC-CD targeted CFSE labeled T cells after co-culture with OV-CAR3(MUC-CD) tumor cells. CFSE labeled CAR$^+$T cells were co-cultured with MUC-CD expressing OV-CAR3 tumor cells at 1:1 ratio for 5 days. Proliferation of CFSE labeled T cells was assessed by FACS demonstrating efficient proliferation of both 4H11z$^+$ and 4H11-28z$^+$ T cells but not control 19-28z$^+$ T cells. (E) CFSE results were further confirmed by absolute T cell numbers assessed on days 2, 4 and 7 following co-culture with OV-CAR3(MUC-CD) tumor cells. (F) FACS analysis of the expression of 4-1BBL on OVCAR3(MUC-CD) cells. OV-CAR3(MUC-CD) cells were stained with anti-human 4-1BBL antibody (thick line) or with isotype control (thin line). FACS analysis demonstrated expression of 4-1BBL on OV-CAR3(MUC-CD) tumor cells. Further FACS analyses failed to reveal expression of the co-stimulatory ligands B7.1, B7.2, or OX-40L.

Figure 14. Eradication of OV-CAR3(MUC-CD) tumors after intra-peritoneal treatment with first and second generation of MUC-CD targeted T cells. (A) Intraperitoneal injection of OV-CAR3(MUC-CD) tumors in untreated SCID-Beige mice results in abdominal distension and nodular peritoneal tumors. SCID-Beige mice were injected intraperitoneally with 3x10^6 OV-CAR3(MUC-CD) cells. At 5 weeks post intraperitoneal injection of OV-CAR3(MUC-CD) tumor cells mice developed ascities as evidenced by a distended abdomen (center panel) when compared to a tumor free mouse (left panel). Post mortem visualization of the peritoneum demonstrates nodular tumor masses (arrows) within the abdominal cavity (right panel). (B) Intraperitoneal injection of 4H11z$^+$ and 4H11-28z$^+$ T cells either delay tumor progression or fully eradicate disease. Kaplan-Meier survival curve of SCID-Beige mice treated with first or second generation of MUC-CD targeted T cells. SCID-Beige mice were infused ip with 3x10^6 OV-CAR3(MUC-CD) tumor cells on day 1 followed by 3x10^7 4H11z$^+$ or 4H11-28z$^+$ T cells on day 2. All untreated mice or mice treated with control 19z1$^+$ T cells developed established tumors and were sacrificed by day 50. In contrast, 27% of mice treated with either 4H11z$^+$or 4H11-28z$^+$ T cells remained without clinical evidence of disease by day 120. *p=0.01 (4H11z compared to 19z1), **p=0.0023 (4H11-28z compared to 19z1), p=0.63 (4H11z compared to 4H11-28z).

Figure 15. MUC-CD targeted 4H11-28z$^+$ T cells successfully traffic to ip OV-CAR3(MUC-CD/GFP-FFLuc) tumors following systemic intravenous infusion resulting in equally efficient anti-tumor efficacy when compared to ip 4H11-28z$^+$ treated tumor bearing mice. (A) Kaplan-Meier survival curve of SCID-Beige mice treated ip or iv with 4H11-28z$^+$ T cells. SCID-Beige mice were injected intraperitoneally with 3x10^6 OV-CAR3(MUC-CD/GFP-FFLuc) tumor cells followed by either iv or ip infusion of 3x10^7 4H11-28z$^+$ T cells. Tumor eradication is enhanced after either ip or iv infusion of 4H11-28z$^+$ T cells when compared to control treated mice. Both ip and iv 4H11-28z$^+$ T cell treated mice exhibited statistically enhanced survival (***p<0.0001 and **p=0.0038, respectively) when compared to 19-28z$^+$ T cell treated control cohorts. Conversely, difference in survival between the ip and iv 4H11-28z$^+$ T cell cohorts was not statistically significant (p=0.22). (B) BLI of tumor progression of representative ip and iv 4H11-28z$^+$ T cell treated mice with ultimately progressive disease following treatment compared to BLI of tumor progression in a representative control 19-28z$^+$ T cell treated mouse. (C) Systemically injected CFSE stained 4H11-28z$^+$ T cells traffic to advanced ip OV-CAR(MUC-CD) tumors. Presence of iv injected CFSE labeled 19-28z$^+$ control T cells (left panel) and 4H11-28z$^+$ T cells (right panel) 1 day following infusion into SCID-Beige mice with advanced OV-CAR(MUC-CD) tumors (injected 7 days earlier), as assessed by FACS analysis of single cell OV-CAR3(MUC-CD) tumor suspensions, reveals a marked population of 4H11-28z$^+$ but not control 19-28z$^+$ T cells within peritoneal OV-CAR3(MUC-CD) tumors.

Figure 16. Eradication of advanced OV-CAR3(MUC-CD) tumors in SCID-Beige mice by ip infusion of 4H11-28z$^+$ T cells. SCID-Beige mice were injected ip with 3x10^6 OV-CAR3(MUC-CD/GFP-FFLuc) tumor cells 7 days prior to ip treatment with 3x10^7 4H11-28z$^+$ T cells. (A) BLI of 4H11-28z$^+$ T cell treated mice with either relapsed disease (middle row) or eradicated disease (bottom row) compared to a representative 19-28z$^+$ T cell treated control mouse. (B) Kaplan-Meier survival curve of SCID-Beige mice with advanced OV-CAR3(MUC-CD/GFP-FFLuc) tumors treated ip with 4H11-28z$^+$ T cells. All 4H11-28z$^+$ T cell treated mice demonstrated enhanced survival when compared to control 19-28z$^+$ T cell treated mice (**p=0.0011), with an overall long-term survival of 25% at day 120.

Figure 17: CD8 leader sequence, CD3 zeta chain intracellular domain sequence, (G4S)3 serine-glycine linker sequence, CD8 transmembrane domain sequence, and CD28 transmembrane + intracellular domains (-STOP) sequence.

Figure 18: SFG_4H11z sequence.

Figure 19: SFG-4H11-28z sequence.

Figure 20: (A) Mouse MUC16-CD Peptide 1 (SEQ ID NO:21), Mouse first Cysteine Loop Peptide 2 (SEQ ID NO:22), and Mouse second Cysteine Loop Peptide 3 (SEQ ID NO:23). (B) Alignment of mouse MUC16 (SEQ ID NO:24)

and human MUC16 (SEQ ID NO:25) amino acid sequences. A cysteine was added to the peptide sequence at the N terminus of Peptide 1 and Peptide 3 for better conjugation with KLH.

Figure 21: ID8 extract with 1:10 dilution of Mouse MUC16 monoclonal Primary Supernatants.

Figure 22: BR5-FVB1 extract with 1:10 dilution of Mouse MUC16 monoclonal Primary Supernatants

Figure 23: Western Blot showing 38 hamster's monoclonal antibody Supernatants on ID8 cell extracts.

Figure 24 (A) Nucleotide sequence encoding 12B10-3G10-V_H (SEQ ID NO:26), (B) 12B10-3G10-V_H Amino Acid sequence (SEQ ID NO:27), (C) Nucleotide sequence encoding 12B10-3G10-V_L (SEQ ID NO:28) (Note the VL has an optional *NotI* site added by the primer for cloning, and (D) 12B10-3G10-V_L Amino Acid sequence (SEQ ID NO:29).

Figure 25: FACS Analysis with Purified 12B10-3G10 mAb on ID8 (mouse), OVCAR-3 (human) and BR5-FVB1 (mouse) cell lines.

DEFINITIONS

[0022] To facilitate understanding of the invention, a number of terms are defined below.

[0023] The terms "purified," "isolated," and grammatical equivalents thereof as used herein, refer to the reduction in the amount of at least one undesirable component (such as cell, protein, nucleic acid sequence, carbohydrate, *etc.*) from a sample, including a reduction by any numerical percentage of from 5% to 100%, such as, but not limited to, from 10% to 100%, from 20% to 100%, from 30% to 100%, from 40% to 100%, from 50% to 100%, from 60% to 100%, from 70% to 100%, from 80% to 100%, and from 90% to 100%. Thus purification results in an "enrichment," *i.e.,* an increase in the amount of a desirable component cell, protein, nucleic acid sequence, carbohydrate, *etc.*).

[0024] The term "antibody" refers to an immunoglobulin *(e.g.,* IgG, IgM, IgA, IgE, IgD, *etc.).* The basic functional unit of each antibody is an immunoglobulin (Ig) mononer (containing only one immunoglobulin ("Ig") unit). Included within this definition are polyclonal antibody, monoclonal antibody, and chimeric antibody.

[0025] The variable part of an antibody is its "V domain" (also referred to as "variable region"), and the constant part is its "C domain" (also referred to as "constant region") such as the kappa, lambda, alpha, gamma, delta, epsilon and mu constant regions. The "variable domain" is also referred to as the "F_v region" and is the most important region for binding to antigens. More specifically, variable loops, three each on the light (V_L) and heavy (VH) chains are responsible for binding to the antigen. These loops are referred to as the "complementarity determining regions" ("CDRs" and "idiotypes.")

[0026] The immunoglobulin (Ig) monomer of an antibody is a "Y"-shaped molecule that contains four polypeptide chains: two light chains and two heavy chains, joined by disulfide bridges.

[0027] Light chains are classified as either (λ) or kappa (κ). A light chain has two successive domains: one constant domain ("C_L") and one variable domain ("V_L"). The variable domain, V_L, is different in each type of antibody and is the active portion of the molecule that binds with the specific antigen. The approximate length of a light chain is 211 to 217 amino acids.

[0028] Each heavy chain has two regions, the *constant region* and the *variable region.* The There are five types of mammalian Ig heavy denoted a α, δ, ε, γ, and μ. The type of heavy chain present defines the *class* of antibody; these chains are found in IgA, IgD, IgE, IgG, and IgM antibodies, respectively. Distinct heavy chains differ in size and composition; α and γ contain approximately 450 amino acids, while μ and ε have approximately 550 amino acids. Each heavy chain has two regions, the constant region ("C_H") and the variable ("V_H") region. The constant region (C_H) is identical in all antibodies of the same isotype, but differs in antibodies of different isotypes. Heavy chains γ, α and δ have a constant region composed of *three* tandem (in a line) Ig domains, and a hinge region for added flexibility. Heavy chains μ and ε have a constant region composed *of four* immunoglobulin domains. The variable region (V_H) of the heavy chain differs in antibodies produced by different B cells, but is the same for all antibodies produced by a single B cell or B cell clone. The variable region of each heavy chain is approximately 110 amino acids long.

[0029] The term "specifically binds" and "specific binding" when made in reference to the binding of two molecules *(e.g.* antibody to an antigen, *etc.*) refer to an interaction of the two molecules that is dependent upon the presence of a particular structure on one or both of the molecules. For example, if an antibody is specific for epitope "A" on the molecule, then the presence of a protein containing epitope A (or free, unlabelled A) in a reaction containing labeled "A" and the antibody will reduce the amount of labeled A bound to the antibody.

[0030] The term "capable of binding" when made in reference to the interaction between a first molecule (such as antibody, polypeptide, glycoprotein, nucleic acid sequence, *etc.*) and a second molecule (such as antigen, polypeptide, glycoprotein, nucleic acid sequence, *etc.*) means that the first molecule binds to the second molecule in the presence of suitable concentration of salts, and suitable temperature, and pH. The conditions for binding molecules may be determined using routine and/or commercially available methods

[0031] The terms "antigen," "immunogen," "antigenic," "immunogenic," "antigenically active," "immunologic," and "immunologically active" when made in reference to a molecule, refer to any substance that is capable of inducing a specific humoral immune response (including eliciting a soluble antibody response) and/or cell-mediated immune response

(including eliciting a CTL response). Antigenic peptides preferably contain at least 5, at least 6, at least 7, at least 8, at least 9, and more preferably at least 10 amino acids. To elicit antibody production, in one embodiment, antigens may be conjugated to keyhole limpet hemocyanin (KLH) or fused to glutathione-S-transferase (GST).

[0032] A "cognate antigen" when in reference to an antigen that binds to an antibody, refers to an antigen that is capable of specifically binding to the antibody.

[0033] In one embodiment, the antigen comprises an epitope. The terms "epitope" and "antigenic determinant" refer to a structure on an antigen, which interacts with the binding site of an antibody or T cell receptor as a result of molecular complementarity. An epitope may compete with the intact antigen, from which it is derived, for binding to an antibody.

[0034] As used herein the terms "portion" and "fragment" when made in reference to a nucleic acid sequence or protein sequence refer to a piece of that sequence that may range in size from 2 contiguous nucleotides and amino acids, respectively, to the entire sequence minus one nucleotide and amino acid, respectively.

[0035] A "subject" that may benefit from the invention's methods includes any multicellular animal, preferably a mammal. Mammalian subjects include humans, non-human primates, murines, ovines, bovines, ruminants, lagomorphs, porcines, caprines, equines, canines, felines, aves, *etc.).* Thus, mammalian subjects are exemplified by mouse, rat, guinea pig, hamster, ferret and chinchilla. The invention's compositions and methods are also useful for a subject "in need of reducing one or more symptoms of a disease, e.g., in need of reducing cancer metastasis and/or in need of reducing one or more symptoms of cancer, includes a subject that exhibits and/or is at risk of exhibiting one or more symptoms of the disease. For Example, subjects may be at risk based on family history, genetic factors, environmental factors, *etc.* This term includes animal models of the disease. Thus, administering a composition (which reduces a disease and/or which reduces one or more symptoms of a disease) to a subject in need of reducing the disease and/or of reducing one or more symptoms of the disease includes prophylactic administration of the composition (*i.e.,* before the disease and/or one or more symptoms of the disease are detectable) and/or therapeutic administration of the composition (*i.e.,* after the disease and/or one or more symptoms of the disease are detectable). The invention's compositions and methods are also useful for a subject "at risk" for disease (such as cancer) refers to a subject that is predisposed to contracting and/or expressing one or more symptoms of the disease. This predisposition may be genetic *(e.g.,* a particular genetic tendency to expressing one or more symptoms of the disease, such as heritable disorders, *etc.*), or due to other factors (*e.g.*, environmental conditions, exposures to detrimental compounds, including carcinogens, present in the environment, *etc.*). The term subject "at risk" includes subjects "suffering from disease," *i.e.,* a subject that is experiencing one or more symptoms of the disease. It is not intended that the present invention be limited to any particular signs or symptoms. Thus, it is intended that the present invention encompass subjects that are experiencing any range of disease, from sub-clinical symptoms to full-blown disease, wherein the subject exhibits at least one of the indicia (*e.g.*, signs and symptoms) associated with the disease.

[0036] "Cancer cell" refers to a cell undergoing early, intermediate or advanced stages of multistep neoplastic progression as previously described (Pitot et al., Fundamentals of Oncology, 15-28 (1978)). This includes cells in early, intermediate and advanced stages of neoplastic progression including "pre-neoplastic cells (*i.e.,* "hyperplastic cells and dysplastic cells), and neoplastic cells in advanced stages of neoplastic progression of a dysplastic cell.

[0037] "Metastatic" cancer cell refers to a cancer cell that is translocated from a primary cancer site (*i.e.,* a location where the cancer cell initially formed from a normal, hyperplastic or dysplastic cell) to a site other than the primary site, where the translocated cancer cell lodges and proliferates.

[0038] "Cancer" refers to a plurality of cancer cells that may or may not be metastatic, such as ovarian cancer, breast cancer, lung cancer, prostate cancer, cervical cancer, pancreatic cancer, colon cancer, stomach cancer, esophagus cancer, mouth cancer, tongue cancer, gum cancer, skin cancer *(e.g.,* melanoma, basal cell carcinoma, Kaposi's sarcoma, *etc.*), muscle cancer, heart cancer, liver cancer, bronchial cancer, cartilage cancer, bone cancer, testis cancer, kidney cancer, endometrium cancer, uterus cancer, bladder cancer, bone marrow cancer, lymphoma cancer, spleen cancer, thymus cancer, thyroid cancer, brain cancer, neuron cancer, mesothelioma, gall bladder cancer, ocular cancer *(e.g.,* cancer of the cornea, cancer of uvea, cancer of the choroids, cancer of the macula, vitreous humor cancer, *etc.*), joint cancer (such as synovium cancer), glioblastoma, lymphoma, and leukemia.

[0039] "Sample" and "specimen" as used herein are used in their broadest sense to include any composition that is obtained and/or derived from a biological source, as well as sampling devices (*e.g.*, swabs), which are brought into contact with biological or environmental samples. "Biological samples" include those obtained from a subject, including body fluids (such as urine, blood, plasma, fecal matter, cerebrospinal fluid (CSF), semen, sputum, and saliva), as well as solid tissue. Biological samples also include a cell (such as cell lines, cells isolated from tissue whether or not the isolated cells are cultured after isolation from tissue, fixed cells such as cells fixed for histological and/or immunohistochemical analysis), tissue (such as biopsy material), cell extract, tissue extract, and nucleic acid *(e.g.,* DNA and RNA) isolated from a cell and/or tissue, and the like. These examples are illustrative, and are not to be construed as limiting the sample types applicable to the present invention.

[0040] "Overexpression of MUC16" by a cell of interest (such as a cancer cell) refers to a higher level of MUC16 protein and/or mRNA that is expressed by the cell of interest compared to a control cell (such as a non-cancerous cell, normal

cell, *etc.*).

[0041] "Internalize" when in reference to a cell refers to entry from the extracellular medium into the cell membrane and/or cytoplasm.

[0042] "Glycosylated" when in reference to a sequence (*e.g.*, an amino acid sequence or nucleotide sequence) refers to a sequence that is covalently linked to one or more saccharides.

[0043] "Pharmaceutical" and "physiologically tolerable " composition refers to a composition that contains pharmaceutical molecules, *i.e.,* molecules that are capable of administration to or upon a subject and that do not substantially produce an undesirable effect such as, for example, adverse or allergic reactions, dizziness, gastric upset, toxicity and the like, when administered to a subject. Preferably also, the pharmaceutical molecule does not substantially reduce the activity of the invention's compositions. Pharmaceutical molecules include "diluent" (*i.e.,* "carrier") molecules and excipients.

[0044] "Immunogenically effective" and "antigenically effective" amount of a molecule interchangeably refer to an amount of the molecule that is capable of inducing a specific humoral immune response (including eliciting a soluble antibody response) and/or cell-mediated immune response (including eliciting a cytotoxic T-lymphocyte (CTL) response).

[0045] "Treating" a disease refers to reducing one or more symptoms (such as objective, subjective, pathological, clinical, sub-clinical, *etc.*) of the disease.

[0046] The terms "reduce," "inhibit," "diminish," "suppress," "decrease," and grammatical equivalents (including "lower," "smaller," *etc.*) when in reference to the level of any molecule *(e.g.,* amino acid sequence, and nucleic acid sequence, antibody, *etc.*), cell, and/or phenomenon (*e.g.*, disease symptom, binding to a molecule, specificity of binding of two molecules, affinity of binding of two molecules, specificity to cancer, sensitivity to cancer, affinity of binding, enzyme activity, *etc.*) in a first sample (or in a first subject) relative to a second sample (or relative to a second subject), mean that the quantity of molecule, cell and/or phenomenon in the first sample (or in the first subject) is lower than in the second sample (or in the second subject) by any amount that is statistically significant using any art-accepted statistical method of analysis. In one embodiment, the quantity of molecule, cell and/or phenomenon in the first sample (or in the first subject) is at least 10% lower than, at least 25% lower than, at least 50% lower than, at least 75% lower than, and/or at least 90% lower than the quantity of the same molecule, cell and/or phenomenon in the second sample (or in the second subject). In another embodiment, the quantity of molecule, cell, and/or phenomenon in the first sample (or in the first subject) is lower by any numerical percentage from 5% to 100%, such as, but not limited to, from 10% to 100%, from 20% to 100%, from 30% to 100%, from 40% to 100%, from 50% to 100%, from 60% to 100%, from 70% to 100%, from 80% to 100%, and from 90% to 100% lower than the quantity of the same molecule, cell and/or phenomenon in the second sample (or in the second subject). In one embodiment, the first subject is exemplified by, but not limited to, a subject that has been manipulated using the invention's compositions and/or methods. In a further embodiment, the second subject is exemplified by, but not limited to, a subject that has not been manipulated using the invention's compositions and/or methods. In an alternative embodiment, the second subject is exemplified by, but not limited to, a subject to that has been manipulated, using the invention's compositions and/or methods, at a different dosage and/or for a different duration and/or via a different route of administration compared to the first subject. In one embodiment, the first and second subjects may be the same individual, such as where the effect of different regimens *(e.g.,* of dosages, duration, route of administration, *etc.*) of the invention's compositions and/or methods is sought to be determined in one individual. In another embodiment, the first and second subjects may be different individuals, such as when comparing the effect of the invention's compositions and/or methods on one individual participating in a clinical trial and another individual in a hospital.

[0047] The terms "increase," "elevate," "raise," and grammatical equivalents (including "higher," "greater," *etc.*) when in reference to the level of any molecule *(e.g.,* amino acid sequence, and nucleic acid sequence, antibody, *etc.*), cell, and/or phenomenon *(e.g.,* disease symptom, binding to a molecule, specificity of binding of two molecules, affinity of binding of two molecules, specificity to cancer, sensitivity to cancer, affinity of binding, enzyme activity, *etc.*) in a first sample (or in a first subject) relative to a second sample (or relative to a second subject), mean that the quantity of the molecule, cell and/or phenomenon in the first sample (or in the first subject) is higher than in the second sample (or in the second subject) by any amount that is statistically significant using any art-accepted statistical method of analysis. In one embodiment, the quantity of the molecule, cell and/or phenomenon in the first sample (or in the first subject) is at least 10% greater than, at least 25% greater than, at least 50% greater than, at least 75% greater than, and/or at least 90% greater than the quantity of the same molecule, cell and/or phenomenon in the second sample (or in the second subject). This includes, without limitation, a quantity of molecule, cell, and/or phenomenon in the first sample (or in the first subject) that is at least 10% greater than, at least 15% greater than, at least 20% greater than, at least 25% greater than, at least 30% greater than, at least 35% greater than, at least 40% greater than, at least 45% greater than, at least 50% greater than, at least 55% greater than, at least 60% greater than, at least 65% greater than, at least 70% greater than, at least 75% greater than, at least 80% greater than, at least 85% greater than, at least 90% greater than, and/or at least 95% greater than the quantity of the same molecule, cell and/or phenomenon in the second sample (or in the second subject). In one embodiment, the first subject is exemplified by, but not limited to, a subject that has

been manipulated using the invention's compositions and/or methods. In a further embodiment, the second subject is exemplified by, but not limited to, a subject that has not been manipulated using the invention's compositions and/or methods. In an alternative embodiment, the second subject is exemplified by, but not limited to, a subject to that has been manipulated, using the invention's compositions and/or methods, at a different dosage and/or for a different duration and/or via a different route of administration compared to the first subject. In one embodiment, the first and second subjects may be the same individual, such as where the effect of different regimens *(e.g.,* of dosages, duration, route of administration, *etc.*) of the invention's compositions and/or methods is sought to be determined in one individual. In another embodiment, the first and second subjects may be different individuals, such as when comparing the effect of the invention's compositions and/or methods on one individual participating in a clinical trial and another individual in a hospital.

[0048] The terms "alter" and "modify" when in reference to the level of any molecule and/or phenomenon refer to an increase or decrease.

[0049] Reference herein to any numerical range expressly includes each numerical value (including fractional numbers and whole numbers) encompassed by that range. To illustrate, and without limitation, reference herein to a range of "at least 50" includes whole numbers of 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, *etc.*, and fractional numbers 50.1, 50.2 50.3, 50.4, 50.5, 50.6, 50.7, 50.8, 50.9, *etc.* In a further illustration, reference herein to a range of "less than 50" includes whole numbers 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, *etc.,* and fractional numbers 49.9, 49.8, 49.7, 49.6, 49.5, 49.4, 49.3, 49.2, 49.1, 49.0, *etc.* In yet another illustration, reference herein to a range of from "5 to 10" includes each whole number of 5, 6, 7, 8, 9, and 10, and each fractional number such as 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, *etc.*

DESCRIPTION OF THE INVENTION

[0050] The invention provides antibodies, and antigen-binding fragments thereof, that specifically bind to a polypeptide, or antigenic portion thereof, wherein the polypeptide is selected from a) MUC16 ectodomain polypeptide, b) MUC16 cytoplasmic domain polypeptide, and c) MUC16 extracellular domain polypeptide that contains a cysteine loop polypeptide. The invention's antibodies and compositions containing them are useful in diagnostic and therapeutic applications for diseases in which MUC16 is overexpressed, such as cancer.

[0051] Using synthetic peptides, the inventors raised novel-specific antibodies to the carboxy-terminal portion of MUC16, retained by the cell, proximal to the putative cleavage site. These antibodies were characterized using fluorescence-activated cell-sorting analysis, enzyme-linked immunoassay, Western blot analysis, and immunohistochemistry. Each of the selected monoclonal antibodies was reactive against recombinant GST-ΔMUC16^{c114} protein and the MUC16 transfected SKOV3 cell line. Three antibodies, 4H11, 9C9, and 4A5 antibodies demonstrated high affinities by Western blot analysis and saturation-binding studies of transfected SKOV3 cells, and displayed antibody internalization. Immunohistochemical positivity with novel antibody 4H11 was similar to OC125, but with important differences, including diffuse positivity in lobular breast cancer and a small percentage of OC125-negative ovarian carcinomas which showed intense and diffuse 4H11 antibody binding.

[0052] The invention's compositions and methods are useful for diagnostic and therapeutic applications, as well as biologic studies such as membrane receptor trafficking and intracellular events. Diagnostic applications include, for example, detection of cancer using immunohistochemical, radiographic imaging, enzyme-linked immunosorbent assay (ELISA), fluorescence-activated cell sorting (FACS), Western blot, and/or immunoprecipitation detection.

[0053] The invention is further described under (A) MUC16, (B) Prior Art Antibodies, (C) Invention's Antibodies, (D) Hybridoma Cell Lines, (E) Conjugates Of The Invention's Antibodies Linked To Cytotoxic Agents And/Or Prodrugs, (F) Detecting Mucl6 Portions And Diagnostic Applications, and (G) Therapeutic Applications.

A. MUC16

[0054] "MUC16," "MUC-16" and "Mucin 16" interchangeably refer to a type I membrane protein that is part of a family of tethered mucins. A schematic of Mucl6 is in Figure 10, and an exemplary human Mucl6 amino acid sequence (SEQ ID NO:13) is shown in Figure 9A. An alignment of mouse MUC16 (SEQ ID NO:24) and human MUC16 (SEQ ID NO:25) amino acid sequences is shown in Figure 20B. The term "type 1 protein" refers to a "membrane protein" that is at least partially embedded in the lipid bilayer of a cell, virus and the like, and that contains a transmembrane domain (TM) sequence embedded in the lipid bilayer of the cell, virus and the like. The portion of the protein on the NH_2-terminal side of the TM domain is exposed on the exterior side of the membrane, and the COOH-terminal portion is exposed on the cytoplasmic side.

[0055] Recently, the sequence of the cDNA-encoding MUC16/CA125 was described by Yin and Lloyd in 2001 and completed by O'Brien in 2002 (10-12). The complete MUC16 protein has various components consisting of a cytoplasmic tail with potential phosphorylation sites, a transmembrane domain, and an external domain proximal to an apparent cleavage site. Distal to the cleavage site, the released external domain contains 16-20 tandem repeats of 156 amino

acids, each with many potential glycosylation sites (11). The overall repeat structure (Figure 10) is well conserved across mammals, but the repeats are not completely identical in exact amino acid composition.

[0056] The MUC16 protein is part of a family of tethered mucins that includes both MUC1 and MUC4 (13). MUC1 is present in a variety of tissues and appears to signal through a beta catenin pathway, interact with EGF receptor, mediates drug resistance and can act as an oncogene (14-17). The MUC4 protein is also expressed in a variety of tissues but is common on neoplasms of the gastrointestinal track (18-20). In contrast, the CA125 antigen has been more restricted in its distribution and is present primarily in gynecologic tissues and overexpressed in Müllerian neoplasms (21). However, the CA125 antigen, recognized by the OC125 antibody, is a heavily glycosylated antigen expressed in the tandem repeat region of the larger MUC16 protein. This glycoprotein is typically shed from a putative cleavage site in the extracellular domain of the MUC16 peptide backbone.

[0057] Thus, "MUC16" protein contains (a) a "cytoplasmic domain," (b)a "transmembrane domain," and (c) a "extracellular domain." The MUC16 extracellular domain contains a cleavage site between a non-glycosylated ectodomain and a large glycosylated ectodomain of tandem repeats.

[0058] The terms "cytoplasmic domain," "cytoplasmic tail," and "CT" are used interchangeably to refer to a protein sequence, and portions thereof, that is on the cytoplasmic side of the lipid bilayer of a cell, virus and the like. Methods for determining the CT of a protein are known in the art Elofsson et al. (2007) Annu. Rev. Biochem. 76:125-140; Bernsel et al. (2005) Protein Science 14:1723-1728).

[0059] The terms "transmembrane domain" and "TM" are used interchangeably to refer to a protein sequence, and portions thereof, that spans the lipid bilayer of a cell, virus and the like. Methods for determining the TM of a protein are known in the art (Elofsson et al. (2007) Annu. Rev. Biochem. 76:125-140; Bernsel et al. (2005) Protein Science 14:1723-1728).

[0060] The terms "ectodomain" and "extracellular domain" are interchangeably used when in reference to a membrane protein to refer to the portion of the protein that is exposed on the extracellular side of a lipid bilayer of a cell, virus and the like. Methods for determining the ectodomain of a protein are known in the art (Singer (1990) Annu. Rev. Cell Biol. 6:247-296 and High et al. (1993) J. Cell Biol. 121:743-750, and McVector software, Oxford Molecular).

[0061] The exemplary Mucl6 of Figure 9 contains (a) a "MUC16 cytoplasmic domain" from amino acid 14476 to 14507, vttrr rkkegeynvq qqcpgyyqsh ldledlq (SEQ ID NO:16), that interacts with the intracellular signal transduction machinery; (b) a "MUC16 transmembrane domain" from amino acid 14452 to 14475, fwaviligl agllgvitcl icgvl (SEQ ID NO:14) that spans the plasma membrane; and (c) a "MUC16 extracellular domain" amino acid 1 to 14392 (SEQ ID NO:13) that contains a cleavage site between an non-glycosylated ectodomain and a large glycosylated ectodomain of tandem repeats. The "MUC16 ectodomain" is exemplified by nfsplar rvdrvaiyee flrmtrngtq lqnftldrss vlvdgyspnr nepltgnsdl p (SEQ ID NO:17) from amino acid 14394 to 14451 of SEQ ID NO:13 of Figure 9A.

[0062] The exemplary MUC16 ectodomain contains both Polypeptide 1 (nfsplar rvdrvaiyee (SEQ ID NO:01), which is from amino acid 14394 to 14410 of SEQ ID NO:13), and Polypeptide 2 (tldrss vlvdgyspnr ne (SEQ ID NO:02), which is from amino acid 14425 to 14442 of SEQ ID NO:13), against which the invention's exemplary antibodies were produced. Polypeptide 3, cgvlvttrr rkkegeynvq qq (SEQ ID NO:03) is from amino acid 14472 to 14492 of SEQ ID NO:13, and contains both a transmembrane domain portion (cgvl) and a cytoplasmic domain portion (vttrr rkkegeynvq qq (SEQ ID NO:18)). Thus, the CGVL is optional in SEQ ID NO:03, as it is part of the transmembrane domain.

[0063] Polypeptide 4 (ksyf sdcqvstfrs vpnrhhtgvd slcnfspl (SEQ ID NO:15), is located in a non-glycosylated portion of the Mucl6 extracellular domain, is from amino acid 14367 to 14398 of SEQ ID NO:13, and contains a cysteine loop polypeptide cqvstfrsvpnrhhtgvdslc (SEQ ID NO:13).

B. Prior Art Antibodies

[0064] The expression of the MUC16/CA125 antigen has long been associated with gynecologic tissues. "CA125," "CA-125," "Cleaved CA125," and "cleaved CA-125," interchangeably refer to the glycosylated external domain of the tethered mucin MUC16, that is distal to the cleavage site (Payne et al., U.S. Pat. No. 7,202,346). This released external domain contains 16-20 tandem repeats of 156 amino acids, each with potential glycosylation sites. An apparent cysteine-based disulfide loop of 19 amino acids is present in all repeats and the N-terminal end contains a hairbrush structure that is heavily 0-glycosylated (11). The deduced size would be 2.5 MD for the protein part, and with added carbohydrates, this could increase to 5 MD (10, 26).

[0065] CA125, though it is not sensitive or specific enough to be used as a general screening tool, is routinely used to monitor patients with ovarian carcinoma. The tests used to measure CA125 are antibody based detection methods, as are the immunohistochemical stains routinely performed for diagnostic purposes. The epitope specificity of 26 antibodies to MUC16 was studied in the first report from the International Society of Oncodevelopmental Biology and Medicine (ISOBM) TD-1 Workshop and the application of 22 antibodies to immunohistochemistry was reported in the second report from the TD-1 workshop (7, 21). The existing antibodies were grouped as OC125-like, M11-like, or OV197-like and all of the known antibodies recognized CA125 epitopes in the repeating, glycosylated elements in the external

domain of the tethered mucin MUC16, distal to the putative cleavage site.

[0066] The vast majority of MUC16-reactive antibodies, including OC125, react with the glycosylation-dependent antigen present exclusively in the cleaved portion of the molecule so the true distribution of MUC16 expression is not known (21). There is currently no antibody available to track the fate of the remaining MUC16 protein fragment after cleavage and CA125 release.

C. Invention's Antibodies

[0067] In order to better explore the biology of human MUC16, the inventors have derived monoclonal antibodies against the extracellular portion of the MUC16-carboxy terminus, proximal to the putative cleavage site, as well as one monoclonal antibody against the internal cytoplasmic domain. In contrast to prior antibodies, these are derived against the peptide backbone of MUC16 and are not directed at complex glycoprotein epitopes. Since these epitopes are proximal to the cleavage site, they are unlikely to be found in the circulation and provide novel targets for diagnostic methods and therapeutic interventions. Data herein demonstrate the identification and characterization of exemplary antibodies developed against the MUC16 peptide backbone.

[0068] The inventors have developed novel antibodies that are directed at the non-cleaved, non-glycosylated peptide backbone of MUC16. These are exemplified by both 4H11 and 9C9 antibodies, which react with peptide sequences in the non-cleaved ectodomain of MUC16 and are detectable on the surface of ovarian cancer cell lines and in paraffin-fixed tissues from human ovarian cancer surgical specimens. The antibodies show high affinity and are readily internalized by ovarian cancer cells when bound to the ectodomain of MUC16. This suggests that the proximal portion of MUC16 has an independent biology from the more distal, cleaved portion of the mucin. It also suggests that the proximal portions of MUC16 could provide convenient targets for diagnostic and therapeutic interventions. Targeting the peptide backbone of MUC16 provides highly specific tissue delivery for genetically engineered cells, liposomes, or antibody conjugates, including conjugates with the invention's antibodies.

[0069] The invention's antibodies, exemplified by antibody 4H11, are useful as tools in immunohistochemistry. Date herein show that 4H11 is relatively specific to high-grade ovarian serous carcinoma. Invasive lobular breast carcinoma is the major exception and shows extensive MUC16 protein as detected by 4H11. Lobular carcinoma of the breast has unique biology which is characterized by a propensity to metastasize to serosal surfaces (27). Since MUC16 is the cognate binding partner of mesothelin, this may have important implications for lobular cancer (28). The discordance rates for OC125 and 4H11 also suggest that 4H11 might provide additional, independent information from OC125 in a subset of ovarian carcinomas. Some tumors that are negative with OC125 retain cytoplasmic and extracellular portions of the MUC16 glycoprotein, portions of the molecule that are likely involved in transduction of signals potentially important in the malignant phenotype.

[0070] Thus, in one embodiment, the invention provides an isolated antibody, or an antigen-binding fragment thereof, that specifically binds to a polypeptide, or antigenic portion thereof, wherein the polypeptide is exemplified by a) MUC16 ectodomain polypeptide (exemplified by NFSPLAR RVDRVAIYEE FLRMTRNGTQ LQNFTLDRSS VLVDGYSPNR NEPLTGNSDL P (SEQ ID NO:17)), b) MUC16 cytoplasmic domain polypeptide (exemplified by VTTRR RKKEGEYNVQ QQ (SEQ ID NO:18), which is contained within each of CGVLVTTRR RKKEGEYNVQ QQ (SEQ ID NO:03) and LVTTRR RKKEGEYNVQ QQ (SEQ ID NO:20)), and c) MUC16 extracellular domain polypeptide that contains a cysteine loop polypeptide CQVSTFRSVPNRHHTGVDSLC (SEQ ID NO:19).

[0071] One advantage of the invention's antibodies is that the antibody internalizes into a cell, thereby being useful in applications for delivery inside a cell, such as disease therapy. "Internalized" when in reference to a molecule that is internalized by a cell refers to passage of the molecule that is in contact with the extracellular surface of a cell membrane across the cell membrane to the intracellular surface of the cell membrane and/or into the cell cytoplasm. Methods for determining internalization are disclosed herein, including the detection of radiolabeled molecule inside the cell (Figure 5B).

[0072] In one embodiment, the invention's antibodies specifically bind to MUC16 ectodomain polypeptide that comprises a polypeptide selected from the group consisting of Polypeptide 1 NFSPLARRVDRVAIYEE (SEQ ID NO:01) and Polypeptide 2 TLDRSSVLVDGYSPNRNE (SEQ ID NO:02). Data herein show that the invention's antibodies specifically bind to GST-ΔMUC16^{c114} (Example 2, Table 1A). The specificity of the invention's antibodies is in contrast to prior art antibodies *(e.g.,* VK8, M11 and OC125 antibodies) that did not bind to GST-4MUC16^{c114} purified protein or cell lysates of the SKOV3-phrGFP-ΔMUC6^{c114} cell line (Example 2, Figure 2).

[0073] In a further embodiment, the invention's antibodies lack specific binding to a glycosylated MUC16 extracellular domain, exemplified by the cleaved CA-125 described in Payne et al., U.S. Pat. No. 7,202,346.

[0074] While not intending to limit the sequence of the V_L and V_H regions of the invention's antibodies, in one embodiment, the antibody specifically binds to the Polypeptide 2 (SEQ ID NO:02) of the MUC16 ectodomain polypeptide, wherein the antibody comprises a variable heavy (V_H) chain encoded by SEQ ID NO:06 *(i.e.,* the antibody 4H11 variable heavy (V_H) chain amino acid sequence of Figure 8), and a variable light (V_L) chain encoded by SEQ ID NO:07 *(i.e.,* the

antibody 4H11 variable light (V_L) chain amino acid sequence of Figure 8). In a particular embodiment, the antibody is chimeric, wherein at least one of the V_L and V_H chains is fused to a human immunoglobulin constant region.

[0075] Also without intending to limit the sequence of the V_L and V_H regions of the invention's antibodies, in one embodiment, the antibody specifically binds to the Polypeptide 2 (SEQ ID NO:02) of the MUC16 ectodomain polypeptide, wherein the antibody comprises a variable heavy (V_H) chain encoded by SEQ ID NO:04 (*i.e.,* the antibody 4A5 variable heavy (V_H) chain nucleotide sequence of Figure 8), and a variable light (V_L) chain encoded by SEQ ID NO:05 *(i.e.,* the antibody 4A5 variable light (V_L) chain nucleotide sequence of Figure 8). In a particular embodiment, the antibody is chimeric wherein at least one of the V_L and V_H chains is covalently linked to a human immunoglobulin constant region.

[0076] Still without intending to limit the sequence of the V_L and V_H regions of the invention's antibodies, in one embodiment, the antibody specifically binds to the Polypeptide 1 (SEQ ID NO:01) of the MUC16 ectodomain polypeptide, wherein the antibody comprises a variable heavy (V_H) chain encoded by SEQ ID NO:08 *(i.e.,* the antibody 9B11 variable heavy (V_H) chain nucleotide sequence of Figure 8), and a variable light (V_L) chain encoded by at least one of SEQ ID NO:09 *(i.e.,* antibody 9B11 variable light ($V_{L.A}$) chain nucleotide sequence of Figure 8), and SEQ ID NO:10 *(i.e.,* the antibody 9B11 variable light ($V_{L.B}$) chain nucleotide sequence of Figure 8). In a particular embodiment, the antibody is chimeric wherein at least one of the V_L and V_H chains is covalently linked to a human immunoglobulin constant region.

[0077] While not intending to restrict the source of antigen to which the invention's antibodies bind, in one embodiment, the MUC16 ectodomain polypeptide is expressed by a cell. Data herein show that the invention's exemplary antibodies bind to SKOV3 cells transduced with phrGFP-ΔMUC16^{c114} (Example 2).

[0078] While not limiting the sequence of antigen to which the invention's antibodies bind, in a further embodiment, the invention's antibodies specifically bind to a MUC16 cytoplasmic domain polypeptide that comprises VTTRR RKKEGEYNVQ QQ (SEQ ID NO:18). In a particular embodiment, the MUC16 cytoplasmic domain polypeptide comprises Polypeptide 3 CGVLVTTRRRKKEGEYNVQQQ (SEQ ID NO:03). In some embodiment, the MUC16 cytoplasmic domain polypeptide is expressed by a cell. For example, data herein show that the invention's exemplary antibody binds to SKOV3 cells transduced with phrGFP-ΔMUC16^{c114} (Example 2). In a particular embodiment, the cell is permeabilized to facilitate internalization of the antibody into the cell so that it comes into contact with its cytoplasmic antigen.

[0079] Still without limiting the sequence of antigen to which the invention's antibodies bind, in a further embodiment, the invention's antibodies bind to a MUC16 extracellular domain polypeptide that contains a cysteine loop polypeptide CQVSTFRSVPNRHHTGVDSLC (SEQ ID NO:19). In a more preferred embodiment, the MUC16 extracellular domain polypeptide comprises Polypeptide 4 KSYF SDCQVSTFRS VPNRHHTGVD SLCNFSPL (SEQ ID NO:15).

[0080] Still without intending to limit the sequence of the V_L and V_H regions of the invention's antibodies, in one embodiment, the antibody specifically binds to Polypeptide 4 (SEQ ID NO:15) of the MUC16 extracellular domain polypeptide, wherein the antibody comprises a variable heavy (V_H) chain encoded by SEQ ID NO:11 (*i.e.,* the antibody 24B3 variable heavy (V_H) chain amino acid sequence of Figure 8), and a variable light (V_L) chain encoded by SEQ ID NO:12 *(i.e.,* the antibody 24B3 variable light (V_L) chain amino acid sequence of Figure 8).

[0081] The invention contemplates chimeric antibodies (see U.S. Pat. No. 7,662,387), monoclonal antibodies, recombinant antibodies, an antigen-binding fragment of a recombinant antibody, a humanized antibody, and an antibody displayed upon the surface of a phage (U.S. Pat. No. 7,202,346). In particular, the invention contemplates antibody fragments that contain the idiotype ("antigen-binding region" or "antigen-binding fragment") of the antibody molecule. For example, such antigen-binding fragments include, but are not limited to, the Fab region, F(ab')2 fragment, pFc' fragment, and Fab' fragments.

[0082] The "Fab region" and "fragment, antigen binding region," interchangeably refer to portion of the antibody arms of the immunoglobulin "Y" that function in binding antigen. The Fab region is composed of one constant and one variable domain from each heavy and light chain of the antibody. Methods are known in the art for the construction of Fab expression libraries (Huse et al., Science, 246:1275-1281 (1989)) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. In another embodiment, Fc and Fab fragments can be generated by using the enzyme papain to cleave an immunoglobulin monomer into two Fab fragments and an Fc fragment. The enzyme pepsin cleaves below the hinge region, so a "F(ab')2 fragment" and a "pFc' fragment" is formed. The F(ab')2 fragment can be split into two "Fab' fragments" by mild reduction.

[0083] The invention also contemplates a "single-chain antibody" fragment, *i.e.,* an amino acid sequence having at least one of the variable or complementarity determining regions (CDRs) of the whole antibody, and lacking some or all of the constant domains of the antibody. These constant domains are not necessary for antigen binding, but constitute a major portion of the structure of whole antibodies. Single-chain antibody fragments are smaller than whole antibodies and may therefore have greater capillary permeability than whole antibodies, allowing single-chain antibody fragments to localize and bind to target antigen-binding sites more efficiently. Also, antibody fragments can be produced on a relatively large scale in prokaryotic cells, thus facilitating their production. Furthermore, the relatively small size of single-chain antibody fragments makes them less likely to provoke an immune response in a recipient than whole antibodies. Techniques for the production of single-chain antibodies are known (U.S. 4,946,778). The variable regions of the heavy and light chains can be fused together to form a "single-chain variable fragment" ("scFv fragment"), which is only half

the size of the Fab fragment, yet retains the original specificity of the parent immunoglobulin.

[0084] The "Fc region" and "Fragment, crystallizable region" interchangeably refer to portion of the base of the immunoglobulin "Y" that function in role in modulating immune cell activity. The Fc region is composed of two heavy chains that contribute two or three constant domains depending on the class of the antibody. By binding to specific proteins, the Fc region ensures that each antibody generates an appropriate immune response for a given antigen. The Fc region also binds to various cell receptors, such as Fc receptors, and other immune molecules, such as complement proteins. By doing this, it mediates different physiological effects including opsonization, cell lysis, and degranulation of mast cells, basophils and eosinophils. In an experimental setting, Fc and Fab fragments can be generated in the laboratory by cleaving an immunoglobulin monomer with the enzyme papain into two Fab fragments and an Fc fragment.

[0085] The invention contemplates polyclonal antibodies and monoclonal antibodies. "Polyclonal antibody" refers to an immunoglobulin produced from more than a single clone of plasma cells; in contrast "monoclonal antibody" refers to an immunoglobulin produced from a single clone of plasma cells. Generic methods are available for making polyclonal and monoclonal antibodies that are specific to a desirable polypeptide. For the production of monoclonal and polyclonal antibodies, various host animals can be immunized by injection with the peptide corresponding to any molecule of interest in the present invention, including but not limited to hamsters, rabbits, mice, rats, sheep, goats, *etc.* For preparation of monoclonal antibodies, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used (See *e.g.*, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). These include, but are not limited to, the hybridoma technique originally developed by Köhler and Milstein (Köhler and Milstein, Nature, 256:495-497 (1975)), techniques using germ-free animals and utilizing technology such as that described in PCT/US90/02545, as well as the trioma technique, the human B-cell hybridoma technique (See *e.g.,* Kozbor et al., Immunol. Today, 4:72 (1983)), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96 (1985)). In some particularly preferred embodiments of the present invention, the present invention provides monoclonal antibodies.

[0086] Also contemplated are chimeric antibodies. As used herein, the term "chimeric antibody" contains portions of two different antibodies, typically of two different species. See, *e.g.:* U.S. Pat. No. 4,816,567 to Cabilly et al.; U.S. Pat. No. 4,978,745 to Shoemaker et al.; U.S. Pat. No. 4,975,369 to Beavers et al.; and U.S. Pat. No. 4,816,397 to Boss et al. Chimeric antibodies include monovalent, divalent or polyvalent immunoglobulins. A monovalent chimeric antibody is a dimer (HL) formed by a chimeric H chain associated through disulfide bridges with a chimeric L chain. A divalent chimeric antibody is tetramer (H_2L_2) formed by two HL dimers associated through at least one disulfide bridge. A polyvalent chimeric antibody can also be produced, for example, by employing a Hc region that aggregates *(e.g.,* IgM H chain).

[0087] The invention also contemplates "humanized antibodies," *i.e.,* chimeric antibodies that have constant regions derived substantially or exclusively from human antibody constant regions, and variable regions derived substantially or exclusively from the sequence of the variable region from a mammal other than a human. Humanized antibodies preferably have constant regions and variable regions other than the complement determining regions (CDRs) derived substantially or exclusively from the corresponding human antibody regions and CDRs derived substantially or exclusively from a mammal other than a human. Thus, in one embodiment, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are generally made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a nonhuman immunoglobulin and all or substantially all of the FR residues are those of a human immunoglobulin sequence. The humanized antibody may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Humanized antibodies may be generated using methods known in the art, *e.g.,* U.S. Pat. No. 5,225,539 to Winter et al., including using human hybridomas (Cote et al., Proc. Natl. Acad. Sci. U.S.A.80:2026-2030 (1983)) or by transforming human B cells with EBV virus *in vitro* (Cole et al., in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, pp. 77-96 (1985)). Additional methods include, for example, generation of transgenic non-human animals which contain human immunoglobulin chain genes and which are capable of expressing these genes to produce a repertoire of antibodies of various isotypes encoded by the human immunoglobulin genes (U.S. Pat. Nos. 5,545,806; 5,569,825 and 5,625,126). Humanized antibodies may also be made by substituting the complementarity determining regions of, for example, a mouse antibody, into a human framework domain (PCT Pub. No. WO92/22653).

[0088] Importantly, early methods for humanizing antibodies often resulted in antibodies with lower affinity than the non-human antibody starting material. More recent approaches to humanizing antibodies address this problem by making changes to the CDRs. See U.S. Patent Application Publication No. 20040162413, hereby incorporated by reference. In some embodiments, the invention's humanized antibodies contain an optimized heteromeric variable region *(e.g.* that

may or may not be part of a full antibody other molecule) having equal or higher antigen binding affinity than a donor heteromeric variable region, wherein the donor heteromeric variable region comprises three light chain donor CDRs, and wherein the optimized heteromeric variable region comprises: a) a light chain altered variable region comprising; i) four unvaried human germline light chain framework regions, and ii) three light chain altered variable region CDRs, wherein at least one of the three light chain altered variable region CDRs is a light chain donor CDR variant, and wherein the light chain donor CDR variant comprises a different amino acid at only one, two, three or four positions compared to one of the three light chain donor CDRs *(e.g.* the at least one light chain donor CDR variant is identical to one of the light chain donor CDRs except for one, two, three or four amino acid differences).

[0089] Chimeric antibodies containing amino acid sequences that are fused to constant regions from human antibodies, or to toxins or to molecules with cytotoxic effect, are known in the art *(e.g.,* U.S. Pat. Nos. 7,585,952; 7,227,002; 7,632,925; 7,501,123; 7,202,346; 6,333,410; 5,475,092; 5,585,499; 5,846,545; 7,202,346; 6,340,701; 6,372,738; 7,202,346; 5,846,545; 5,585,499; 5,475,092; 7,202,346; 7,662,387; 6,429,295; 7,666,425; and 5,057,313).

[0090] Antibodies that are specific for a particular antigen may be screened using methods known in the art *(e.g.,* U.S. Pat. No. 7,202,346) and disclosed herein. For example, In the production of antibodies, screening for the desired antibody can be accomplished by radioimmunoassay, ELISA (enzyme-linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (e.g., using colloidal gold, enzyme or radioisotope labels), Western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays, *etc.*), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, *etc.*

[0091] In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention. As is well known in the art, the immunogenic peptide should be provided free of the carrier molecule used in any immunization protocol. For example, if the peptide was conjugated to KLH, it may be conjugated to BSA, or used directly, in a screening assay.

[0092] In one embodiment, the invention's antibodies are monoclonal antibodies produced by a hybridoma cell line. In a particular embodiment, the monoclonal antibody specifically binds to a MUC16 ectodomain polypeptide that comprises Polypeptide 1 (SEQ ID NO:01), as exemplified by the antibody selected from the group consisting of 9B11.20.16, 10A2, 2F4, 23D3, 30B 1, and 31B2 (Tables 1 and 2). In a preferred embodiment, the antibody is 9B 11.

[0093] In another embodiment, the monoclonal antibody specifically binds to a MUC16 ectodomain polypeptide that comprises Polypeptide 2 (SEQ ID NO:02), wherein the antibody is exemplified by 4H11.2.5, 13H1, 29G9, 9C9.21.5.13, 28F8, 23G12, 9C7.6, 11B6, 25G4, 5C2.17, 4C7, 26B2, 4A5.37, 4A2, 25H3, and 28F7.18.10 (Tables 1 and 2). In a preferred embodiment, the antibody is exemplified by 4H11.2.5, 4A5.37, 9C9.21.5.13, 28F7.18.10, 9C7.6, and 5C2.17.

[0094] In a further embodiment, the monoclonal antibody specifically binds to a MUC16 cytoplasmic domain polypeptide that comprises Polypeptide 3 CGVLVTTRRRKKEGEYNVQQQ (SEQ ID NO:03), wherein the antibody is exemplified by 31A3.5.1, 19D1, 10F6, 22E10, 22F1, 3H8, 22F11, 4D7, 24G12, 19G4, 9A5, 4C2, 31C8, 27G4, and 6H2 (Tables 1 and 2). In a preferred embodiment, the antibody is 31A3.5.1.

[0095] In another embodiment, the monoclonal antibody specifically binds to a MUC16 extracellular domain polypeptide that comprises Polypeptide 4 KSYF SDCQVSTFRS VPNRHHTGVD SLCNFSPL (SEQ ID NO:15), wherein the antibody is exemplified by 24B3 and 9C7 (Table 2).

[0096] The invention's antibodies and methods for their use (both diagnostic and therapeutic) are disease specific. "Specificity" of a method and/or molecule for disease, such as "specificity for cancer" which is interchangeably used with "cancer specificity", refers to the proportion (*e.g.*, percentage, fraction, etc.) of negatives (*i.e.,* healthy individuals not having disease) that are correctly identified, *i.e.,* the percentage of healthy subjects who are correctly identified as not having disease. Specificity may be calculated according to the following equation:

$$\text{Specificity} = \text{number of true negatives} / (\text{number of true negatives} + \text{number of false positives}).$$

[0097] Thus, in some embodiments, the invention's compositions and/or methods have a "cancer specificity" greater than 50%, including any numerical value from 51% to 100%, such as 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, and 99%. While a 100% specificity is most desirable, *i.e.,* not predicting anyone from the healthy group as having cancer, it is not necessary. Data herein demonstrate the invention's cancer specificity (Table 3).

[0098] In alternative embodiments, specificity is expressed (together with sensitivity) as a statistical measure of the performance of a binary classification test, such as using a Receiver Operator Characteristic (ROC) curve". For any test,

there is usually a trade-off between specificity and sensitivity. For example: in cancer screening tests of human subjects, it is undesirable to risk falsely identifying healthy people as having cancer (low specificity), due to the high costs. These costs are both physical (unnecessary risky procedures) and financial. This trade-off can be represented graphically using a ROC curve. "Receiver Operator Characteristic curve" and "ROC curve" refer to a plot of the true positive rate (AKA sensitivity) versus true negative rate (AKA 1-specificity). The measured result of the test is represented on the x axis while the y axis represents the number of control *(e.g.,* healthy) or case *(e.g.,* cancer) subjects. For any given cut point (each point along the x axis) a sensitivity and specificity of the assay can be measured. The range of sensitivity and specificity for any given assay can range from 0% to 100%, depending on the selected cut point. For this reason, in some preferred embodiments, the AUC is used as the standard measure of an assay's specificity and/or sensitivity. The "area under the curve" ("AUC") for the ROC curve plot is equal to the probability that a classifier will rank a randomly chosen positive instance higher than a randomly chosen negative one. Thus, AUC is a general measure of a tests ability to successfully discriminate between case *(e.g.,* cancer) and control (*e.g.*, healthy) subjects. Random chance would generate an AUC of 0.5. Therefore, in one embodiment, useful tests preferably have AUC's greater than 0.50, including any value from 0.51 to 1.00, such as from 0.55 to 1.00, from 0.60 to 1.00, from 0.65 to 1.00, from 0.70 to 1.00, from 0.75 to 1.00, from 0.80 to 1.00, from 0.85 to 1.00, from 0.90 to 1.00, from 0.95 to 1.00, and most preferably 1.00. AUC values greater than 0.50 include 0.51, 0.52, 0.52, 0.54, 0.55, 0.56, 0.57, 0.58, 0.59, 0.60, 0.61, 0.62, 0.63, 0.64, 0.65, 0.66, 0.67, 0.68, 0.69, 0.70, 0.71, 0.72, 0.73, 0.74, 0.75, 0.76, 0.77, 0.78, 0.79, 0.80, 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, and 0.99.

[0099] The invention's antibodies and methods for their use (both diagnostic and therapeutic) are disease sensitive. "Sensitivity" of a method and/or molecule for disease, such as "sensitivity for cancer" which is interchangeably used with "cancer sensitivity," refers to the proportion (*e.g.*, percentage, fraction, etc.) of positives (*i.e.,* individuals having cancer) that are correctly identified as such *(e.g.* the percentage of people with cancer who are identified as having the condition). Sensitivity may be calculated according to the following equation; Sensitivity = number of true positives / (number of true positives + number of false negatives).

[0100] Thus, in some embodiments, the invention's compositions and/or methods have a "disease sensitivity," such as "cancer sensitivity," greater than 50%, including any numerical value from 51% to 100%, such as 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, and 99%. While a 100% sensitivity is most desirable (*i.e.,* predicting all subjects from the cancer group as having cancer), it is not necessary.

[0101] In alternative embodiments, the invention's compositions and/or methods have a "disease sensitivity," such as "cancer sensitivity," equal to or lower than 50%, including any numerical value from 0% to 50%, such as 1%, 2%, 3%, 4%, 6%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, and 49%.

[0102] In some embodiments, sensitivity is expressed (together with specificity) as a statistical measure of the performance of a binary classification test, such as using AUC of a ROC curve, as discussed above with respect to specificity.

D. Hybridoma Cell Lines

[0103] In addition to the invention's novel antibodies, the invention also provides hybridoma cell lines that produce these antibodies. "Hybridoma cell" refers to a cell line produced by fusing a specific antibody-producing B cell with a myeloma (B cell cancer) cell that is selected for its ability to grow in tissue culture and for an absence of antibody chain synthesis. The antibodies produced by the hybridoma cell are all of a single specificity and are therefore monoclonal antibodies (in contrast to polyclonal antibodies).

[0104] In a particular embodiment, the invention provides hybridoma cell lines that produce a monoclonal antibody that specifically binds to a polypeptide, or antigenic portion thereof, selected from the group consisting of a) MUC16 ectodomain polypeptide *(e.g.,* NFSPLAR RVDRVAIYEE FLRMTRNGTQ LQNFTLDRSS VLVDGYSPNR NEPLTGNSDL P (SEQ ID NO:17)), b) MUC16 cytoplasmic domain polypeptide (*e.g.*, VTTRR RKKEGEYNVQ QQ (SEQ ID NO:18)), and c) MUC16 extracellular domain polypeptide that contains a cysteine loop polypeptide CQVSTFRSVPNRHHTGVD-SLC (SEQ NO:19). The MUC16 polypeptide SEQ ID NO:18 is contained within LVTTRR RKKEGEYNVQ QQ (SEQ ID NO:20). Thus, SEQ ID NO:20 contains both a transmembrane domain amino acid (L) and a cytoplasmic domain portion VTTRR RKKEGEYNVQ QQ (SEQ ID NO:18), *i.e.,* the L is optional, as it is part of the transmembrane domain. The MUC16 polypeptide SEQ ID NO:18 is also contained within CGVLVTTRR RKKEGEYNVQ QQ (SEQ ID NO:03). Thus, SEQ ID NO:03 contains both a transmembrane domain portion (CGVL) and a cytoplasmic domain portion VTTRR RKKEGEYNVQ QQ (SEQ NO:18), *i.e.,* the CGVL is optional, as it is part of the transmembrane domain.

E. Conjugates Of The Invention's Antibodies Linked To Cytotoxic Agents And/Or Prodrugs

[0105] The invention contemplates conjugate antibodies. A "conjugate" antibody refers to an antibody of the present invention covalently linked to a cytotoxic agent and/or a prodrug of a cytotoxic agent.

[0106] "Cytotoxic agent" refers any agent that is capable of reducing the growth of, and/or killing, a target cell. A "prodrug" represents an analog of a cytotoxic agent that substantially lacks cytotoxic activity until subjected to an activation step. Activation steps may include enzymatic cleavage, a chemical activation step such as exposure to a reductant, or a physical activation step such as photolysis.

[0107] The covalent linkage between the invention's antibodies and the cytotoxic agent or prodrug can include cleavable linkages such as disulfide bonds, which may advantageously result in cleavage of the covalent linkage within the reducing environment of the target cell. Such conjugates are useful as tumor-cell specific therapeutic agents.

[0108] In one embodiment, the cytotoxic agent is a small drug molecule (Payne et al., U.S. Pat. No. 7,202,346). In another embodiment, the cytotoxic agent a maytansinoid, an analog of a maytansinoid, a prodrug of a maytansinoid, or a prodrug of an analog of a maytansinoid (U.S. Pat. Nos. 6,333,410; 5,475,092; 5,585,499; 5,846,545; 7,202,346). In another embodiment, the cytotoxic agent may be a taxane (see U.S. Pat. Nos. 6,340,701 & 6,372,738 & 7,202,346) or CC-1065 analog (see U.S. Pat. Nos. 5,846,545; 5,585,499; 5,475,092 & 7,202,346).

[0109] In another embodiment, the cytotoxic agent is exemplified by an auristatin, a DNA minor groove binding agent, a DNA minor groove alkylating agent, an enediyne, a duocarmycin, a maytansinoid, and a vinca alkaloid (U.S. Pat. No. 7,662,387).

[0110] In a further embodiment, the cytotoxic agent is an anti-tubulin agent (U.S. Pat. No. 7,662,387). In yet another embodiment, the cytotoxic agent is exemplified by dimethylvaline-valine-dolaisoleuine-dolaproine-phenylalanine-p-phenylenediamine (AFP), dovaline-valine-dolaisoleunine-dolaproine-phenylalanine (MMAF), and monomethyl auristatin E (MAE) (U.S. Pat. No. 7,662,387).

[0111] In an additional embodiment the toxic agent is exemplified by radioisotope emitting radiation, immunomodulator, lectin, and toxin (U.S. Pat. No. 6,429,295). In particular, the radioisotope emitting radiation is an alpha-emitter selected from the group consisting of ^{21}2Bi, ^{213}Bi, and ^{211}At, or a beta-emitter selected from the group consisting of ^{186} Re and ^{90}Y, or a gamma-emitter ^{131}I (U.S. Pat. No. 7,666,425).

[0112] In an alternative embodiment, the toxin is exemplified by ricin, the A-chain of ricin, and pokeweed antiviral protein (U.S. Pat. No. 5,057,313).

[0113] In yet another embodiment, the cytotoxic agent is an anti-cancer drug selected from the group consisting of methotrexate, 5-fluorouracil, cycloheximide, daunomycin, doxorubicin, chlorambucil, trenimon, phenylenediamine mustard, adriamycin, bleomycin, cytosine arabinoside or Cyclophosphamide (U.S. Pat. No. 5,057,13).

F. Detecting Mucl6 Portions And Diagnostic Applications

[0114] The invention provides a method for detecting a disease that comprises overexpression of MUC16 in a subject, wherein the method comprises a) providing i) a sample from a subject, and ii) any one or more of the invention's antibodies, b) contacting the sample with the antibody under conditions for specific binding of the antibody with its cognate antigen, and c) detecting an increased level of binding of the antibody to the sample compared to a control sample lacking the disease, thereby detecting the disease in the subject. Generic methods for detecting disease using antibodies are known in the art (Payne et al., U.S. Pat. No. 7,202,346). The invention's methods are particularly useful in detecting cancer, such as ovarian cancer and breast cancer.

[0115] The invention's methods are not limited to a particular approach to detecting binding of the invention's antibodies to their antigens. In one embodiment, detecting binding to the invention's antibodies typically involves using antibodies that are labeled with a detectable moiety, such as radioisotope (*e.g.*, ^{3}H, ^{14}C, ^{32}P, ^{35}S and/or ^{125}I) fluorescent or chemiluminescent compound *(e.g.,* fluorescein isothiocyanate, rhodamine, and/or luciferin) and/or an enzyme *(e.g.,* alkaline phosphatase, beta-galactosidase and/or horseradish peroxidase).

[0116] Methods for conjugating antibodies to a detectable moiety are known in the art (*e.g.,* Hunter, et al., Nature 144:945 (1962); David, e at., Biochemistry 13:1014 (1974); Pain, et al., J. Immunol. Meth. 40:219 (1981); and Nygren, J. Histochem. and Cytochem. 30:407 (1982).

[0117] Thus, the invention's antibodies may be employed in immunoassays, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays, including immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), fluorescence-activated cell sorting (FACS), and Western blots.

[0118] For example, with respect to immunohistochemical detection, data herein demonstrate that antibody 4H11 is useful in detecting high-grade ovarian serous carcinoma, lobular cancer (28), and a subset of ovarian carcinomas that are negative with OC125 and that retain cytoplasmic and extracellular portions of the MUC16 glycoprotein.

[0119] The antibodies of the invention also are useful for radiographic *in vivo* imaging, wherein an antibody labeled with a detectable moiety such as a radio-opaque agent or radioisotope is administered to a subject, preferably into the

bloodstream, and the presence and location of the labeled antibody in the host is assayed. This imaging technique is useful in the staging and treatment of malignancies.

[0120] The invention's antibodies are additionally useful as affinity purification agents. In this process, the antibodies are immobilized on a suitable support, such a Sephadex resin or filter paper, using methods well known in the art, to capture and purify molecules that contain antigens that specifically bind to the invention's antibodies.

G. Therapeutic Applications

[0121] The invention provides methods for treating a disease that comprises overexpression of MUC16, comprising administering to a subject having the disease a therapeutically effective amount of any one or more of the invention's antibodies. Generic methods for treating disease with antibodies are known in the art (Payne et al., U.S. Pat. No. 7,202,346). The invention's methods are particularly useful in treating cancer, such as ovarian cancer and breast cancer. These methods are also applicable to primary cancer, metastatic cancer, and recurrent cancer.

[0122] The term "administering" to a subject means providing a molecule to a subject. This may be done using methods known in the art (e.g., Erickson et al., U.S. Patent 6,632,979; Furuta et al., U.S. Patent 6,905,839; Jackobsen et al., U.S. Patent 6,238,878; Simon et al., U.S. Patent 5,851,789). The invention's compositions may be administered prophylactically (*i.e.,* before the observation of disease symptoms) and/or therapeutically (*i.e.,* after the observation of disease symptoms). Administration also may be concomitant with (*i.e.,* at the same time as, or during) manifestation of one or more disease symptoms. Also, the invention's compositions may be administered before, concomitantly with, and/or after administration of another type of drug or therapeutic procedure (*e.g.*, surgery). Methods of administering the invention's compositions include, without limitation, administration in parenteral, oral, intraperitoneal, intranasal, topical and sublingual forms. Parenteral routes of administration include, for example, subcutaneous, intravenous, intramuscular, intrastemal injection, and infusion routes.

[0123] In one embodiment, the invention's compositions comprise a lipid for delivery as liposomes. Methods for generating such compositions are known in the art (Borghouts et al. (2005). J Pept Sci 11, 713-726; Chang et al. (2009) PLoS One 4, e4171; Faisal et al. (2009) Vaccine 27, 6537-6545; Huwyleret al. (2008) Int J Nanomedicine 3, 21-29; Song et al. (2008) Int J Pharm 363, 155-161; Voinea et al. J Cell Mol Med 6, 465-474).

[0124] Antibody treatment of human beings with cancer is known in the art, for example in U.S. Pat. Nos. 5,736,137; 6,333,410; 5,475,092; 5,585,499; 5,846,545; 7,202,346; 6,340,701; 6,372,738; 7,202,346; 5,846,545; 5,585,499; 5,475,092; 7,202,346; 7,662,387; 7,662,387; 6,429,295; 7,666,425; 5,057,313.

[0125] The invention's antibodies may be administered with pharmaceutically acceptable carriers, diluents, and/or excipients. Examples of suitable carriers, diluents and/or excipients include: (1) Dulbecco's phosphate buffered saline, pH about 7.4, containing about 1 mg/ml to 25 mg/ml human serum albumin, (2) 0.9% saline (0.9% w/v NaCl), and (3) 5% (w/v) dextrose.

[0126] The invention's antibodies are typically administered in a therapeutic amount. The terms "therapeutic amount," "pharmaceutically effective amount," "therapeutically effective amount," and "biologically effective amount," are used interchangeably herein to refer to an amount that is sufficient to achieve a desired result, whether quantitative or qualitative. In particular, a pharmaceutically effective amount is that amount that results in the reduction, delay, and/or elimination of undesirable effects (such as pathological, clinical, biochemical and the like) that are associated with disease. For example, a "therapeutic amount that reduces cancer" is an amount that reduces, delays, and/or eliminates one or more symptoms of cancer.

[0127] For example, specific "dosages" of a ""therapeutic amount" will depend on the route of administration, the type of subject being treated, and the physical characteristics of the specific subject under consideration. These factors and their relationship to determining this amount are well known to skilled practitioners in the medical, veterinary, and other related arts. This amount and the method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent medication and other factors, which those skilled in the art will recognize. The dosage amount and frequency are selected to create an effective level of the compound without substantially harmful effects.

[0128] When present in an aqueous dosage form, rather than being lyophilized, the antibody typically will be formulated at a concentration of about 0.1 mg/ml to 100 mg/ml.

[0129] Depending on the type and severity of the disease, about 0.015 to 15 mg of antibody/kg of patient weight is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs.

[0130] The methods of the present invention can be practiced *in vitro, in vivo,* or *ex vivo.*

EXPERIMENTAL

[0131] The following examples serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

EXAMPLE 1

Materials And Methods

[0132] The following is a brief description of the exemplary materials and methods used in the subsequent Examples.

Cell Cultures:

[0133] OVCAR3, SKOV3, and A2780 cell lines were obtained through the American Type Culture Collection (ATCC, Manassas, VA) and sustained in culture according to the ATCC literature. For the creation of MUC16+ transfected cell lines, the carboxyterminus portion of the MUC16 cDNA was introduced as green fluorescent protein fusion proteins using the Vitality phrGFP vector expression system (Stratagene, La Jolla, CA). Stable cell lines were selected using geneticin (G418, Invitrogen, Grand Island, NY) in their respective culture media and isolated by expression of Green Fluorescence Protein. Stable transfectants were routinely maintained in G418 in their culture media respectively. The $\Delta MUC16^{c114}$ transfectants have cell surface expression of MUC16 protein from the putative cleavage site to the carboxyterminus (AA 1776 to 1890) (12).

Monoclonal Preparation:

[0134] Using the MUC16 sequence, peptide sequences encoding elements of the $\Delta MUC16^{c114}$ amino acid sequence were synthesized at the Memorial Sloan-Kettering Cancer Center (MSKCC) Microchemistry Core Facility. The inventors synthesized 3 polypeptides (Figure 1) and modified Polypeptide 1 and Polypeptide 2 with a cysteine at the N-terminus for better conjugation to KLH. Equal concentrations of the KLH-conjugated peptides were mixed and then used as the immunogen for 5 BALB/c mice. The inventors selected 1 of the 5 mice whose serum showed the highest reactivity to individual peptides by ELISA, and the MSKCC Monoclonal Antibody Core Facility performed the fusion and selected the antibodies using standard protocols. After 10 days of fusion, supernatants were selected and screened for reactivity by ELISA against the individual synthetic peptides.

ELISA:

[0135] Sandwich ELISA was performed to see the positivity of the antibodies to individual peptides and GST-$\Delta MUC16^{c114}$ fusion protein following routine core facility protocol for ELISA assay.

FACS Analyses:

[0136] Adherent target cells were removed by 0.05% Trypsin and 0.1% EDTA, washed, and counted by a hemocytometer. Cells were distributed into multiple Eppendorf tubes with at least 0.5-1 x 10^6 cells per tube. Cells were washed with phosphate buffered saline (PBS) containing 1% FCS and 0.025% Sodium Azide (FACS buffer). For internal FACS staining, cells in the Eppendorf tubes were permeabilized with 1:10 diluted FACS Permeabilizing Solution 2 (BD Biosciences, San Jose, CA) for 10 minutes at room temperature and then washed twice with ice cold FACS buffer. Then they were incubated either without (for second antibody control) or with 1 μg/tube of bioreactive supernatants of mouse MUC16 monoclonals for 30 minutes on ice. For surface FACS staining, cells were incubated either without (for second antibody control) or with 1 μg/tube of bioreactive supernatants of MUC16 monoclonals (9B11.20.16, 9C9.21.5.13 and 4H11.2.5), Mouse anti-human OC125 (M3519), Mouse anti-human M11 (M3520) (DakoCytomation, Dako North America Inc., Carpinteria, CA) or VK8 (kindly provided by Dr. Beatrice Yin and Dr. Ken Lloyd, MSKCC, New York, NY) for 30 minutes on ice. Cells in Eppendorf tubes were also surface stained with 1 μg/tube of non-specific isotype matched control mouse antibodies (13C4 for IgG1 and 4E11 for IgG2b monoclonals obtained from MSKCC Monoclonal Core Facility) and incubated on ice for 30 minutes. All cells were washed three times with FACS buffer. Cells were incubated with 1 μg/tube of second antibody Goat anti-mouse IgG1-PE or IgG2b-PE for 30 minutes on ice and then washed three times with FACS buffer. The cells were analyzed by a FACS Calibur machine at the MSKCC Flow Cytometry Core Facility.

Western Blot Analysis:

[0137] Stable cell lines were cultured in 10 cm dishes in their respective culture media and incubated with 5% CO_2 at 37°C for 3 days. They were washed twice with ice cold PBS to remove the serum-containing media. Adherent cells were scraped with 1-2 ml of ice cold PBS, and the cells were spun down in an Eppendorf tube at 4°C in an Eppendorf centrifuge. Supernatant was discarded, and the cells were lysed with 0.2 ml of modified Ripa lysis buffer (20 mM Tris-HCL; pH 7.4; 150 mM NaCl; 1% NP-40; 1 mM Na3VO4; 1 mM PMSF; 1 mM DTT; 10 μg/ml leupeptin; and 10 μg/ml aprotinin) for 30 minutes on ice and spun at 4°C for 10 minutes. The soluble solution was separated into a tube and the debris pellet was discarded. Protein concentration was measured using the Bio-Rad Protein Assay (BioRaD Laboratories, Hercules, CA). Equal amounts of proteins (GST-MUC16-CD-fusion protein or stable cell line extracts) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membrane using a BioRad transfer apparatus in a cold room at 4°C. The membranes were blocked with 3% bovine serum albumin (BSA) in PBS with 0.1% Tween-20 (PBST) at 4°C overnight. Membranes were probed with primary antibody (1:1000 dilution) for 1 hr at room temperature and then washed three times with PBST. Then the membranes were stained with corresponding second antibody, anti-Mouse IgG Horse Radish Peroxidase (HRP) linked whole antibody from sheep (GE Healthcare, UK) (1:5000 dilution), for 1 hr at room temperature. Membranes were washed three times with PBST and developed with a Western Lightning® chemiluminescence reagent (ECL, Perkin Elmer, Waltham, MA) for 1-5 minutes at room temperature, and the signals were developed on Kodak BioMax Film.

[0138] Binding and internalization studies with monoclonal antibodies and OVCAR3 and SKOV3 stable transfectants:

[0139] Purified monoclonal antibodies were labeled with ^{131}I using the iodogen method and purified by size exclusion chromatography (22). Saturation binding studies were performed with radiolabeled antibodies using substrates of intact OVCAR-3 cells. Briefly, 10 test solutions were prepared (in triplicate) and they contained increasing amounts of the radioiodinated antibodies, 3-500 000 cells in a total volume of 500 μL of PBS (0.2 % BSA; pH 7.4). The cells were isolated by rapid filtration through a glass fiber membrane and washed with ice cold tris buffered saline. Cells were counted in a gamma counter with standards of total activity added. For each concentration of radiolabeled antibody, non-specific binding was determined in the presence of 100 nM of the unmodified antibody. The data were analyzed with a least squares regression method (Origin, Microcal, Software Inc., Northampton, MA) to determine the K_d and B_{max} values, and a Scatchard transformation was performed.

[0140] Antibody cell internalization studies were performed with ^{131}I-4H11 and ^{13}I-OC125 monoclonal antibodies and SKOV3-phrGFP-ΔMUC16^{c334} stable transfected cells. Briefly, radiolabeled antibody (370 MBq/mg, 100 kcpm) in 2 mL of medium was added to SKOV3 cells plated in a 6-well plate. The plates were incubated at 37°C for up to 24 hours. At various time points, the medium was removed from three wells and the cells washed with 2 x 2 mL PBS. Cell surface bound activity was then stripped and collected with 2 x 2 mL of an ice cold acid wash (100 mM acetic acid 100 mM glycine; pH 3.0). The cells were then dissolved with 2 x 1 ml 1M NaOH and collected. At the end of the study all samples were counted with a gamma counter together with standards, representing the initial amount of radioactivity added. All the media samples were analyzed by ITLC-SG with mobile phases of 5% TCA to determine unbound ^{131}I.

Tissue microarray (TMA):

[0141] Tissue microarrays were either constructed within our institution or bought from a commercial laboratory if not available internally. Briefly, core-needle biopsies of pre-existing paraffin-embedded tissue were obtained from the so-called donor blocks and then relocated into a recipient paraffin-arrayed "master" block by using the techniques by Kononen et al. and subsequently modified by Hedvat et al (23-24). A manually operated Tissue Arrayer MTA-1 from Beecher Instruments Inc. (Sun Prairie, WI) was used to produce sample circular spots (cores) that measured 0.6 to 1.0 mm in diameter. The cores were arrayed 0.3 to 0.4 mm apart from each other. A layer of control tissues was strategically laid around the actual tissue microarrays in order to avoid edging effects. The specific composition of each tissue microarray is delineated below. Slides of tissue microarrays for ovarian cancer, prostate cancer, adenocarcinoma of the lung, mucinous neoplasms of the pancreas, and invasive ductal and invasive lobular breast carcinoma were prepared by cutting 4 um sections from formalin-fixed paraffin-embedded tissue. Normal adult and fetal tissue microarrays were obtained from a commercial source (Biomax, US). OVCAR3 cells were used as positive controls.

Immunohistochemistry:

[0142] Immunohistochemistry was performed on the tissue microarrays with both standard OC125 (Ventana, Tuscon, AZ) and the novel monoclonal antibodies. Sections of the tissue microarrays were cut at 4 microns, placed on Superfrost/Plus microscope slides (Fisher brand) and baked in a 60° oven for at least 60 minutes. The slides were then deparaffinized and hydrated to distilled water, soaked in citrate buffer at pH 6.00 for 30 minutes at 97° C, washed in running water for 2-5 minutes, incubated for 5 minutes in 3% hydrogen peroxide diluted in distilled water. Slides were

washed in distilled water for 1 minute, transferred to a bath of phosphate buffered saline (PBS), pH 7.2, for two changes of 5 minutes each and placed in 0.05% BSA diluted in PBS for a minimum of 1 minute. After drying around tissue sections, normal serum was applied at a 1:20 dilution in 2% BSA/PBS and incubated for a minimum of 10 minutes at room temperature in a humidity chamber. The serum was then suctioned off without allowing the sections to dry, and approximately 150 lambda of novel antibody at a dilution of 1:1000 was placed on the tissue. The slide was incubated overnight (approximately 15-18 hours) at 4° C in a humidity chamber. Primary antibody was washed off using three changes of PBS for 10 minutes each. Secondary antibody, biotinylated α-mouse from Vector laboratories (Burlingame, Ca), was applied at 1:500 dilution in 1% BSA/PBS and incubated for 45-60 minutes at room temperature in humidity chamber. The antibody was washed off again using three changes of PBS as above. Slides were then transferred to a bath of diaminobenzidine (DAB), diluted in PBS for 5-15 minutes. The slides were then washed in tap water for 1 minute, counterstained using Harris modified hematoxylin (Fisher), decolorized with 1% acid alcohol and blue in ammonia water, dehydrated with 3 changes each of 95% ethanol, 100% ethanol and xylene for 2 minutes each and coverslipped with permanent mounting medium.

Immunohistochemistry scoring:

[0143] Commercially available antibodies, such as OC125 and M11, target complex glycosylation-dependent epitopes. Our hypothesis is that glycosylation may be tissue specific; therefore, it was important to examine the utility of the peptide-directed antibodies in paraffin-fixed tissues and survey the prevalence of MUC16 expression. The three candidate antibodies, 4H11, 9C9 and 4A5, were characterized using OVCAR3 cell line pellets. Of the three, the 4H11 antibody showed the strongest, most diffuse and consistent staining pattern at multiple dilutions, with the least amount of background staining and, therefore, was optimized for use in human tissues in the pathology core facility.

[0144] Using 4H11, the inventors stained and scored positivity using tissue microarrays from high-stage, high-grade ovarian serous carcinomas (Figure 2), these tumors being the most common type of ovarian cancer, representing approximately 80-85% of all ovarian carcinomas in Western industrialized nations (25). To test the specificity of the novel antibody, the inventors also stained tissue microarrays of cancers of the prostate, lung, breast, and pancreas and compared their staining intensities with that of OC125 monoclonal antibody (Figure 6A-D). To determine whether there would be any cross-reactivity with normal human tissues, the antibodies were also tested on normal human adult and fetal TMAs.

[0145] All of the stained sections were reviewed by a reference pathologist (KJP). A subset of cores for which there was equivocal staining was also independently scored by a second pathologist (RAS) to ensure consistency in scoring methods. Only cytoplasmic and/or membranous staining was considered positive. If a portion of the cell showed membranous staining, that was considered partial staining. A scoring system was devised to provide a semiquantitative assessment of staining distribution and intensity in individual cores. At the same time, it was designed to be useful for comparing the staining distribution and intensity between OC125 and the novel antibodies. The score incorporated the percentage of cells, the intensity and pattern of the staining according to the following standards: score 0: no staining; score 1: <5% strong or weak; score 2: 5-50% strong or weak; score 3: 51-75% strong or 51-100% weak; score 4: 76-99% strong; and score 5: 100% strong staining (Figure 3). The pathologist first reviewed all tissue microarrays stained with OC125 and scored each core. Then the same cores stained with the novel antibodies were scored 1 to several days after OC125 without reference to the previous results. Direct comparison of the scoring between the stains for each core was made only after all of the scoring was completed. The same process was used for all non-ovarian tissue microarrays. After comparison, core staining was determined to be concordant, equivocal, or discordant based on the point differentials. Concordant cores differed by 0 to 1 point, equivocal cores differed by 2 points, and discordant cores differed by 3 to 5 points. The one exception to this rule was when the difference of 1 point was between a score of 0 and 1, in which case, the differences were considered equivocal. This was in order to truly separate negative cases from even focally positive ones.

EXAMPLE 2

Generation and characterization of anti-MUC16 monoclonal antibodies

[0146] MUC16-directed monoclonal antibodies were isolated by ELISA-based screening using both the individual peptides and recombinant GST-ΔMUC16^{c114} protein followed by sequential subcloning for single cell clones. **Tables 1A and 1B:** MUC16-carboxyterminus monoclonal antibodies showing their reactivity to GST-ΔMUC16^{c114} western, FACS analysis on OVCAR3 wild type cells

Table 1A

ELISA Hybridom a Sups (1:1).	Peptide 1			ELISA Hybridoma Sups (1:1)	Peptide 2			EISA Hybridoma Sups (1:1)	Peptide 3		
	(1:10) GST-MucDO Western +/-	OVCAR3 FACS +/-	Isotype		(1:10) GST-MucCD Western +/-	(1:1) OVCAR3 FACS +/-	Isotype		(1:10) GST MucCD Western +/-	(1:1) OVCAR3 FACS +/-	Isotype
10A2	+	-	IgG1JgM	13H1	Weak	-	IgG1	222E10	+	-	IgG2b
23D4	-	-	missing	28F8	+	+	Igg1,JgM	22F11	Weak	-	IgM
2F4	Weak	-	IgG1JgM	11B6	-	-	IgM	19G4	Weak	-	IgG1JgM
9B11	Weak	-	IgG1)	4C7	+	-	IgG1	31A3	Weak	-	IgG1
23D3	Weak	+/-	IgG1JgG2b	28F7	+	+	IgG1	4C2	+	-	IgG1JgM
30B1	-	-	IgG1	9C7	+	+	IgG1	27G4	+	-	IgM
31B2	+	-	IgM	9C9	+	+	IgG1,IgG2b	19D1	+	-	IgG2b
				4H11	+	+	IgG2bJgM	22F1	-	-	IgG2bJgM
				4A2	-	-	IgG1	4D7	+	-	IgG3
				4A5	+	+		9A5	-	-	IgM
				29G9	-	-	IgG1	31C8	-	-	IgG2b
				5C2	+	+	IgG1	6H2	Weak	-	IgG1JgM
				23G12	-	-	IgG1,IgG2a	10F6	-	-	IgG1
				25G4	-	-	IgG1JgM	3H8	+	-	IgG1JgM
				26B2	-	+	IgG1JgG2bJgM	24G12	-	-	IgG1JgM
				25H3	-	-	IgG1JgM				

Table 1B

Peptide 1			Peptide 2			Peptide 3		
	OVCAR3 FACS +/-	Isotype		OVCAR3 FACS +/-	Isotype		OVCAR3 FACS +/-	Isotype
9B11.20.16	+/-	IgG1	9C9.21.5.13	+	IgG2b	31A3.5.1	-	IgG1
			4H11.2.5	+	IgG2b			
			9C7.6	+	IgG1			
			5C2.17	+	IgG1			
			4A5.37	+	IgG1			
			28F7.18.10	+	IgG1			

Table 2: Antibodies specific for exemplary portions of MUC16

1. Muc16 Polypeptide 1:			
14394	14410	(MUC16 sequence)	
NFSPLARRVDRVAIYEE (SEQ ID NO:01)			17 aa
Mouse monoclonals which are specific to this peptide are:			
9B11.20.16 (IgG1)			
10A2 (IgG1, IgM)			
2F4 (IgG1, IgM)			
23D3 (IgG1, IgG2b)			
30B1 (IgG1)			
31B2 (IgM)			
2. Muc16 Polypeptide 2:			
14425	14442	(MUC16 sequence)	
TLDRSSVLVDGYSPNRNE (SEQ ID NO:02)			18 aa
Mouse monoclonals which are specific to this peptide are:			
4H11.2.5 (IgG2b)	13H1 (IgG1)	2909 (IgG1)	
9C9.21.5.13 (IgG2b)	28F8 (IgG1, IgM)	23G12 (IgG1, IgG2a)	
9C7.6 (IgG1)	11B6 (IgM)	25G4 (IgG1, IgM)	
SC2.17 (IgG1)	4G7 (IgG1)	26B2 (IgG1, IgG2b, IgM)	
4A5.37 (IgG1)	4A2 (IgG1)	25H3 (IgG1, IgM)	
28F7.18.10 (IgG1)			
3. Muc16 Polypeptide 3 (SEQ ID NO:03)			
14472	14492	(MUC16 sequence)	
CGVLVTTRRRKKEGEYNVQQQ			21 aa
Mouse monoclonals which are specific to this peptide are:			
31A3.5.1 (IgG1)	19D1 (IgG2b)	10F6 (IgG1)	
2.2E10 (IgG2b)	22F1 (IgG2b, IgM)	3H8 (IgG1, IgM)	
22F11 (IgM)	4D7 (IgG3)	24G12 (IgG1, IgM)	
19G4 (IgG1, IgM)	9A5 (IgM)		
4C2 (IgG1, IgM)	31C8 (IgG2b)		
27G4 (IgM)	6H2 (IgG1, IgM)		
14452	14475		
FWAVILIGLAGLLGLITCLICGVL (SEX ID NO:14) is Transmembrane regions			24 aa
4. Muc16 Polypeptide 4 (SEQ ID NO:15) containing a cysteine loop polypeptide (SEQ NO:19)			
14367		14398 (MUC16 sequence)	
KSYFSDCQVSTFRSVPNRHHTGVDSLCNFSPL (SEQ ID NO:15)			32 aa
S - S			
Mouse monoclonals which are specific to this peptide are:			
24B3 (IgM)			
9C7(IgM)			

4F12	IgM kappa
6H6	IgM kappa
25C2	IgM kappa
6E8	IgM kappa
2A3	IgM, IgG1, IgG2b, kappa

(continued)

3. Muc16 Polypeptide 3 (SEQ ID NO:03)

2G4	IgM IgG1, kappa
4C8	IgM, kappa
2A6	IgG1 kappa
24G12	IgG1 kappa
15O5	IgG1 kappa
6E2	IgM, IgG1, IgG3, IgG2a, kappa
7E6	IgM, kappa, lambda
7G11	IgM kappa
20C3	IgG1, IgG2b
9A3	IgM kappa
15B6	IgM kappa
19O3	IgM kappa
5H6	IgM, IgG1, IgG2b, kappa
24A12	IgM kappa

2D10	IgG3, IgM kappa
5B2	IgM, IgG3, IgG2b, IgG2a, IgG1, kappa
8B6	IgG2a, IgG3. kappa
5A11	IgM, kappa
7D11	light kappa only
9F10	IgM, kappa
15D10	IgM, kappa
18D2	IgM, kappa
13A11	IgM, kappa
1A9	IgM, kappa
3B2	IgM, kappa
24F6	IgM, kappa
24E4	IgM, kappa
5A1	IgG2a, IgM, kappa
7B9	IgM, kappa
22F4	IgM, kappa

[0147] The identified monoclonal antibodies are listed in Table 1A and Table 2. Each of the selected monoclonal antibodies was reactive against GST-ΔMUC16^{c114}. The commercial MUC16-directed antibodies (OC125, M11, or VK8) did not bind to GST-ΔMUC16^{c114} in ELISA or Western blotting. The clones were tested in FACS against OVCAR3 ovarian cancer cells and in Western blot analysis against GST-ΔMUC16^{c114} (Table 1B), and selected purified monoclonal antibodies were isolated.

[0148] The inventors used the OVCAR3 wild type and the SKOV3 cells transduced with phrGFP-ΔMUC16^{c114} to characterize the selected antibodies by FACS analysis. All of the selected monoclonal antibodies bound to both cell lines while commercial VK8, M11 and OC125 antibodies bound to the OVCAR3 cells but not to the SKOV3-phrGFP-

ΔMUC16^{c114} cell line. The antibodies against Polypeptide 3 required permeabilization since it is an internal epitope (Figure 7).

[0149] Western blot analysis using the GST-ΔMUC16^{c114} purified protein showed strong binding with 4H11 and 9C9 antibodies (Figure 4A), while the other selected antibodies showed less binding. The SKOV3-phrGFP-ΔMUC16^{c114} transfectant was also positive by Western blot analysis using 4H11 and 9C9 antibodies (Figure 4B). As before, the commercial antibodies did not interact with the GST-ΔMUC16^{c114} purified protein or cell lysates of the SKOV3-phrGFP-ΔMUC16^{c114} cell line.

[0150] The binding of six monoclonal antibodies against OVCAR3 MUC16 were examined in affinity binding studies. Three antibodies-9C7, 5C2 and 28F7-showed only modest levels of binding compared to the nonspecific binding of these antibodies to the OVCAR3 cells, which carry large numbers of MUC16 binding sites. In contrast, 4H11, 9C9, and 4A5 monoclonal antibodies showed highly specific binding affinity, as shown in Figure 5A, with binding affinities of 6.8-8.6 nM against the cell surface epitopes of OVCAR3 cells. The inventors also examined the internalization of antibody bound to cell surface MUC16 protein. The inventors examined internalization in the transfected SKOV3-phrGFP-ΔMUC16^{c334} cell line which bears the carboxy terminus of MUC16, including the 4H11 epitope and a single degenerate tandem repeat sequence to interact with the OC125 antibody. The commercial antibodies OC125, M11, and VK8 all bind to the cell surface of this transduced cell line. The ^{131}I-labeled 4H11 showed rapid internalization at a high level, whereas ^{131}I-labeled OC125 antibody was internalized at a much lower rate (Figure 5B).

EXAMPLE 3

Immunohistochemistry results:

[0151] Given their highly specific binding affinities, the antibodies 9C9, 4A5, and 4H11 were characterized for utility in immunohistochemistry using OVCAR3 cell lines. Of the three, the 4H11 antibody was selected to be optimized for use in human tissues based on its robust, sensitive and specific staining pattern as compared to the other two antibodies.

A. Ovary

[0152] Two high-stage, high-grade ovarian serous carcinoma tissue microarray slides composed of 419 cores, representing primary, metastatic and recurrent tumors from 40 patients were stained with both OC125 and 4H11 monoclonal antibodies (Figure 2). The OC125 tissue microarrays showed 279 (66%) cores with 3-5 staining, 99 (24%) with 1-2 staining, and 41 (10%) with no staining. The 4H11 tissue microarrays showed 236 (56%) with 3-5 staining, 91 (22%) with 1-2 staining, and 92 (22%) with no staining. The two antibodies were concordant in 233 (56%) cores, equivocal in 161 (38%), and discordant in 25 (6%). Of the 25 discordant cores, 12 (48% of discordant cases, 3% of all cases) showed greater 4H11 positivity than OC125. Nine were discordant by a difference of 4 points, and 3 were discordant by a difference of 5 points. There was a total of 186 discordant and equivocal cores together, 48 (26%) of which showed greater staining with 4H11 than OC125. The staining pattern of both 4H11 and OC125 was cytoplasmic and membranous, although the membranous pattern of OC125 was stronger and better defined than 4H11 in the majority of cases. Discordant cases demonstrated higher levels of 4H11 than other cases.

B. Breast Cancer

[0153] A variety of other tissues were also examined for 4H11 staining to test the antibody's specificity. Of the 50 cores of invasive ductal carcinomas of the breast (number of patients unavailable), only 2 (4%) showed a score of 4 or greater 4H11 staining and none had scores of 3-5 for OC125 staining. The staining pattern with OC125 was mostly apical/luminal with some granular cytoplasmic staining. Some tumors with intracytoplasmic lumina also picked up the OC125 stain. 4H11 showed a more diffuse cytoplasmic blush without membranous accentuation.

[0154] In contrast, the invasive lobular breast carcinoma tissue microarray (composed of 179 cores with viable tumor, number of patients unavailable) had frequent MUC16 staining with 4H11. In this tissue microarray, 168 cores (94%) showed no staining for OC125, 5 (3%) showed 1-2 staining, and only 6 (3%) showed a staining intensity of 3. 4H11 staining was different in its distribution pattern, with 49 (27%) showing no staining, 81 (45%) showing 1-2 staining, and 49 (27%) showing 3-4 staining. Neither OC125 nor 4H11 had cores with a staining intensity of 5. The staining pattern was of cytoplasmic, luminal/membranous, or intraluminal for both OC125 and 4H11. The intraluminal pattern was strong and intense for both stains and highlighted the intracytoplasmic lumen that is commonly present in lobular carcinomas. The concordance rates were 34% concordant, 43% equivocal, and 23% discordant. Of the equivocal and discordant cases, there was none in which the OC125 was greater than the 4H11. All 42 discordant cases and 76 of 77 equivocal cases had 4H11 greater than OC125. There was also focal luminal staining with 4H11 in benign breast ducts and lobular carcinoma in situ.

C. Lung, pancreatic and prostatic adenocarcinomas

[0155] Tumors from other organs were not reactive with either antibody. The lung adenocarcinoma TMA had 237 cores from 86 patients containing viable tumor. In the pancreatic TMA there were 92 cores from 21 patients containing pancreatic mucinous tumors, including intraductal papillary mucinous neoplasms (IPMN) and invasive ductal carcinomas. In the prostate cancer TMA there were 169 cores (number of patients not available). None of these cancer tissue microarrays had significant binding to either OC125 or 4H11. This information is summarized in Table 3.

Table 3. Staining intensity of OC125 as compared to 4H11 in tissue microarrays

	OC125 vs. 4H11 staining intensity score (%)					
Site	**0**		**1-2**		**3-5**	
	OC125	**4H11**	**OC125**	**41111**	**OC125**	**41111**
Ovary high grade serous	10	28	24	22	66	56
Breast invasive ductal	68	78	32	18	0	4
Breast invasive lobular	94	27	3	45	3	27
Lung adenocarcinoma	63	77	24	18	13	5
Pancreas mucinous neoplasms	98	88	2	10	0	2
Prostate adenocarcinoma	0	0	0	0	0	0
Score 0: 0% staining; 1: <5% strong or weak; 2: 5-50% strong or weak; 3: 51-75% strong or 51-100% weak; 4 76-99% strong 5: 100% -ong						

D. Normal Tissues

[0156] There was no staining with OC125 or 4H11 in normal adult colon, rectum, ectocervix, small intestine, ovary, liver, pancreatic ducts, spleen, kidney, and skin. OC125 and 4H11 both stained endocervical glands (OC125 luminal, 4H11 weak cytoplasmic), esophageal glands (luminal), bronchial epithelium (OC125 luminal, 4H11 intracytoplasmic granules), and thymic corpuscles (cytoplasmic). 4H11 demonstrated weak to moderate staining of the gastric glands, particularly at the crypts, with an intracytoplasmic granular pattern. Other organs that showed punctuate intracytoplasmic staining with 4H11 only were prostate, seminiferous tubules of the testes, and the islet cells of the pancreas. The staining in the pancreatic islets cells was particularly strong and consistent. There was also nonspecific staining of liver, kidney and brain with 4H11. There were no cases that stained with OC125 and not 4H11.

[0157] Similarly, there was no staining with either OC125 or 4H11 in fetal heart, gallbladder, colon, small intestine, liver, rectum, adrenal, thyroid, spleen, skin, bone, epididymis, brain, lung, muscle, smooth muscle, kidney, eye, umbilical cord, and placenta. OC125 only stained thymic corpuscles in a pattern similar to that in adult tissue. 4H11 stained both fetal pancreatic endocrine cells and endocervical glands in a similar pattern to that of their adult counterparts. Islet cells showed a granular cytoplasmic pattern, and endocervical glands showed a linear luminal pattern, which was more similar to the OC125 pattern in the adult tissue.

EXAMPLE 4

Successful eradication of established peritoneal ovarian tumors in SCID-Beige mice following adoptive transfer of T cells genetically targeted to the MUC16 antigen.

[0158] **Purpose:** Most patients diagnosed with ovarian cancer will ultimately die from their disease. For this reason, novel approaches to the treatment of this malignancy are needed. Adoptive transfer of a patient's own T cells, genetically modified *ex vivo* through the introduction of a gene encoding an chimeric antigen receptor (CAR), an artificial T cell receptor, targeted to a tumor associated antigen, is a novel and promising approach to cancer therapy applicable to the treatment of ovarian cancer.

[0159] **Experimental design:** We have generated several CARs targeted to the retained extracellular domain of MUC16, termed MUC-CD, an antigen highly expressed on a majority of ovarian carcinomas. We investigate the *in vitro* biology of human T cells retrovirally transduced to express these CARs by co-culture assays on artificial antigen presenting cells (AAPCs) generated from NIH3T3 fibroblasts genetically modified to express the target MUC-CD antigen, as well as by cytotoxicity assays utilizing the human OV-CAR3(MUC-CD) ovarian tumor cell line and primary patient tumor cells.

Finally, we assess the *in vivo* anti-tumor efficacy of MUC-CD targeted T cells in a SCID-Beige orthotopic, xenogeneic OV-CAR3(MUC-CD) murine tumor model.

[0160] Exemplary sequences used in this work are in Figure 17-19.

[0161] **Results:** CAR modified MUC-CD targeted T cells derived from both healthy donors and ovarian cancer patients exhibited efficient *in vitro* cytolytic activity against both human ovarian cell lines as well as primary ovarian carcinoma cells. MUC-CD targeted T cells may be further expanded *ex vivo* through multiple cycles of co-culture on 3T3(MUC-CD/B7.1) AAPCs. Expanded MUC-CD targeted T cells infused into SCID-Beige mice bearing intraperitoneal human OV-CAR3(MUC-CD) tumors either delayed progression or fully eradicated tumor even in the setting of advanced disease.

[0162] **Conclusion:** These promising pre-clinical studies justify further investigation of MUC-CD targeted T cells as a potential therapeutic approach in the clinical setting treating patients with high risk MUC-16^{+} ovarian carcinomas.

INTRODUCTION

[0163] Ovarian cancer is the sixth most common cancer worldwide and the seventh leading cause of cancer-related deaths in women (1, 2). Despite multimodality therapy with surgery and chemotherapy, most patients with ovarian carcinomas have a poor prognosis. For this reason, alternative approaches to treating this disease are urgently needed.

[0164] Infusion of a patient's own T cells genetically targeted *ex vivo* to antigens expressed on the surface of tumor cells is a promising novel approach to the adoptive immunotherapy of cancer, and one which has only recently been explored in earnest in the clinical setting. T cells may be genetically modified to target tumor associated antigens through the retroviral introduction of genes encoding artificial T cell receptors termed chimeric antigen receptors (CARs). Genetic engineering of T cells to express artificial T cell receptors that direct cytotoxicity toward a tumor cell presents a means to enhance immune recognition and elimination of cancer cells. CARs are most commonly composed of a single chain fragment length antibody (scFv), derived from a murine monoclonal antibody targeting a given tumor associated antigen, fused to a transmembrane domain (typically CD8, CD28, OX-40, and 4-1BB), fused to the TCR ζ chain cytoplasmic signaling domain (3-13). When used to reprogram T-cell specificity, these fusion receptors permit recognition of native antigen. When expressed by the T cells, the resulting construct, upon engagement with the targeted antigen, induces T cell activation, proliferation, and lysis of targeted cells. These fusion receptors transduce a functional antigen-dependent co-stimulatory signal in primary T cells, permitting sustained T-cell proliferation when both endogenous TCR and a chimeric receptor for stimulatory signaling are engaged. To date, preclinical studies utilizing CAR-modified T cells have demonstrated promising results in a wide variety of malignancies (3, 4, 11, 14-18). More recently this approach been investigated clinically in the form of phase I trials (6, 19-21). These genetic approaches offer a means to enhance immune recognition and elimination of cancer cells.

[0165] Ovarian carcinomas appear to be relatively immunogenic tumors capable of inducing an endogenous immune response based on the fact that long-term prognosis of patients is markedly influenced by the degree and quality of the endogenous immune response to the tumor. Specifically, it has been well documented that the presence of endogenous effector T cells within the ovarian cancer tumor microenvironment directly correlates to prolonged patient survival (22-25). In contrast, increasing numbers of immune suppressive $CD4^{+}CD25^{hi}$ regulatory T cells (Tregs) within the tumor, which in turn presumably abrogate the anti-tumor activity of infiltrating effector T cells, correlates with shorter patient survival (26-29). In fact, it appears that it is the ratio of Tregs to effector T cells within the tumor microenvironment which ultimately dictates whether the endogenous immune response to the cancer is of benefit or detriment to the patient (24, 28). In this setting, the ability to generate and subsequently expand a population of tumor targeted effector T cells *ex vivo* which are subsequently infused back into the patient, may in turn skew the Treg to effector T cell ratio to one more favorable to eradicating the disease.

[0166] Mucins are important biomolecules for cellular homeostasis and protection of epithelial surfaces. Changes to expression of mucins in ovarian cancer might be exploited in diagnosis, prognosis and treatment (1). MUC16 is one such mucin which is over expressed on most ovarian carcinomas and is an established surrogate serum marker (CA-125) for the detection and progression of ovarian cancers (30-33). MUC16 is a high-glycosylated mucin composed of a large cleaved and released domain, termed CA-125, consisting of multiple repeat sequences, and a retained domain (MUC-CD) which includes a residual non-repeating extracellular fragment, a transmembrane domain, and a cytoplasmic tail (34). Since the antigen is otherwise only expressed at low levels in the uterus, endometrium, fallopian tubes, ovaries, and serosa of the abdominal and thoracic cavities, MUC16 is a potentially attractive target for immune-based therapies.

[0167] However, the fact that most of the extracellular domain of MUC16 is cleaved and secreted limits the utility of MUC16 as a target antigen on ovarian carcinomas. In fact, to date, all reported MAbs to MUC16 bind to epitopes present on the large secreted CA-125 fraction of the glycoprotein, with none known to bind to the retained extra-cellular fraction (MUC-CD) of the antigen (35-37). Since the MUC-CD fraction of the antigen is retained on cell surface, generating T cells specific to this portion of MUC16 may largely overcome the limitation of MUC16 as a target for adoptive cellular immunotherapy. To this end, we have previously generated a series of murine MAbs specific to the retained MUC-CD extracellular domain (38). Utilizing a hybridoma which expresses one such MAb, 4H11, we have successfully constructed

several CARs specific to the MUC-CD antigen. This invention provides a nucleic acid encoding a chimeric T cell receptor, composed of, at least a zeta chain, a signaling region and a binding element that specifically interacts with a selected target as well as the chimeric T cell receptor comprising a zeta chain portion, a signaling region and a binding element.

[0168] In this report, we demonstrate highly efficient retroviral transduction of these MUC-CD targeted CARs into human T cells with resulting T cells able to specifically target and lyse MUC-CD$^+$ tumor cells *in vitro.* Furthermore, we demonstrate efficient MUC-CD targeted T cell expansion *in vitro* through repeated co-culture on NIH (3T3) fibroblasts genetically modified to express MUC-CD and the co-stimulatory ligand B7.1 (CD80). Successful expansion of modified T cells allowed us to subsequently generate sufficient T cell numbers to conduct *in vivo* studies in immune compromised SCID-Beige mice bearing established intraperitoneal MUC-CD$^+$ human ovarian tumors. Significantly, in these studies we demonstrate marked anti-tumor efficacy of MUC-CD targeted T cells, both following direct intraperitoneal as well as intravenous injection when compared to either untreated mice, or mice treated with T cells bearing a CAR targeted to an irrelevant antigen. In addition, we demonstrate significant cytotoxicity of 4H11-28z$^+$ patient's T cells and healthy donor's T cells targeting primary ascites-derived ovarian carcinoma cells from cancer patients.

[0169] To our knowledge this is the first report wherein T cells genetically targeted to the MUC16 antigen demonstrate marked anti-tumor efficacy against MUC16$^+$ tumors either *in vitro* or *in vivo.* These data serve as a rationale for proposing future clinical trials utilizing this approach in patients with high risk ovarian carcinomas.

MATERIALS AND METHODS

Cell lines and T cells

[0170] The OV-CAR3 tumor cell line was cultured in RPMI 1640 (Invitrogen, Grand Island, NY) supplemented with 10% heat-inactivated FBS, nonessential amino acids, HEPES buffer, pyruvate, and BME (Invitrogen). The PG13 and gpg29 retroviral producer cell lines were cultured in DMEM (Invitrogen) supplemented with 10% FCS, and NIH-3T3 artificial antigen-presenting cells (AAPC), described previously (3), were cultured in DMEM supplemented with 10% heat-inactivated donor calf serum. T cells were obtained from peripheral blood of healthy donors under IRB approved protocol #95-054, in BD Vacutainer CPT tubes (Becton Dickinson, Franklin Lakes, NJ) as per the manufacturer's instructions. All media were supplemented with 2 mmol/L L-glutamine (Invitrogen), 100 units/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). T cells were cultured RPMI 1640 media as above supplemented with 20 IU/ml IL-2 (Novartis Pharmaceuticals, East Hanover, NJ) and where indicated, medium was supplemented with 10 ng/mL interleukin 15 (R&D Systems, Minneapolis, MN).

Isolation of patients ascites-derived cancer cells

[0171] Primary human ascites-derived cancer cells were obtained from ovarian cancer patients undergoing surgery for newly diagnosed advanced serous ovarian carcinoma under IRB approved protocol #97-134. The tumor cells were isolated from ascitic fluid of patients by centrifugation at 600g for 10 min at room temperature. Cells were washed once with 1x PBS and cultured in RPMI 1640 media supplemented with 10% FBS for future analysis.

Generation of the MUC-CD targeted 4H11z and 4H11-28z CARs

[0172] The heavy and light chain variable regions of the 4H11 monoclonal antibody were derived from the hybridoma cell line 4H11. Utilizing cDNA generated from 4H11 RNA we isolated the V_H coding region by RACE PCR utilizing modified primers as described elsewhere (39, 40). The V_L chain variable region was cloned by standard PCR utilizing modified primers as described by Orlandi et al (41, 42). The resulting V_H and V_L fragments were subcloned into the TopoTA PCR 2.1 cloning vector (Invitrogen) and sequenced. The V_H and V_L fragments were subsequently ligated to a $(Gly_4Ser)_3$ spacer domain, generating the 4H11 scFv and fused to the human CD8 leader peptide (CD8L) by overlapping PCR (9, 41). In order to construct the MUC-CD targeted 4H11 CARs, the coding region of the CD8L-4H11 scFv was fused to the human CD8 hinge and transmembrane domains (to generate the 4H11z CAR), or alternatively to the CD28 transmembrane and cytoplasmic signaling domains (to generate the 4H11-28z CAR), fused to the T cell receptor CD3-ζ signaling domain (3, 9, 43). The resulting CAR constructs were subsequently sub-cloned into the modified MMLV retroviral vector SFG (44). VSV-G preudotyped retroviral supernatants derived from transduced gpg29 fibroblasts were used to construct stable PG13 gibbon ape leukemia virus (GaLV) envelope-pseudotyped retroviral producing cell lines (41).

Retroviral gene transfer

[0173] Isolated healthy donor peripheral blood mononuclear cells (PBMCs) were activated with phytohemagglutinin

(PHA) at 2μg/ml (Sigma. St. Louis, MO) and retrovirally transduced on retronectin coated non-tissue culture plates (45). Briefly, six-well non-tissue culture plates (BD Biosciences, San Jose, CA) were coated with RetroNectin (RN) (Takara Biomedicals, Otsu, Japan) as per manufacturer's instructions. Forty-eight hours after PHA activation, aliquots of $1x10^6$ T cells in 1 ml of supplemented RPMI medium were placed in each well of the RN-coated plates, along with 1 ml of SFG retroviral supernatant. T cells were centrifuged daily for 3 consecutive days with fresh retroviral supernatant added daily at 2000g at 30°C for 1hr (45). Gene transfer was assessed on day 7 by FACS.

[0174] In order to generate the relevant NIH-3T3 murine fibroblast artificial antigen presenting cells, a MUC-CD construct encoding the retained extracellular, transmembrane and cytoplasmic domains of the MUC-16 antigen was initially subcloned into SFG retroviral vector, SFG(MUC-CD). 3T3(MUC-CD) AAPCs were generated by retroviral transduction of SFG(MUC-CD) into wild-type NIH-3T3 fibroblasts, while 3T3(MUC-CD/B7.1) AAPCs were generated by retroviral transduction of previously established 3T3(B7.1) fibroblasts (41, 46). Highly enriched cell lines were isolated by FACS.

[0175] To generate the OV-CAR3(MUC-CD) and OV-CAR3(MUC-CD/GFP-FFLuc) cell lines, we retrovirally transduced the WT OV-CAR3 human ovarian cancer cell line with SFG(GFP-FFLuc) as described previously (47) and/or SFG(MUC-CD) VSV-G pseudotyped retroviral supernatants derived from gpg29 fibroblasts as described elsewhere (44). Resulting tumor cells were sorted by FACS for either MUC-CD expression alone for the OVCAR3(MUC-CD) cell line, or dual MUC-CD and GFP expression for the OVCAR3(MUC-CD/GFP-FFLuc) cell line. MUC-CD expression by FACS was assessed using the 4H11 MAb.

In vitro analyses of CAR$^+$ human T cells

[0176] To assess *in vitro* expansion and cytokine release upon stimulation, transduced T cells were co-cultured for 7 days after retroviral transduction in 6-well tissue culture plates (BD Biosciences) on confluent NIH 3T3 AAPCs in RPMI medium supplemented with 10% FBS in the absence of supplemented cytokines. In order to generate sufficient numbers of CAR-modified T cells for *in vivo* studies, transduced T cells were co-cultured on B7.1$^+$ AAPCs (3T3(MUC-CD/B7.1)) in RPMI medium supplemented with 20 IU IL-2/mL and 10 ng/mL IL-15 as described previously (3, 43). Patients T cells were activated and expanded with human CD3/CD28 beads (DYNAL®, Invitrogen, Carlsbad, CA) following manufacturer's recommendations.

Western Blot analysis of CAR expression

[0177] Western blot analysis of T-cell lysates under reducing conditions with 0.1 mol/L DTT (Sigma) was performed as previously described (46). Briefly, transduced T cells were washed in PBS and resuspended in radioimmunoprecipitation assay (RIPA) buffer (Boston BioProducts, Worcester, MA) with mini complete protease inhibitor as per the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN). Resulting proteins were separated on 12% SDS-PAGE mini gels (Bio-Rad, Hercules, CA) after the addition of 6X reducing loading buffer (Boston BioProducts, Worcester, MA) and heating at 100°C for 10 min. Separated proteins were subsequently transferred to Immobilon membranes and probed using an anti-human CD3ζ chain monoclonal antibody (BD Biosciences). Antibody binding was detected by probing the blot with goat anti-mouse horse radish peroxidase-conjugated antibody followed by luminescent detection using Western Lighting Chemiluminescence Reagent Plus (Perkin-Elmer Life Sciences, Boston, MA) as per the manufacturer's instructions.

Cytotoxicity assays

[0178] *In vitro* modified T cell cytotoxicity was assessed using the DELFIA® EuTDA assay (PerkinElmer LAS, Inc, Boston, MA) following manufacturer's recommendations. Cytotoxocity was assessed at 2 hours at effector T cell to target OV-CAR3(MUC-CD) or primary tumor cells (E:T) at indicated ratios. Effector T cells in these assays represent the number of CD8$^+$ CAR* T cells.

Cytokine detection assays

[0179] Cytokine assays were performed as per manufacturer's specifications using a multiplex Human Cytokine Detection assay to detect IL-2 and IFNγ (Millipore Corporation, Billerica, MA) utilizing the Luminex IS100 system. Cytokine concentrations were assessed using IS 2.3 software (Luminex Corp., Austin, TX).

In vivo SCID-Beige mouse tumor models

[0180] In all *in vivo* studies, 8-12 week-old FOX CHASE C.B.-17 (SCID-Beige mice) (Taconic, Hudson, NY) were initially injected ip with either 3 x 10^6 OV-CAR3(MUC-CD), or for bioluminescent imaging (BLI) studies 3 x 10^6 OV-

CAR3(MUC-CD/GFP-FFLuc) tumor cells. Subsequently, $3x10^7$ CAR^+ T cells were injected ip or iv on day 1 or 7 following tumor injection as indicated. Mice were monitored for distress as assessed by increasing abdominal girth, ruffled fur, and decreased response to stimuli. Distressed mice were euthanized. All murine studies were done in context of an Institutional Animal Care and Use Committee-approved protocol (#00-05-065).

Bioluminescent imaging (BLI) of OVCAR3(MUC-CD/GFP-FFLuc) tumor cells in SCID-Beige mice

[0181] BLI was performed using Xenogen IVIS imaging system with Living Image software (Xenogen; Alameda, CA). Briefly, OVCAR3(MUC-CD/GFP-FFLuc) tumor bearing mice were injected by ip with D-luciferin (150 mg/kg; Xenogen) suspended in 200μl PBS and imaged under 2% isoflurane anesthesia after 10 min. Image acquisition was done on a 25-cm field of view at medium binning level for 0.5-min exposure time (3, 43).

Flow cytometry

[0182] All flow cytometric analyses of T cells and tumor cells was performed using a FACScan cytometer with Cellquest software (BD Biosciences). T cells were analyzed using CAR-specific polyclonal goat Alexa Fluor 647 antibody (Molecular probes, Eugene, OR) phycoerythrin-labeled anti-human CD4, CD8, B7.1 (Caltag Laboratories, Burlingame, CA), B7.2 (Invitrogen, Camarillo, CA), 4-1BBL, and OX40 antibodies (Ancell Corporation, Bayport, MN). 3T3(MUC-CD) and OV-CAR3(MUC-CD) cells were stained with Alexa Fluor 647 labeled 4H11 antibody (generated and labeled in the MSKCC monoclonal antibody core facility).

CFSE labeling of CARP T cells

[0183] CAR^+ T cells were stained with CFSE using the CellTrace™ CFSE cell proliferation kit following manufacturer's recommendations (Molecular Probes, Eugene, OR). Proliferation of CFSE labeled T cells was analyzed by FACS. For detection of CFSE labeling T cells *in vivo,* ovarian tumors were macerated through 40 [im cell strainer (BD Falcon, Franklin Lakes, NJ) and washed twice with 2% FBS/PBS before antibody staining and FACS analysis.

Statistics

[0184] Survival data assessed by log-rank analysis using GraphPad Prism software (GraphPad Prism software, San Diego, CA). Cytokine data were analyzed by Student's one-tailed t-test.

RESULTS

[0185] We have constructed SFG retroviral vectors encoding first (4H11z) and second generation (4H11-28z) CARs targeted to the MUC-CD antigen using the 4H11 hybridoma which generates a MAb specific to the MUC-CD antigen (Figure 11A). We confirmed expression of appropriately sized CAR proteins by Western blot analysis of resulting PG-13 retroviral producer cells (SFG-4H11z and SFG-4H11-28z) probed with a ζ-chain specific antibody (data not shown).

[0186] In order to assess the function of the first generation 4H11z CAR, healthy donor T cells isolated from peripheral blood were retrovirally transduced to express the 4H11z and control 19z1 CARs (Figure 11B). Function of the 4H11z CAR was assessed by proliferation of 4H11z transduced T cells following co-culture on 3T3(MUC-CD/B7.1) AAPCs. Results demonstrate specific proliferation of 4H11z transduced T cells, when compared to 19z1 modified T cells (Figure 11C). These data are consistent 4H11z CAR mediated specific binding to the MUC-CD antigen and subsequent T cell activation.

[0187] Since most malignancies fail to express co-stimulatory ligands, we further modified the 4H11z CAR to express the CD28 transmembrane and cytoplasmic co-stimulatory signaling domains, constructing the second generation 4H11-28z CAR (Figure 11A). To assess whether the 4H11-28z CAR, when expressed by human T cells, was capable of generating both a primary activating signal (termed "signal 1") through the ζ chain, as well as a co-stimulatory signal (termed "signal 2") through the CD28 cytoplasmic domain, which in turn allows for efficient T cell proliferation in the absence of exogenous co-stimulatory ligands, we compared T cell proliferation following co-culture on either 3T3(MUC-CD) or 3T3(MUC-CD/B7.1) AAPCs in the absence of exogenous cytokines. As expected, the second generation $4H11\text{-}28z^+$ T cells markedly expanded when compared to $4H11z^+$ T cells upon co-culture with 3T3(MUC-CD) AAPCs. In contrast, both $4H11z^+$ and $4H11\text{-}28z^+$ T cells expanded similarly on 3T3(MUC-CD/B7.1) AAPCs (Figure 12A). Co-stimulation mediated by the 4H11-28z CAR was further verified by analysis of day 2 tissue culture supernatants from co-culture experiments on 3T3(MUC-CD) AAPCs demonstrating enhanced IL-2 secretion, a cytokine typically secreted in the context of T cell co-stimulation, when compared to control $19z1^+$ and $19\text{-}28z^+$T cells and first generation $4H11z^+$ T cells (Figure 12B). Secretion of IFNγ was comparable between $4H11z^+$ and 4H11-28z activated T cells.

[0188] We next assessed the ability of MUC-CD targeted T cells to expand following weekly re-stimulations through co-culture on 3T3(MUC-CD/B7.1) AAPCs in the context of exogenous IL-2 and IL-15 (3). Both 4H11z$^+$ and 4H11-28z$^+$ T cells expanded greater than 2 logs over 3 weeks (Figure 12C). T cells transduced with the 4H11-28z were further analyzed by FACS for CAR expression 7 days after initial activation on AAPCs and following two subsequent co-stimulations on AAPCs demonstrating an expected enrichment of the CAR$^+$ T cell fraction (Figure 12D). Similar data was generated with expanded 4H11z$^+$ T cells (data not shown).

[0189] *In vitro cytotoxicity and proliferation of MUC-CD targeted T cells following co-culture with OV-CAR3(MUC-CD) and freshly isolated ascites derived ovarian tumor cells.*

[0190] In order to assess the ability of 4H11z$^+$ and 4H11-28z T cells to target and lyse human ovarian carcinoma tumors, we utilized the human OV-CAR3 cell line which was genetically modified to express the MUC-CD antigen thereby better reflecting the majority of clinical ovarian tumor samples which express the 4H11-targeted MUC-CD antigen (48). We initially verified specific lysis by MUC-CD targeted T cells demonstrating similar significant cytotoxic activity of 4H11z and 4H11-28z CAR modified T cells targeting OV-CAR3(MUC-CD) tumor cells when compared control T cells expressing the irrelevant first and second generation CD19-targeted 19z1 and 1928z CARs (Figure 13A). Healthy donor T cells modified to express the 4H11-28z CAR similarly exhibited lysis of freshly isolated ascites derived MUC-CD$^+$ ovarian carcinoma cells when compared to 19-28z transduced T cells (Figure 13B). Moreover, patient's peripheral blood T cells modified to express the 4H11-28z CAR similarly lysed autologous primary MUC-CD$^+$ tumor cells derived from the same ascites sample when compared to T cells modified to express the control 19-28z CAR (Figure 13C).

[0191] We further assessed the ability of 4H11z$^+$ and 4H11-28z$^+$ T cells from healthy donors to proliferate following co-culture on OV-CAR3(MUC-CD) as assessed by FACS of CFSE labeled T cells, as well as absolute T cells numbers over 7 days following co-culture with tumor (Figures 13D and E). Surprisingly, we found that both 4H11z$^+$ and 4H11-28z T cells expanded equally well following co-culture with OV-CAR3(MUC-CD) tumor cells suggesting the ability of this tumor cell line to co-stimulate T cells through expression of a co-stimulatory ligand. To address this possibility, we conducted further FACS analyses of OV-CAR3(MUC-CD) tumor cells demonstrating expression of the co-stimulatory 4-1BBL ligand (Figure 13F), but not the B7.1, B7.2, or OX-40L co-stimulatory ligands (data not shown).

[0192] *In vivo anti-tumor activity of MUC-CD targeted T cells in SCID-Beige mice.*

[0193] To assess the *in vivo* anti-tumor activity of 4H11z$^+$ and 4H11-28z$^+$ T cells, we next generated an orthotopic xenotransplant ovarian cancer tumor model by ip injection of OV-CAR3(MUC-CD) tumor cells into SCID-Beige mice. If left untreated, these mice developed marked ascites and multiple nodular peritoneal tumors by 3 weeks following tumor cell injection (Figure 14A). All untreated tumor bearing mice had to be euthanized by 7 weeks following tumor cell injection due to evidence of distress.

[0194] To assess the *in vivo* anti-tumor efficacy of MUC-CD-targeted T cells, SCID-Beige mice were injected ip with OV-CAR3(MUC-CD/GFP-FFLuc) tumor cells on day 1 followed by ip injection of 4H11z$^+$ or 4H11-28z$^+$ T cells on day 2. For negative controls, tumor bearing mice were either untreated or treated with T cells modified to express the irrelevant CD19-targeted CAR. Collectively, we found that 27% of all mice treated with MUC-CD targeted T cells (3/11 mice) remained alive without clinical evidence of disease 120 days out from tumor injection with no statistically significant difference in survival when comparing the 4H11z$^+$ and 4H11-28z$^+$ T cell treated cohorts (Figure 14B). In contrast, both MUC-CD-targeted T cell treated cohorts demonstrated statistically significant enhanced survival when compared to untreated and 19z1$^+$ T cell treated control cohorts.

[0195] To assess whether systemically infused MUC-CD-targeted T cells successfully traffic to ip tumors, we next compared ip to iv infusion of 4H11-28z$^+$ T cells in SCID-Beige mice bearing ip OV-CAR3(MUC-CD/GFP-FFLuc) tumors. Both ip and iv 4H11-28z$^+$ T cell treated mice exhibited statistically enhanced survival when compared to untreated or 19-28z$^+$ T cell treated control cohorts as assessed by overall survival (Figure 15A) as well as by BLI of tumor progression (Figure 15B). Furthermore, we found overall survival between the ip and iv treated groups to be statistically equivalent by log rank analysis. These data imply successful trafficking of iv infused 4H11-28z$^+$ T cells to peritoneal tumors. We further confirmed trafficking of iv infused CFSE labeled 4H11-28z T cells to the peritoneum by FACS analysis of single cell suspensions of macerated OV-CAR3(MUC-CD) tumors (Figure 15C).

[0196] *In vivo anti-tumor activity of MUC-CD targeted T cells in SCID-Beige mice bearing well established OV-CAR3(MUC-CDIGFP-FFLuc) tumors.*

[0197] To further assess whether 4H11-28z$^+$ T cells were able to eradicate more clinically relevant tumor burdens, we next treated SCID-Beige mice bearing well established ip OV-CAR3(MUC-CD/GFP-FFLuc) tumor injected 7 days prior to adoptive T cell therapy. Once more, we found that therapy with MUC-CD targeted T cells markedly eradicated BLI evident disease in all treated mice (Figure 16A) with 5 of 8 treated mice eventually developing relapsed progressive disease, and 3 mice remaining disease free as assessed by BLI imaging (not shown) out to 120 days post-tumor cell infusion (Figure 16B). These data demonstrate potent *in vivo* anti-tumor activity mediated by MUC-CD targeted T cells even in the setting of advanced disease.

DISCUSSION

[0198] Based on extensive analyses of patient tumor samples, ovarian carcinomas appear to be relatively immunogenic tumors. Specifically, researchers have found there to be a direct correlation between prognosis following surgery and chemotherapy and the quantity of tumor infiltrating effector T cells (TILs) in pretreatment tumor samples (25, 49, 50). Furthermore, others have described an inverse correlation between prognosis following therapy and pre-treatment levels of Tregs within the tumor, which in turn presumably inhibit the anti-tumor function of tumor specific effector TILs (26, 28, 51). Both of these findings imply a role for an endogenous effector T cell response to tumor in controlling disease progression both prior to and following initial therapy and strongly support the contention that ovarian carcinomas may be susceptible to killing by adoptive infusion of autologous T cells targeted to ovarian tumor cell antigens.

[0199] While endogenous effector TILs are one source for presumably tumor specific T cells, an alternative approach to adoptive T cell therapy is to isolate autologous peripheral blood T cells which in turn may be genetically modified *ex vivo* to target tumor cell antigens. One such genetic approach is to retrovirally transduce patient T cells with CARs targeted to surface exposed antigens either unique to or over-expressed by the tumor. To this end, promising preclinical studies utilizing this approach in other malignancies have recently been translated into the clinical setting (6, 16, 19, 52). Similarly, we have previously generated CARs targeted to the CD19 antigen expressed on normal B cells as well as most B cell malignancies and are currently conducting clinical trials treating patients with relapsed B cell chronic lymphocytic leukemia and acute lymphoblastic leukemias with autologous T cell modified to express a CD19 specific CAR (53).

[0200] Application of this approach to ovarian carcinomas requires the identification to suitable target antigens expressed on the tumor cell surface. Significantly, other investigators have studied this approach in both the pre-clinical and clinical setting (4, 11, 54-57). Specifically, several groups have demonstrated significant anti-tumor responses to subcutaneous human ovarian carcinoma cell line tumors in immune compromised mice following intratumoral and/or intravenous infusion of T cells expressing CARs specific to the mesothelin and Lewis-Y antigens overexpressed on these tumor cell lines (56, 58, 59). Furthermore, Kershaw et al recently published the results of a phase I clinical trial treating patients with relapsed ovarian carcinomas with autologous T cells modified to express a CAR specific to the alpha-folate receptor (6). The authors of this study found that therapy with targeted T cells was well tolerated, but noted a lack of anti-tumor response in these studies related to poor persistence of modified T cells over time as well as a yet undefined T cell inhibitory factor in the serum of several treated patients.

[0201] In our studies, we have chosen to target the MUC-16 glycoprotein which is over-expressed on a majority of ovarian carcinomas (1, 30, 32, 33). The utility of MUC-16 as a target antigen for adoptive T cell therapy is compromised by the fact that most of the extracellular portion of this molecule is cleaved by the tumor cell, secreted, and may be detected in the serum as the CA-125 tumor marker. However, following cleavage of this secreted fraction of MUC-16, there remains a residual extracellular fraction of the glycoprotein, termed MUC-CD, which is retained on the tumor surface and is therefore an attractive target for immune-based therapies. To this end, we utilized a series of murine hybridomas generated to the MUC-CD antigen to construct CARs specific to MUC-CD. Of these CARs, we identified a CAR generated from the 4H11 murine hybridoma termed 4H11z, which, when expressed in human T cells, following co-culture on 3T3(MUC-CD/B7.1) AAPCs, resulted in rapid destruction of AAPC monolayers as well as marked modified T cell expansion. Significantly, the antigen to the 4H11 antibody is highly expressed on a majority of pre-treatment ovarian carcinoma surgical tumor samples obtained from patients treated at our institution as assessed by immunohistochemistry (48).

[0202] Optimal T cell activation requires both a primary T cell receptor mediated signal, "signal 1," along with a co-stimulatory "signal 2." Classically, this co-stimulatory signal may be provided by ligation of either B7.1 (CD80) or B7.2 (CD86) on the target cell with the T cell co-stimulatory receptor CD28. Alternatively, co-stimulation may be generated by ligation of 4-1BBL or OX-40L on the target cell with the respective 4-1BB or OX40 co-stimulatory receptors on the T cell (12, 60, 61). Since most tumor cells fail to express co-stimulatory ligands, we and others have previously demonstrated that second generation CARs further incorporating the cytoplasmic signaling domains the co-stimulatory receptors CD28, 4-1BB, and/or OX40 resulted in CARs capable of providing both signal 1 and signal 2 to the T cell upon binding to cognate antigen in the absence of exogenous co-stimulatory ligands (7-10, 12, 13, 15, 16, 62-65). To this end, we constructed a second generation CAR from the 4H11z CAR incorporating the transmembrane and cytoplasmic signaling domain of CD28 as described elsewhere (3, 9, 43). Consistent with previous studies, we found that T cells transduced to express the resulting 4H11-28z CAR, but not the first generation 4H11z CAR, efficiently expanded upon co-culture with 3T3(MUC-CD) fibroblasts in the absence of exogenous co-stimulation consistent with the ability of the 4H11-28z CAR to deliver both signal 1 and signal 2 to the T cell. This conclusion is further supported by the finding that 4H11-28z$^+$ T cells secreted significantly higher levels of IL-2, a cytokine indicative of T cell co-stimulation, upon co-culture on 3T3(MUC-CD) fibroblasts when compared to T cells transduced to express the first generation 4H11z CAR.

[0203] We next assessed the ability of 4H11z$^+$ and 4H11-28z$^+$ T cells to target and lyse human ovarian carcinoma tumor cells. To this end, we initially utilized the OV-CAR3 human ovarian cancer cell line. Since the OV-CAR3 tumor

cell line binds the 4H11 antibody weakly, we further genetically modified the cell line to express MUC-CD (OV-CAR3 (MUC-CD)) to better mimic the clinical setting wherein a majority of clinical ovarian carcinoma tumor specimens highly express the 4H11 MUC-CD antigen (48). We demonstrated that human T cells modified to express either 4H11z or 4H11-28z eradicated OV-CAR3(MUC-CD) tumor cells *in vitro,* and surprisingly observed that both 4H11z$^+$ and 4H11-28z T cells expanded following co-culture with tumor *in vitro.* To define the etiology of this unanticipated 4H11z$^+$ T cell expansion, we further assessed whether OV-CAR3(MUC-CD) tumor cells expressed co-stimulatory ligands, and found that this tumor cell line expressed 4-1BBL, consistent with our experimental findings as well as with previously published reports demonstrating 4-1BBL expression by a variety of carcinoma cell lines (66-68). In order to further validate the clinical relevance of these findings, we subsequently demonstrated specific *in vitro* lysis of primary ascites-derived tumor cells isolated from untreated ovarian carcinoma patients by both healthy donor allogeneic 4H11-28z$^+$ T cells as well as more significantly autologous 4H11-28z$^+$patient peripheral blood T cells. These data strongly support the contention that treatment with autologous 4H11-based CAR$^+$ T cells have promise in future clinical applications.

[0204] In order to assess the *in vivo* relevance of our *in vitro* findings, we next generated a murine orthotopic OV-CAR3(MUC-CD) tumor model in SCID-Beige mice. We injected mice i.p. with OV-CAR3(MUC-CD) tumor cells and the following day infused 4H11z$^+$, 4H11-28z$^+$, and control 19z1$^+$ T cells i.p. This treatment approach resulted in a significant but similar delay to tumor progression and long-term survival in both the 4H11z$^+$ and 4H11-28z$^+$ T cell treated cohorts when compared to untreated mice or mice treated with control T cells targeted to the irrelevant CD19 antigen. We next compared ip to iv treatment with 4H11-28z$^+$ T cells of orthotopic OV-CAR3(MUC-CD/GFP-FFLuc) bearing mice, and found similar statistically significant survivals of mice over time with either direct ip infusion of T cells or systemic iv infusion of targeted T cells. Significantly, iv treated mice by day 1 following treatment, exhibited successful trafficking of targeted T cells to the peritoneum. These data suggests that adoptive therapy with targeted T cells may be equally efficacious following either a direct infusion into the peritoneum or through systemic iv infusion. These findings further support the future clinical potential of this approach in treating patients both with local relapse of disease as well as metastatic relapse to sites outside of the peritoneum.

[0205] Finally, we assessed the ability of 4H11-28z$^+$ T cells to eradicate more established disease by delaying modified T cell ip infusion by 7 days, when mice had greater established tumor burdens as assessed by bioluminescent imaging. This experimental setting better reflects the initial clinical setting wherein this adoptive T cell approach would be utilized. Significantly, despite the setting of markedly established disease, 4H11-28z$^+$ T cells retained the ability to lyse larger tumor burdens, delay relapse of tumor, and in a significant percentage of mice, fully eradicate disease.

[0206] In the studies presented here, we have consistently utilized mixed populations of CD4$^+$ and CDS$^+$ CAR$^+$ T cells to assess both *in vitro* and *in vivo* anti-tumor activity. To this end, ongoing studies will address the role of isolated CD4$^+$ and CD8$^+$ CAR$^+$ T cell subsets in the successful eradication of disease in this SCID-Beige OV-CAR3(MUC-CD) tumor model. The results of these studies may have implications to translating this therapeutic approach to the clinical setting. Furthermore, we acknowledge the limitations associated with the presented SCID-Beige tumor model. Namely, this is a xenotransplant model in an immune compromised mouse. To this end, ongoing studies in or laboratory are focused on generating a more clinically relevant syngeneic immune competent tumor model to better define the biology and anti-tumor efficacy of MUC-CD targeted CAR-modified T cells in the context of an intact immune system.

[0207] In conclusion, herein we present the first published data demonstrating the feasibility of targeting MUC-16, an antigen over-expressed on a majority of ovarian carcinomas, through adoptive therapy with genetically modified T cells targeted to the retained MUC-CD portion of the MUC-16 antigen. Further, this report is the first to demonstrate efficient targeting of T cells in an orthotopic, clinically relevant, murine model of ovarian cancer, demonstrating efficacy both by ip and iv infusion of modified T cells. Finally, these data support the further translation of this approach to the clinical setting in the form of a phase I clinical trial in patients with persistent or relapsed ovarian carcinomas following initial therapy with surgery and chemotherapy. [jfl]

EXAMPLE 5

Raising Mouse MUC16 monoclonal antibodies in mice and hamsters.

[0208] We selected 3 different regions of mouse MUC16 genome for which monoclonal antibodies were generated in mouse and hamster. The selected regions of the mouse MUC16 are Peptide 1 (SEQ ID NO:21, ecto region of cytoplasmic domain), Peptide 2 (SEQ ID NO:22, first cysteine loop) and Peptide 3 (SEQ ID NO:23, second cysteine loop) (Figure 20A) and its comparison with human MUC16 is shown in Figure 20B. A cysteine was added to the peptide sequence at the N terminus of Peptide 1 (SEQ ID NO:21) and Peptide 3 (SEQ ID NO:23) for better conjugation with KLH. Individual peptides were conjugated to KLH using Promega kit. These 3 conjugated peptides were pooled and immunized into 5 mice and 4 hamsters. 5 immunizations with a 3 week interval for each immunization were administered. Sera from these animals were tested by ELISA for their specific reactivity with individual peptides (SEQ ID NO:21, 22 and 23). Positive selected animals were allowed to rest for a month and then i.v. boosted with pooled peptides immunogen (SEQ ID

NO:21, 22 and 23) and harvested the spleens after 4 days. Splenocytes were mixed with hybridoma partners and plated into microtiter plates at various clonal densities. Plates were cultured at 37°C 5% CO^2 for 10 days and then selected the clones. Supernatants from these selected clones were tested by ELISA for their specific reactivity with individual peptides (SEQ ID NO:21, 22 and 23). Positive clonal sups were tested by FACS, western blot and imaging using 2 mouse cell lines (ID8 and BR5-FVB1) and a human cell line (OVCAR-3).

[0209] **Table 4** shows the summary of mouse and hamster monoclonal antibodies against mouse MUC16 peptide antigens Peptide 1 (SEQ ID NO:21), Peptide 2 (SEQ ID NO:22), and Peptide 3 (SEQ ID NO:23). A very strong antigenic response was seen with Peptide 1 (SEQ ID NO:21).

Table 4

Mouse MUC16		Mouse mAbs	Frozen Mouse mAb	
peptide 1		46	16 (3-IgG1; 8-IgG2b; 1-IgM; 4-Unkown isotype)	Animals not iv boosted with peptide 2
Peptide 2		0	0	
peptide 3		6	6 (4-IgG1; 2-IgM)	
Peptide 1,2,3		0	0	
Peptide 1,2		0	0	
peptide 2,3		0	0	
No Peptide		0	0	
Mouse MUC16		Hamster mAbs	Frozen Hamster mAb	
peptide 1		69	21	
Peptide 2		6	6	
peptide 3		7	7	
peptide 1,2,3		2	1	
Peptide 1,2		1	1	
Peptide 2,3		1	0	
No Peptide		10	2	

[0210] Details of mouse and hamster mAbs against Peptide 1 (SEQ ID NO:21), Peptide 2 (SEQ ID NO:22), and Peptide 3 (SEQ ID NO:23 are listed in Table 5 and Table 6 respectively.

Table 5

isotype	PEPTIDE	Fusion Well	Cloned	Clones			
-	1	**01D01**					
-	1	**09F07**					
IgG 1	1	**16A09**	**no success**				
-	1	**21A07**					
-	1	**24G10**					
IgG 1	1	**10C04**	**yes**	**10C4-3H5**	10C4-1F2	10C4-2H8	10C4-1G7
IgG 1	1	**17F02**	**yes**	**17F2-3G5**	17F2-3F6	17F2-2F9	17F2-1E11
IgG 2b	1	**01A08**					
IgG 2b	1	**01F08**					
IgG 2b	1	**12B10**	**yes**	12B10-3F7	**12B10-3G10**	12B10-2F6	12B10-2F10
IgG 2b	1	**17H10**					
IgG 2b	1	**18D05**					
IgG 2b	1	**23B12**					
IgG 2b	1	**25E09**		25E9-3	**25E9-5**	25E9-13	25E9-16
IgM	1	**16F12**					
IgG 1	3	**04A06**	**no success**				
IgG 1	3	**05D01**	**no success**				
IgG 1	3	**21B08**	**yes**	**21B8-1H11**	21B8-3G6	21B8-3H9	21B8-1G8
IgG 1	3	**21E01**	**yes**	21E1-1E3	21E1-1G9	21E1-2G7	21E1-3G12
IgM	3	**08A02**					
IgM	3	**13E05**					

Table 6:

Hamster mAb	Peptide	Cloned			
01H03					
02F02	1				
04E 4					
04G07	1				
04H01	3	4H1-2E1	4H1-2E3	4H1-3E1	4H1-3H3
06A08	1				
06F02	1				
07F08	3				
07H05	2				
09A05					
09E 1	3				
09F08	1				
09H10					
10G06	1				
10H11	1				
11B10	1				
12F09	2				
15A08	1	15A8-2E2	15A8-2E10	15A8-2E11	15A8-3D2
15H08	3				
19B05	1				
21H04	3				
22B05	2	22B5-1F6	22B5-3G9	22B5-2G8	22B5-3F11
22D11	3				
23G12	1				
25E 8	1				
27H09	3				
28B12	1&2&3				
28C12	2				
30H02	1				
31A11	2				
31C01	2				
33H06	1&2				
34F10	1				
34H05	1				
36C01	1				
36C11					
36E 4	1				
37E 10	1				
10H11	1				

[0211] Hamster antibody 22B05 recognizes mouse (SEQ ID NO:22) and also the corresponding human sequence (SEQ ID NO:15).

[0212] Western blot analysis using mouse IDS and BR5-FVB1 cell extracts were also performed for all the selected monoclonal antibodies as shown in Figure 21 and Figure 22 respectively.

[0213] Among the mouse MUC16 monoclonal antibodies, we selected 12B10-3G10 subclone mouse mAb for further screening. Similarly, hamster monoclonal antibodies, 15A8-2E10, 22B5-2G8 and 4H1-2E1 subclones were selected for further screening.

[0214] Immunohistochemical analysis was performed with paraffin and cryosections of ID8 (mouse), OVCAR-3 (human), BR5-FVB1 (mouse) cell lines and 13.5 days of Embryo. Paraffin or cryosections were probed with mouse 12B10 mAb, hamster 15A8, hamster 22B5 and hamster 4E1 mAbs to see the early development of mouse MUC16 (Figure 23)

[0215] 12B10-3G10 sub clone were further analyzed for single chain Fv fragments. Figure 24 shows 12B10-3G10 V_H and V_L DNA and Amino Acids sequences. Bioreactive supernatants and purified 12B10-3G10 were generated for animal studies and other characterization studies. FACS analysis was performed with purified 12B10-3G10 on ID8, OVCAR3 and BR5-FVB1 cells showing over 90% positivity to both mouse and human MUC16 ecto-domain fragment (Figure 25).

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[0216]

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[0217]

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[0218] Each and every publication and patent mentioned in the above specification is herein incorporated by reference in its entirety for all purposes. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, the invention as claimed should not be unduly

limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art and in fields related thereto are intended to be within the scope of the following claims.

[0219] The present invention will now be further defined by way of the following numbered clauses:

1. An isolated antibody, or an antigen-binding fragment thereof, that specifically binds to a MUC16 polypeptide or to an antigenic portion thereof, wherein the MUC16 polypeptide is selected from the group consisting of

a) TLDRKSVFVDGYSQNRDD (SEQ ID NO:21),

b) KSYFSDCQVLAFRSVSNNNNHTGVDSLCNFSPL (SEQ ID NO:22), and

c) SLYSNCRLASLRPKKNGTATGVNAICSYHQN (SEQ ID NO:23).

2. The antibody of Clause 1, wherein the antibody is selected from the group consisting of a monoclonal antibody, a chimeric antibody, a recombinant antibody, an antigen-binding fragment of a recombinant antibody, a humanized antibody, and an antibody displayed upon the surface of a phage.

3. The antibody of Clause 2, wherein the antibody is a monoclonal antibody.

4. The antibody of Clause 3, wherein the monoclonal antibody is prodticed by hybridoma cells selected from the group consisting of 12B10-3G10, 10C4-3H5, 10C4-1F2, 10C4-2H8, 10C4-1G7, 17F2-3G5, 17F2-3F6, 17F2-2F9, 17F2-1E11, 12B10-3F7, 12B10-2F6, 12B10-2F10, 25E9-3, 25E9-5, 25E9-1, 25E9-16, 21B8-1H11, 21B8-3G6, 21B8-3H9, 21B8-1G8, 21E1-1E3, 21E1-1G9, 21E1-2G7, 21E1-3G12, 4H1-2E1, 4H1-2E3, 4H1-3E1, 4H1-3H3, 15A8-2E2, 15A8-2E10, 15A8-2E11, 15A8-3D2, 22B5-1 F6, 22B5-3G9, 22B5-2G8, and 22B5-3F11.

5. The antibody of Clause 1, wherein the MUC16 polypeptide is TLDRKSVFVDGYSQNRDD (SEQ ID NO-.21).

6. The antibody of Clause 5, wherein the antibody comprises a variable heavy (VH) chain sequence SEQ ID NO:27, and a variable light (V_L) chain sequence SEQ ID NO:29.

7. The antibody of Clause 6, wherein the antibody is a monoclonal antibody produced by hybridoma cell 12B10-3G10.

8. The antibody of Clause 1, wherein the antigen-binding fragment is selected from the group consisting of a Fab fragment, a F(ab')2 fragment, and a Fv fragment.

9. The antibody of Clause 1, wherein the antibody, or antigen-binding fragment thereof, is covalently linked to a cytotoxic agent or a prodrug of a cytotoxic agent.

10. The antibody of Clause 1, wherein the antibody specifically binds to human MUC16 (SEQ ID NO:25).

11. The antibody of Clause 1, wherein the antibody internalizes into a cell.

12. The antibody of Clause 1, wherein the antibody lacks specific binding to a glycosylated MUC16 extracellular domain.

13. A composition comprising (a) the antibody, or antigen-binding fragment thereof, of Clause 1, and (b) a pharmaceutically acceptable carrier.

14. A hybridoma cell that produces an antibody, or an antigen-binding fragment thereof, that specifically binds to a MUC16 polypeptide or to an antigenic portion thereof, wherein the MUC16 polypeptide is selected from the group consisting of

a) TLDRKSVFVDGYSQNRDD (SEQ ID NO:21),

b) KSYFSDCQVLAFRSVSNNNNHTGVDSLCNFSPL (SEQ ID NO:22), and

c) SLYSNCRLASLRPKKNGTATGVNAICSYHQN (SEQ TD NO:23).

15. An isolated nucleotide sequence comprising a polynucleotide that encodes at least one of a variable heavy (VH) chain sequence and the variable light (VL) chain sequence of an antibody that specifically binds to a MUC16 polypeptide, wherein the MUC16 polypeptide is selected from the group consisting of

a) TLDRKSVFVDGYSQNRDD (SEQ ID NO:21),

b) KSYFSDCQVLAFRSVSNNNNHTGVDSLCNFSPL (SEQ ID NO:22), and

c) SLYSNCRLASLRPKKNGTATGVNAICSYHQN (SEQ ID NO:23).

16. The nucleotide sequence of Clause 15, wherein the MUC16 polypeptide is TLDRKSVFVDGYSQNRDD (SEQ ID NO:21).

17. The nucleotide sequence of Clause 16, wherein the polynucleotide encoding the variable heavy (VH) chain sequence comprises SEQ ID NO:26, and wherein the polynucleotide encoding the variable light (VL) chain sequence comprises SEQ ΓÛ NO:28.

18. A method for producing an antibody that specifically binds to a MUC16 polypeptide or to an antigenic portion thereof, comprising administering to a subject an immunologically effective amount of a MUC16 polypeptide selected from the group consisting of

a) TLDRKS VFVDGYS QNRDD (SEQ ID NO:21),

b) KSYFSDCQVLAFRSVSNN NHTGVDSLCNFSPL (SEQ ID NO:22), and

c) SLYSNCRLASLRPKKNGTATGVNAICSYHQN (SEQ ID NO:23).

19. A method for identifying a subject as having disease, comprising determining the level, in a sample from the subject, of specific binding of the antibody of Clause 1 with the MUC16 polypeptide or with the antigenic portion thereof, wherein detecting an altered level of the specific binding relative to a control sample identifies the subject as having disease.

20. The method of Clause 19, wherein the disease is cancer.

21. The method of Clause 20, wherein the cancer is selected from the group consisting of ovarian cancer and breast cancer.

22. The method of Clause 19, further comprising detecting an altered level of binding of the antibody to the sample compared to a control sample.

23. The method of Clause 19, wherein the detecting is selected from the group consisting of immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), fluorescence-activated cell sorting (FACS), Western blot, immunoprecipitation, and radiographic imaging.

24. A method for reducing one or more symptoms of disease comprising administering to a subject in need thereof a therapeutically effective amount of the antibody of Clause 1.

25. The method of Clause 24, wherein the disease is cancer.

26. The method of Clause 25, wherein the cancer is selected from the group consisting of ovarian cancer and breast cancer.

27. The method of Clause 24, further comprising detecting a reduction in one or more symptoms of the disease after the administration.

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Thapi, Dharmarao

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acgttcggtg gagggaccaa gctggagatc aaacgg 216

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tcctgcaagg cttctggtta ctcatttact ggctacttta tgaactgggt gaagcagacc 120

catggaaaga gccttgagtg gattggacgt attaatcctt acaatggtgc tactttctac 180

aatcagaagt tcacgggcaa ggccacaatg actgtagaca aatcctctac cacagcccac 240

atggagctcc tgagcctgac atctgaggac tctgcagtct attattgtgg aaaggggaat 300

tactacggcc cctttgatta ctggggccaa gggaccacgg tcaccgtctc ctca 354

<210> 12
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<213> Artificial Sequence

<220>
<223> Synthetic

<400> 12
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attaattgca gggcaagtaa gagcattagc aaatatttag cctggtatca aaagaaacct 120

gggaaaacta ataagcttct tatctactct ggatccactt tgcaatctgg aattccatca 180

aggttcagtg gcagtggatc tggtacagat ttcactctca ccatcagtag cctggagcct 240

gaagattttg caatgtatta ctgtcaacag cataatgaat acccgtggac gttcggtgga 300

gggaccaagc tggagatcaa acgggcggcc gca 333

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<212> PRT
<213> Homo sapiens

<400> 13

Met Leu Lys Pro Ser Gly Leu Pro Gly Ser Ser Ser Pro Thr Arg Ser
1 5 10 15

Leu Met Thr Gly Ser Arg Ser Thr Lys Ala Thr Pro Glu Met Asp Ser
20 25 30

Gly Leu Thr Gly Ala Thr Leu Ser Pro Lys Thr Ser Thr Gly Ala Ile
35 40 45

Val Val Thr Glu His Thr Leu Pro Phe Thr Ser Pro Asp Lys Thr Leu
50 55 60

Ala Ser Pro Thr Ser Ser Val Val Gly Arg Thr Thr Gln Ser Leu Gly
65 70 75 80

Val Met Ser Ser Ala Leu Pro Glu Ser Thr Ser Arg Gly Met Thr His
85 90 95

Ser Glu Gln Arg Thr Ser Pro Ser Leu Ser Pro Gln Val Asn Gly Thr
100 105 110

Pro Ser Arg Asn Tyr Pro Ala Thr Ser Met Val Ser Gly Leu Ser Ser
115 120 125

Pro Arg Thr Arg Thr Ser Ser Thr Glu Gly Asn Phe Thr Lys Glu Ala

```
    130                 135                 140

Ser Thr Tyr Thr Leu Thr Val Glu Thr Thr Ser Gly Pro Val Thr Glu
145                 150                 155                 160

Lys Tyr Thr Val Pro Thr Glu Thr Ser Thr Thr Glu Gly Asp Ser Thr
                165                 170                 175

Glu Thr Pro Trp Asp Thr Arg Tyr Ile Pro Val Lys Ile Thr Ser Pro
            180                 185                 190

Met Lys Thr Phe Ala Asp Ser Thr Ala Ser Lys Glu Asn Ala Pro Val
        195                 200                 205

Ser Met Thr Pro Ala Glu Thr Thr Val Thr Asp Ser His Thr Pro Gly
    210                 215                 220

Arg Thr Asn Pro Ser Phe Gly Thr Leu Tyr Ser Ser Phe Leu Asp Leu
225                 230                 235                 240

Ser Pro Lys Gly Thr Pro Asn Ser Arg Gly Glu Thr Ser Leu Glu Leu
                245                 250                 255

Ile Leu Ser Thr Thr Gly Tyr Pro Phe Ser Ser Pro Glu Pro Gly Ser
            260                 265                 270

Ala Gly His Ser Arg Ile Ser Thr Ser Ala Pro Leu Ser Ser Ser Ala
        275                 280                 285

Ser Val Leu Asp Asn Lys Ile Ser Glu Thr Ser Ile Phe Ser Gly Gln
    290                 295                 300

Ser Leu Thr Ser Pro Leu Ser Pro Gly Val Pro Glu Ala Arg Ala Ser
305                 310                 315                 320

Thr Met Pro Asn Ser Ala Ile Pro Phe Ser Met Thr Leu Ser Asn Ala
                325                 330                 335

Glu Thr Ser Ala Glu Arg Val Arg Ser Thr Ile Ser Ser Leu Gly Thr
            340                 345                 350

Pro Ser Ile Ser Thr Lys Gln Thr Ala Glu Thr Ile Leu Thr Phe His
        355                 360                 365

Ala Phe Ala Glu Thr Met Asp Ile Pro Ser Thr His Ile Ala Lys Thr
    370                 375                 380
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Leu Ala Ser Glu Trp Leu Gly Ser Pro Gly Thr Leu Gly Gly Thr Ser
385                 390                 395                 400

Thr Ser Ala Leu Thr Thr Thr Ser Pro Ser Thr Thr Leu Val Ser Glu
                405                 410                 415

Glu Thr Asn Thr His His Ser Thr Ser Gly Lys Glu Thr Glu Gly Thr
            420                 425                 430

Leu Asn Thr Ser Met Thr Pro Leu Glu Thr Ser Ala Pro Gly Glu Glu
        435                 440                 445

Ser Glu Met Thr Ala Thr Leu Val Pro Thr Leu Gly Phe Thr Thr Leu
    450                 455                 460

Asp Ser Lys Ile Arg Ser Pro Ser Gln Val Ser Ser Ser His Pro Thr
465                 470                 475                 480

Arg Glu Leu Arg Thr Thr Gly Ser Thr Ser Gly Arg Gln Ser Ser Ser
                485                 490                 495

Thr Ala Ala His Gly Ser Ser Asp Ile Leu Arg Ala Thr Thr Ser Ser
            500                 505                 510

Thr Ser Lys Ala Ser Ser Trp Thr Ser Glu Ser Thr Ala Gln Gln Phe
        515                 520                 525

Ser Glu Pro Gln His Thr Gln Trp Val Glu Thr Ser Pro Ser Met Lys
    530                 535                 540

Thr Glu Arg Pro Pro Ala Ser Thr Ser Val Ala Ala Pro Ile Thr Thr
545                 550                 555                 560

Ser Val Pro Ser Val Val Ser Gly Phe Thr Thr Leu Lys Thr Ser Ser
                565                 570                 575

Thr Lys Gly Ile Trp Leu Glu Glu Thr Ser Ala Asp Thr Leu Ile Gly
            580                 585                 590

Glu Ser Thr Ala Gly Pro Thr Thr His Gln Phe Ala Val Pro Thr Gly
        595                 600                 605

Ile Ser Met Thr Gly Gly Ser Ser Thr Arg Gly Ser Gln Gly Thr Thr
    610                 615                 620

His Leu Leu Thr Arg Ala Thr Ala Ser Ser Glu Thr Ser Ala Asp Leu
625                 630                 635                 640
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Thr Leu Ala Thr Asn Gly Val Pro Val Ser Val Ser Pro Ala Val Ser
                645                 650                 655

Lys Thr Ala Ala Gly Ser Ser Pro Pro Gly Gly Thr Lys Pro Ser Tyr
            660                 665                 670

Thr Met Val Ser Ser Val Ile Pro Glu Thr Ser Ser Leu Gln Ser Ser
        675                 680                 685

Ala Phe Arg Glu Gly Thr Ser Leu Gly Leu Thr Pro Leu Asn Thr Arg
    690                 695                 700

His Pro Phe Ser Ser Pro Glu Pro Asp Ser Ala Gly His Thr Lys Ile
705                 710                 715                 720

Ser Thr Ser Ile Pro Leu Leu Ser Ser Ala Ser Val Leu Glu Asp Lys
                725                 730                 735

Val Ser Ala Thr Ser Thr Phe Ser His His Lys Ala Thr Ser Ser Ile
            740                 745                 750

Thr Thr Gly Thr Pro Glu Ile Ser Thr Lys Thr Lys Pro Ser Ser Ala
        755                 760                 765

Val Leu Ser Ser Met Thr Leu Ser Asn Ala Ala Thr Ser Pro Glu Arg
    770                 775                 780

Val Arg Asn Ala Thr Ser Pro Leu Thr His Pro Ser Pro Ser Gly Glu
785                 790                 795                 800

Glu Thr Ala Gly Ser Val Leu Thr Leu Ser Thr Ser Ala Glu Thr Thr
                805                 810                 815

Asp Ser Pro Asn Ile His Pro Thr Gly Thr Leu Thr Ser Glu Ser Ser
            820                 825                 830

Glu Ser Pro Ser Thr Leu Ser Leu Pro Ser Val Ser Gly Val Lys Thr
        835                 840                 845

Thr Phe Ser Ser Ser Thr Pro Ser Thr His Leu Phe Thr Ser Gly Glu
    850                 855                 860

Glu Thr Glu Glu Thr Ser Asn Pro Ser Val Ser Gln Pro Glu Thr Ser
865                 870                 875                 880

Val Ser Arg Val Arg Thr Thr Leu Ala Ser Thr Ser Val Pro Thr Pro
                885                 890                 895
```

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Val Phe Pro Thr Met Asp Thr Trp Pro Thr Arg Ser Ala Gln Phe Ser
            900                 905                 910

Ser Ser His Leu Val Ser Glu Leu Arg Ala Thr Ser Ser Thr Ser Val
        915                 920                 925

Thr Asn Ser Thr Gly Ser Ala Leu Pro Lys Ile Ser His Leu Thr Gly
    930                 935                 940

Thr Ala Thr Met Ser Gln Thr Asn Arg Asp Thr Phe Asn Asp Ser Ala
945                 950                 955                 960

Ala Pro Gln Ser Thr Thr Trp Pro Glu Thr Ser Pro Arg Phe Lys Thr
                965                 970                 975

Gly Leu Pro Ser Ala Thr Thr Thr Val Ser Thr Ser Ala Thr Ser Leu
            980                 985                 990

Ser Ala Thr Val Met Val Ser Lys  Phe Thr Ser Pro Ala  Thr Ser Ser
        995                 1000                1005

Met Glu  Ala Thr Ser Ile Arg  Glu Pro Ser Thr Thr  Ile Leu Thr
    1010                1015                1020

Thr Glu  Thr Thr Asn Gly Pro  Gly Ser Met Ala Val  Ala Ser Thr
    1025                1030                1035

Asn Ile  Pro Ile Gly Lys Gly  Tyr Ile Thr Glu Gly  Arg Leu Asp
    1040                1045                1050

Thr Ser  His Leu Pro Ile Gly  Thr Thr Ala Ser Ser  Glu Thr Ser
    1055                1060                1065

Met Asp  Phe Thr Met Ala Lys  Glu Ser Val Ser Met  Ser Val Ser
    1070                1075                1080

Pro Ser  Gln Ser Met Asp Ala  Ala Gly Ser Ser Thr  Pro Gly Arg
    1085                1090                1095

Thr Ser  Gln Phe Val Asp Thr  Phe Ser Asp Asp Val  Tyr His Leu
    1100                1105                1110

Thr Ser  Arg Glu Ile Thr Ile  Pro Arg Asp Gly Thr  Ser Ser Ala
    1115                1120                1125

Leu Thr  Pro Gln Met Thr Ala  Thr His Pro Pro Ser  Pro Asp Pro
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    1130                1135                1140

Gly Ser  Ala Arg Ser Thr Trp  Leu Gly Ile Leu Ser  Ser Ser Pro
1145                1150                1155

Ser Ser  Pro Thr Pro Lys Val  Thr Met Ser Ser Thr  Phe Ser Thr
1160                1165                1170

Gln Arg  Val Thr Thr Ser Met  Ile Met Asp Thr Val  Glu Thr Ser
1175                1180                1185

Arg Trp  Asn Met Pro Asn Leu  Pro Ser Thr Thr Ser  Leu Thr Pro
1190                1195                1200

Ser Asn  Ile Pro Thr Ser Gly  Ala Ile Gly Lys Ser  Thr Leu Val
1205                1210                1215

Pro Leu  Asp Thr Pro Ser Pro  Ala Thr Ser Leu Glu  Ala Ser Glu
1220                1225                1230

Gly Gly  Leu Pro Thr Leu Ser  Thr Tyr Pro Glu Ser  Thr Asn Thr
1235                1240                1245

Pro Ser  Ile His Leu Gly Ala  His Ala Ser Ser Glu  Ser Pro Ser
1250                1255                1260

Thr Ile  Lys Leu Thr Met Ala  Ser Val Val Lys Pro  Gly Ser Tyr
1265                1270                1275

Thr Pro  Leu Thr Phe Pro Ser  Ile Glu Thr His Ile  His Val Ser
1280                1285                1290

Thr Ala  Arg Met Ala Tyr Ser  Ser Gly Ser Ser Pro  Glu Met Thr
1295                1300                1305

Ala Pro  Gly Glu Thr Asn Thr  Gly Ser Thr Trp Asp  Pro Thr Thr
1310                1315                1320

Tyr Ile  Thr Thr Thr Asp Pro  Lys Asp Thr Ser Ser  Ala Gln Val
1325                1330                1335

Ser Thr  Pro His Ser Val Arg  Thr Leu Arg Thr Thr  Glu Asn His
1340                1345                1350

Pro Lys  Thr Glu Ser Ala Thr  Pro Ala Ala Tyr Ser  Gly Ser Pro
1355                1360                1365
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Lys Ile  Ser Ser Ser Pro Asn  Leu Thr Ser Pro Ala  Thr Lys Ala
    1370                 1375                 1380

Trp Thr  Ile Thr Asp Thr Thr  Glu His Ser Thr Gln  Leu His Tyr
    1385                 1390                 1395

Thr Lys  Leu Ala Glu Lys Ser  Ser Gly Phe Glu Thr  Gln Ser Ala
    1400                 1405                 1410

Pro Gly  Pro Val Ser Val Val  Ile Pro Thr Ser Pro  Thr Ile Gly
    1415                 1420                 1425

Ser Ser  Thr Leu Glu Leu Thr  Ser Asp Val Pro Gly  Glu Pro Leu
    1430                 1435                 1440

Val Leu  Ala Pro Ser Glu Gln  Thr Thr Ile Thr Leu  Pro Met Ala
    1445                 1450                 1455

Thr Trp  Leu Ser Thr Ser Leu  Thr Glu Glu Met Ala  Ser Thr Asp
    1460                 1465                 1470

Leu Asp  Ile Ser Ser Pro Ser  Ser Pro Met Ser Thr  Phe Ala Ile
    1475                 1480                 1485

Phe Pro  Pro Met Ser Thr Pro  Ser His Glu Leu Ser  Lys Ser Glu
    1490                 1495                 1500

Ala Asp  Thr Ser Ala Ile Arg  Asn Thr Asp Ser Thr  Thr Leu Asp
    1505                 1510                 1515

Gln His  Leu Gly Ile Arg Ser  Leu Gly Arg Thr Gly  Asp Leu Thr
    1520                 1525                 1530

Thr Val  Pro Ile Thr Pro Leu  Thr Thr Thr Trp Thr  Ser Val Ile
    1535                 1540                 1545

Glu His  Ser Thr Gln Ala Gln  Asp Thr Leu Ser Ala  Thr Met Ser
    1550                 1555                 1560

Pro Thr  His Val Thr Gln Ser  Leu Lys Asp Gln Thr  Ser Ile Pro
    1565                 1570                 1575

Ala Ser  Ala Ser Pro Ser His  Leu Thr Glu Val Tyr  Pro Glu Leu
    1580                 1585                 1590

Gly Thr  Gln Gly Arg Ser Ser  Ser Glu Ala Thr Thr  Phe Trp Lys
    1595                 1600                 1605
```

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Pro Ser  Thr Asp Thr Leu Ser  Arg Glu Ile Glu Thr  Gly Pro Thr
    1610                 1615                 1620

Asn Ile  Gln Ser Thr Pro Pro  Met Asp Asn Thr Thr  Thr Gly Ser
    1625                 1630                 1635

Ser Ser  Ser Gly Val Thr Leu  Gly Ile Ala His Leu  Pro Ile Gly
    1640                 1645                 1650

Thr Ser  Ser Pro Ala Glu Thr  Ser Thr Asn Met Ala  Leu Glu Arg
    1655                 1660                 1665

Arg Ser  Ser Thr Ala Thr Val  Ser Met Ala Gly Thr  Met Gly Leu
    1670                 1675                 1680

Leu Val  Thr Ser Ala Pro Gly  Arg Ser Ile Ser Gln  Ser Leu Gly
    1685                 1690                 1695

Arg Val  Ser Ser Val Leu Ser  Glu Ser Thr Thr Glu  Gly Val Thr
    1700                 1705                 1710

Asp Ser  Ser Lys Gly Ser Ser  Pro Arg Leu Asn Thr  Gln Gly Asn
    1715                 1720                 1725

Thr Ala  Leu Ser Ser Ser Leu  Glu Pro Ser Tyr Ala  Glu Gly Ser
    1730                 1735                 1740

Gln Met  Ser Thr Ser Ile Pro  Leu Thr Ser Ser Pro  Thr Thr Pro
    1745                 1750                 1755

Asp Val  Glu Phe Ile Gly Gly  Ser Thr Phe Trp Thr  Lys Glu Val
    1760                 1765                 1770

Thr Thr  Val Met Thr Ser Asp  Ile Ser Lys Ser Ser  Ala Arg Thr
    1775                 1780                 1785

Glu Ser  Ser Ser Ala Thr Leu  Met Ser Thr Ala Leu  Gly Ser Thr
    1790                 1795                 1800

Glu Asn  Thr Gly Lys Glu Lys  Leu Arg Thr Ala Ser  Met Asp Leu
    1805                 1810                 1815

Pro Ser  Pro Thr Pro Ser Met  Glu Val Thr Pro Trp  Ile Ser Leu
    1820                 1825                 1830

Thr Leu  Ser Asn Ala Pro Asn  Thr Thr Asp Ser Leu  Asp Leu Ser
    1835                 1840                 1845
```

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His Gly  Val His Thr Ser Ser  Ala Gly Thr Leu Ala  Thr Asp Arg
    1850                 1855                 1860

Ser Leu  Asn Thr Gly Val Thr  Arg Ala Ser Arg Leu  Glu Asn Gly
    1865                 1870                 1875

Ser Asp  Thr Ser Ser Lys Ser  Leu Ser Met Gly Asn  Ser Thr His
    1880                 1885                 1890

Thr Ser  Met Thr Tyr Thr Glu  Lys Ser Glu Val Ser  Ser Ser Ile
    1895                 1900                 1905

His Pro  Arg Pro Glu Thr Ser  Ala Pro Gly Ala Glu  Thr Thr Leu
    1910                 1915                 1920

Thr Ser  Thr Pro Gly Asn Arg  Ala Ile Ser Leu Thr  Leu Pro Phe
    1925                 1930                 1935

Ser Ser  Ile Pro Val Glu Glu  Val Ile Ser Thr Gly  Ile Thr Ser
    1940                 1945                 1950

Gly Pro  Asp Ile Asn Ser Ala  Pro Met Thr His Ser  Pro Ile Thr
    1955                 1960                 1965

Pro Pro  Thr Ile Val Trp Thr  Ser Thr Gly Thr Ile  Glu Gln Ser
    1970                 1975                 1980

Thr Gln  Pro Leu His Ala Val  Ser Ser Glu Lys Val  Ser Val Gln
    1985                 1990                 1995

Thr Gln  Ser Thr Pro Tyr Val  Asn Ser Val Ala Val  Ser Ala Ser
    2000                 2005                 2010

Pro Thr  His Glu Asn Ser Val  Ser Ser Gly Ser Ser  Thr Ser Ser
    2015                 2020                 2025

Pro Tyr  Ser Ser Ala Ser Leu  Glu Ser Leu Asp Ser  Thr Ile Ser
    2030                 2035                 2040

Arg Arg  Asn Ala Ile Thr Ser  Trp Leu Trp Asp Leu  Thr Thr Ser
    2045                 2050                 2055

Leu Pro  Thr Thr Thr Trp Pro  Ser Thr Ser Leu Ser  Glu Ala Leu
    2060                 2065                 2070

Ser Ser  Gly His Ser Gly Val  Ser Asn Pro Ser Ser  Thr Thr Thr
```

2075 2080 2085

Glu Phe Pro Leu Phe Ser Ala Ala Ser Thr Ser Ala Ala Lys Gln
2090 2095 2100

Arg Asn Pro Glu Thr Glu Thr His Gly Pro Gln Asn Thr Ala Ala
2105 2110 2115

Ser Thr Leu Asn Thr Asp Ala Ser Ser Val Thr Gly Leu Ser Glu
2120 2125 2130

Thr Pro Val Gly Ala Ser Ile Ser Ser Glu Val Pro Leu Pro Met
2135 2140 2145

Ala Ile Thr Ser Arg Ser Asp Val Ser Gly Leu Thr Ser Glu Ser
2150 2155 2160

Thr Ala Asn Pro Ser Leu Gly Thr Ala Ser Ser Ala Gly Thr Lys
2165 2170 2175

Leu Thr Arg Thr Ile Ser Leu Pro Thr Ser Glu Ser Leu Val Ser
2180 2185 2190

Phe Arg Met Asn Lys Asp Pro Trp Thr Val Ser Ile Pro Leu Gly
2195 2200 2205

Ser His Pro Thr Thr Asn Thr Glu Thr Ser Ile Pro Val Asn Ser
2210 2215 2220

Ala Gly Pro Pro Gly Leu Ser Thr Val Ala Ser Asp Val Ile Asp
2225 2230 2235

Thr Pro Ser Asp Gly Ala Glu Ser Ile Pro Thr Val Ser Phe Ser
2240 2245 2250

Pro Ser Pro Asp Thr Glu Val Thr Thr Ile Ser His Phe Pro Glu
2255 2260 2265

Lys Thr Thr His Ser Phe Arg Thr Ile Ser Ser Leu Thr His Glu
2270 2275 2280

Leu Thr Ser Arg Val Thr Pro Ile Pro Gly Asp Trp Met Ser Ser
2285 2290 2295

Ala Met Ser Thr Lys Pro Thr Gly Ala Ser Pro Ser Ile Thr Leu
2300 2305 2310

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Gly Glu  Arg Arg Thr Ile Thr  Ser Ala Ala Pro Thr  Thr Ser Pro
    2315                 2320                 2325

Ile Val  Leu Thr Ala Ser Phe  Thr Glu Thr Ser Thr  Val Ser Leu
    2330                 2335                 2340

Asp Asn  Glu Thr Thr Val Lys  Thr Ser Asp Ile Leu  Asp Ala Arg
    2345                 2350                 2355

Lys Thr  Asn Glu Leu Pro Ser  Asp Ser Ser Ser Ser  Ser Asp Leu
    2360                 2365                 2370

Ile Asn  Thr Ser Ile Ala Ser  Ser Thr Met Asp Val  Thr Lys Thr
    2375                 2380                 2385

Ala Ser  Ile Ser Pro Thr Ser  Ile Ser Gly Met Thr  Ala Ser Ser
    2390                 2395                 2400

Ser Pro  Ser Leu Phe Ser Ser  Asp Arg Pro Gln Val  Pro Thr Ser
    2405                 2410                 2415

Thr Thr  Glu Thr Asn Thr Ala  Thr Ser Pro Ser Val  Ser Ser Asn
    2420                 2425                 2430

Thr Tyr  Ser Leu Asp Gly Gly  Ser Asn Val Gly Gly  Thr Pro Ser
    2435                 2440                 2445

Thr Leu  Pro Pro Phe Thr Ile  Thr His Pro Val Glu  Thr Ser Ser
    2450                 2455                 2460

Ala Leu  Leu Ala Trp Ser Arg  Pro Val Arg Thr Phe  Ser Thr Met
    2465                 2470                 2475

Val Ser  Thr Asp Thr Ala Ser  Gly Glu Asn Pro Thr  Ser Ser Asn
    2480                 2485                 2490

Ser Val  Val Thr Ser Val Pro  Ala Pro Gly Thr Trp  Thr Ser Val
    2495                 2500                 2505

Gly Ser  Thr Thr Asp Leu Pro  Ala Met Gly Phe Leu  Lys Thr Ser
    2510                 2515                 2520

Pro Ala  Gly Glu Ala His Ser  Leu Leu Ala Ser Thr  Ile Glu Pro
    2525                 2530                 2535

Ala Thr  Ala Phe Thr Pro His  Leu Ser Ala Ala Val  Val Thr Gly
    2540                 2545                 2550
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Ser Ser  Ala Thr Ser Glu Ala  Ser Leu Leu Thr Thr  Ser Glu Ser
    2555                 2560                 2565

Lys Ala  Ile His Ser Ser Pro  Gln Thr Pro Thr Thr  Pro Thr Ser
    2570                 2575                 2580

Gly Ala  Asn Trp Glu Thr Ser  Ala Thr Pro Glu Ser  Leu Leu Val
    2585                 2590                 2595

Val Thr  Glu Thr Ser Asp Thr  Thr Leu Thr Ser Lys  Ile Leu Val
    2600                 2605                 2610

Thr Asp  Thr Ile Leu Phe Ser  Thr Val Ser Thr Pro  Pro Ser Lys
    2615                 2620                 2625

Phe Pro  Ser Thr Gly Thr Leu  Ser Gly Ala Ser Phe  Pro Thr Leu
    2630                 2635                 2640

Leu Pro  Asp Thr Pro Ala Ile  Pro Leu Thr Ala Thr  Glu Pro Thr
    2645                 2650                 2655

Ser Ser  Leu Ala Thr Ser Phe  Asp Ser Thr Pro Leu  Val Thr Ile
    2660                 2665                 2670

Ala Ser  Asp Ser Leu Gly Thr  Val Pro Glu Thr Thr  Leu Thr Met
    2675                 2680                 2685

Ser Glu  Thr Ser Asn Gly Asp  Ala Leu Val Leu Lys  Thr Val Ser
    2690                 2695                 2700

Asn Pro  Asp Arg Ser Ile Pro  Gly Ile Thr Ile Gln  Gly Val Thr
    2705                 2710                 2715

Glu Ser  Pro Leu His Pro Ser  Ser Thr Ser Pro Ser  Lys Ile Val
    2720                 2725                 2730

Ala Pro  Arg Asn Thr Thr Tyr  Glu Gly Ser Ile Thr  Val Ala Leu
    2735                 2740                 2745

Ser Thr  Leu Pro Ala Gly Thr  Thr Gly Ser Leu Val  Phe Ser Gln
    2750                 2755                 2760

Ser Ser  Glu Asn Ser Glu Thr  Thr Ala Leu Val Asp  Ser Ser Ala
    2765                 2770                 2775

Gly Leu  Glu Arg Ala Ser Val  Met Pro Leu Thr Thr  Gly Ser Gln
    2780                 2785                 2790
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```
Gly Met  Ala Ser Ser Gly Gly  Ile Arg Ser Gly Ser  Thr His Ser
    2795                 2800                 2805

Thr Gly  Thr Lys Thr Phe Ser  Ser Leu Pro Leu Thr  Met Asn Pro
    2810                 2815                 2820

Gly Glu  Val Thr Ala Met Ser  Glu Ile Thr Thr Asn  Arg Leu Thr
    2825                 2830                 2835

Ala Thr  Gln Ser Thr Ala Pro  Lys Gly Ile Pro Val  Lys Pro Thr
    2840                 2845                 2850

Ser Ala  Glu Ser Gly Leu Leu  Thr Pro Val Ser Ala  Ser Ser Ser
    2855                 2860                 2865

Pro Ser  Lys Ala Phe Ala Ser  Leu Thr Thr Ala Pro  Pro Thr Trp
    2870                 2875                 2880

Gly Ile  Pro Gln Ser Thr Leu  Thr Phe Glu Phe Ser  Glu Val Pro
    2885                 2890                 2895

Ser Leu  Asp Thr Lys Ser Ala  Ser Leu Pro Thr Pro  Gly Gln Ser
    2900                 2905                 2910

Leu Asn  Thr Ile Pro Asp Ser  Asp Ala Ser Thr Ala  Ser Ser Ser
    2915                 2920                 2925

Leu Ser  Lys Ser Pro Glu Lys  Asn Pro Arg Ala Arg  Met Met Thr
    2930                 2935                 2940

Ser Thr  Lys Ala Ile Ser Ala  Ser Ser Phe Gln Ser  Thr Gly Phe
    2945                 2950                 2955

Thr Glu  Thr Pro Glu Gly Ser  Ala Ser Pro Ser Met  Ala Gly His
    2960                 2965                 2970

Glu Pro  Arg Val Pro Thr Ser  Gly Thr Gly Asp Pro  Arg Tyr Ala
    2975                 2980                 2985

Ser Glu  Ser Met Ser Tyr Pro  Asp Pro Ser Lys Ala  Ser Ser Ala
    2990                 2995                 3000

Met Thr  Ser Thr Ser Leu Ala  Ser Lys Leu Thr Thr  Leu Phe Ser
    3005                 3010                 3015

Thr Gly  Gln Ala Ala Arg Ser  Gly Ser Ser Ser Ser  Pro Ile Ser
```

3020 3025 3030

Leu Ser Thr Glu Lys Glu Thr Ser Phe Leu Ser Pro Thr Ala Ser
3035 3040 3045

Thr Ser Arg Lys Thr Ser Leu Phe Leu Gly Pro Ser Met Ala Arg
3050 3055 3060

Gln Pro Asn Ile Leu Val His Leu Gln Thr Ser Ala Leu Thr Leu
3065 3070 3075

Ser Pro Thr Ser Thr Leu Asn Met Ser Gln Glu Glu Pro Pro Glu
3080 3085 3090

Leu Thr Ser Ser Gln Thr Ile Ala Glu Glu Glu Gly Thr Thr Ala
3095 3100 3105

Glu Thr Gln Thr Leu Thr Phe Thr Pro Ser Glu Thr Pro Thr Ser
3110 3115 3120

Leu Leu Pro Val Ser Ser Pro Thr Glu Pro Thr Ala Arg Arg Lys
3125 3130 3135

Ser Ser Pro Glu Thr Trp Ala Ser Ser Ile Ser Val Pro Ala Lys
3140 3145 3150

Thr Ser Leu Val Glu Thr Thr Asp Gly Thr Leu Val Thr Thr Ile
3155 3160 3165

Lys Met Ser Ser Gln Ala Ala Gln Gly Asn Ser Thr Trp Pro Ala
3170 3175 3180

Pro Ala Glu Glu Thr Gly Ser Ser Pro Ala Gly Thr Ser Pro Gly
3185 3190 3195

Ser Pro Glu Met Ser Thr Thr Leu Lys Ile Met Ser Ser Lys Glu
3200 3205 3210

Pro Ser Ile Ser Pro Glu Ile Arg Ser Thr Val Arg Asn Ser Pro
3215 3220 3225

Trp Lys Thr Pro Glu Thr Thr Val Pro Met Glu Thr Thr Val Glu
3230 3235 3240

Pro Val Thr Leu Gln Ser Thr Ala Leu Gly Ser Gly Ser Thr Ser
3245 3250 3255

```
Ile Ser  His Leu Pro Thr Gly  Thr Thr Ser Pro Thr  Lys Ser Pro
    3260                 3265                 3270

Thr Glu  Asn Met Leu Ala Thr  Glu Arg Val Ser Leu  Ser Pro Ser
    3275                 3280                 3285

Pro Pro  Glu Ala Trp Thr Asn  Leu Tyr Ser Gly Thr  Pro Gly Gly
    3290                 3295                 3300

Thr Arg  Gln Ser Leu Ala Thr  Met Ser Ser Val Ser  Leu Glu Ser
    3305                 3310                 3315

Pro Thr  Ala Arg Ser Ile Thr  Gly Thr Gly Gln Gln  Ser Ser Pro
    3320                 3325                 3330

Glu Leu  Val Ser Lys Thr Thr  Gly Met Glu Phe Ser  Met Trp His
    3335                 3340                 3345

Gly Ser  Thr Gly Gly Thr Thr  Gly Asp Thr His Val  Ser Leu Ser
    3350                 3355                 3360

Thr Ser  Ser Asn Ile Leu Glu  Asp Pro Val Thr Ser  Pro Asn Ser
    3365                 3370                 3375

Val Ser  Ser Leu Thr Asp Lys  Ser Lys His Lys Thr  Glu Thr Trp
    3380                 3385                 3390

Val Ser  Thr Thr Ala Ile Pro  Ser Thr Val Leu Asn  Asn Lys Ile
    3395                 3400                 3405

Met Ala  Ala Glu Gln Gln Thr  Ser Arg Ser Val Asp  Glu Ala Tyr
    3410                 3415                 3420

Ser Ser  Thr Ser Ser Trp Ser  Asp Gln Thr Ser Gly  Ser Asp Ile
    3425                 3430                 3435

Thr Leu  Gly Ala Ser Pro Asp  Val Thr Asn Thr Leu  Tyr Ile Thr
    3440                 3445                 3450

Ser Thr  Ala Gln Thr Thr Ser  Leu Val Ser Leu Pro  Ser Gly Asp
    3455                 3460                 3465

Gln Gly  Ile Thr Ser Leu Thr  Asn Pro Ser Gly Gly  Lys Thr Ser
    3470                 3475                 3480

Ser Ala  Ser Ser Val Thr Ser  Pro Ser Ile Gly Leu  Glu Thr Leu
    3485                 3490                 3495
```

```
Arg Ala  Asn Val Ser Ala Val  Lys Ser Asp Ile Ala  Pro Thr Ala
    3500                 3505                 3510

Gly His  Leu Ser Gln Thr Ser  Ser Pro Ala Glu Val  Ser Ile Leu
    3515                 3520                 3525

Asp Val  Thr Thr Ala Pro Thr  Pro Gly Ile Ser Thr  Thr Ile Thr
    3530                 3535                 3540

Thr Met  Gly Thr Asn Ser Ile  Ser Thr Thr Thr Pro  Asn Pro Glu
    3545                 3550                 3555

Val Gly  Met Ser Thr Met Asp  Ser Thr Pro Ala Thr  Glu Arg Arg
    3560                 3565                 3570

Thr Thr  Ser Thr Glu His Pro  Ser Thr Trp Ser Ser  Thr Ala Ala
    3575                 3580                 3585

Ser Asp  Ser Trp Thr Val Thr  Asp Met Thr Ser Asn  Leu Lys Val
    3590                 3595                 3600

Ala Arg  Ser Pro Gly Thr Ile  Ser Thr Met His Thr  Thr Ser Phe
    3605                 3610                 3615

Leu Ala  Ser Ser Thr Glu Leu  Asp Ser Met Ser Thr  Pro His Gly
    3620                 3625                 3630

Arg Ile  Thr Val Ile Gly Thr  Ser Leu Val Thr Pro  Ser Ser Asp
    3635                 3640                 3645

Ala Ser  Ala Val Lys Thr Glu  Thr Ser Thr Ser Glu  Arg Thr Leu
    3650                 3655                 3660

Ser Pro  Ser Asp Thr Thr Ala  Ser Thr Pro Ile Ser  Thr Phe Ser
    3665                 3670                 3675

Arg Val  Gln Arg Met Ser Ile  Ser Val Pro Asp Ile  Leu Ser Thr
    3680                 3685                 3690

Ser Trp  Thr Pro Ser Ser Thr  Glu Ala Glu Asp Val  Pro Val Ser
    3695                 3700                 3705

Met Val  Ser Thr Asp His Ala  Ser Thr Lys Thr Asp  Pro Asn Thr
    3710                 3715                 3720

Pro Leu  Ser Thr Phe Leu Phe  Asp Ser Leu Ser Thr  Leu Asp Trp
    3725                 3730                 3735
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Asp Thr  Gly Arg Ser Leu Ser  Ser Ala Thr Ala Thr  Thr Ser Ala
    3740                 3745                 3750

Pro Gln  Gly Ala Thr Thr Pro  Gln Glu Leu Thr Leu  Glu Thr Met
    3755                 3760                 3765

Ile Ser  Pro Ala Thr Ser Gln  Leu Pro Phe Ser Ile  Gly His Ile
    3770                 3775                 3780

Thr Ser  Ala Val Thr Pro Ala  Ala Met Ala Arg Ser  Ser Gly Val
    3785                 3790                 3795

Thr Phe  Ser Arg Pro Asp Pro  Thr Ser Lys Lys Ala  Glu Gln Thr
    3800                 3805                 3810

Ser Thr  Gln Leu Pro Thr Thr  Thr Ser Ala His Pro  Gly Gln Val
    3815                 3820                 3825

Pro Arg  Ser Ala Ala Thr Thr  Leu Asp Val Ile Pro  His Thr Ala
    3830                 3835                 3840

Lys Thr  Pro Asp Ala Thr Phe  Gln Arg Gln Gly Gln  Thr Ala Leu
    3845                 3850                 3855

Thr Thr  Glu Ala Arg Ala Thr  Ser Asp Ser Trp Asn  Glu Lys Glu
    3860                 3865                 3870

Lys Ser  Thr Pro Ser Ala Pro  Trp Ile Thr Glu Met  Met Asn Ser
    3875                 3880                 3885

Val Ser  Glu Asp Thr Ile Lys  Glu Val Thr Ser Ser  Ser Ser Val
    3890                 3895                 3900

Leu Arg  Thr Leu Asn Thr Leu  Asp Ile Asn Leu Glu  Ser Gly Thr
    3905                 3910                 3915

Thr Ser  Ser Pro Ser Trp Lys  Ser Ser Pro Tyr Glu  Arg Ile Ala
    3920                 3925                 3930

Pro Ser  Glu Ser Thr Thr Asp  Lys Glu Ala Ile His  Pro Ser Thr
    3935                 3940                 3945

Asn Thr  Val Glu Thr Thr Gly  Trp Val Thr Ser Ser  Glu His Ala
    3950                 3955                 3960

Ser His  Ser Thr Ile Pro Ala  His Ser Ala Ser Ser  Lys Leu Thr
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3965 3970 3975

Ser Pro Val Val Thr Thr Ser Thr Arg Glu Gln Ala Ile Val Ser
3980 3985 3990

Met Ser Thr Thr Thr Trp Pro Glu Ser Thr Arg Ala Arg Thr Glu
3995 4000 4005

Pro Asn Ser Phe Leu Thr Ile Glu Leu Arg Asp Val Ser Pro Tyr
4010 4015 4020

Met Asp Thr Ser Ser Thr Thr Gln Thr Ser Ile Ile Ser Ser Pro
4025 4030 4035

Gly Ser Thr Ala Ile Thr Lys Gly Pro Arg Thr Glu Ile Thr Ser
4040 4045 4050

Ser Lys Arg Ile Ser Ser Ser Phe Leu Ala Gln Ser Met Arg Ser
4055 4060 4065

Ser Asp Ser Pro Ser Glu Ala Ile Thr Arg Leu Ser Asn Phe Pro
4070 4075 4080

Ala Met Thr Glu Ser Gly Gly Met Ile Leu Ala Met Gln Thr Ser
4085 4090 4095

Pro Pro Gly Ala Thr Ser Leu Ser Ala Pro Thr Leu Asp Thr Ser
4100 4105 4110

Ala Thr Ala Ser Trp Thr Gly Thr Pro Leu Ala Thr Thr Gln Arg
4115 4120 4125

Phe Thr Tyr Ser Glu Lys Thr Thr Leu Phe Ser Lys Gly Pro Glu
4130 4135 4140

Asp Thr Ser Gln Pro Ser Pro Pro Ser Val Glu Glu Thr Ser Ser
4145 4150 4155

Ser Ser Ser Leu Val Pro Ile His Ala Thr Thr Ser Pro Ser Asn
4160 4165 4170

Ile Leu Leu Thr Ser Gln Gly His Ser Pro Ser Ser Thr Pro Pro
4175 4180 4185

Val Thr Ser Val Phe Leu Ser Glu Thr Ser Gly Leu Gly Lys Thr
4190 4195 4200

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Thr Asp  Met Ser Arg Ile Ser  Leu Glu Pro Gly Thr  Ser Leu Pro
    4205                 4210                 4215

Pro Asn  Leu Ser Ser Thr Ala  Gly Glu Ala Leu Ser  Thr Tyr Glu
    4220                 4225                 4230

Ala Ser  Arg Asp Thr Lys Ala  Ile His His Ser Ala  Asp Thr Ala
    4235                 4240                 4245

Val Thr  Asn Met Glu Ala Thr  Ser Ser Glu Tyr Ser  Pro Ile Pro
    4250                 4255                 4260

Gly His  Thr Lys Pro Ser Lys  Ala Thr Ser Pro Leu  Val Thr Ser
    4265                 4270                 4275

His Ile  Met Gly Asp Ile Thr  Ser Ser Thr Ser Val  Phe Gly Ser
    4280                 4285                 4290

Ser Glu  Thr Thr Glu Ile Glu  Thr Val Ser Ser Val  Asn Gln Gly
    4295                 4300                 4305

Leu Gln  Glu Arg Ser Thr Ser  Gln Val Ala Ser Ser  Ala Thr Glu
    4310                 4315                 4320

Thr Ser  Thr Val Ile Thr His  Val Ser Ser Gly Asp  Ala Thr Thr
    4325                 4330                 4335

His Val  Thr Lys Thr Gln Ala  Thr Phe Ser Ser Gly  Thr Ser Ile
    4340                 4345                 4350

Ser Ser  Pro His Gln Phe Ile  Thr Ser Thr Asn Thr  Phe Thr Asp
    4355                 4360                 4365

Val Ser  Thr Asn Pro Ser Thr  Ser Leu Ile Met Thr  Glu Ser Ser
    4370                 4375                 4380

Gly Val  Thr Ile Thr Thr Gln  Thr Gly Pro Thr Gly  Ala Ala Thr
    4385                 4390                 4395

Gln Gly  Pro Tyr Leu Leu Asp  Thr Ser Thr Met Pro  Tyr Leu Thr
    4400                 4405                 4410

Glu Thr  Pro Leu Ala Val Thr  Pro Asp Phe Met Gln  Ser Glu Lys
    4415                 4420                 4425

Thr Thr  Leu Ile Ser Lys Gly  Pro Lys Asp Val Ser  Trp Thr Ser
    4430                 4435                 4440
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Pro Pro  Ser Val Ala Glu Thr  Ser Tyr Pro Ser Ser  Leu Thr Pro
    4445                 4450                 4455

Phe Leu  Val Thr Thr Ile Pro  Pro Ala Thr Ser Thr  Leu Gln Gly
    4460                 4465                 4470

Gln His  Thr Ser Ser Pro Val  Ser Ala Thr Ser Val  Leu Thr Ser
    4475                 4480                 4485

Gly Leu  Val Lys Thr Thr Asp  Met Leu Asn Thr Ser  Met Glu Pro
    4490                 4495                 4500

Val Thr  Asn Ser Pro Gln Asn  Leu Asn Asn Pro Ser  Asn Glu Ile
    4505                 4510                 4515

Leu Ala  Thr Leu Ala Ala Thr  Thr Asp Ile Glu Thr  Ile His Pro
    4520                 4525                 4530

Ser Ile  Asn Lys Ala Val Thr  Asn Met Gly Thr Ala  Ser Ser Ala
    4535                 4540                 4545

His Val  Leu His Ser Thr Leu  Pro Val Ser Ser Glu  Pro Ser Thr
    4550                 4555                 4560

Ala Thr  Ser Pro Met Val Pro  Ala Ser Ser Met Gly  Asp Ala Leu
    4565                 4570                 4575

Ala Ser  Ile Ser Ile Pro Gly  Ser Glu Thr Thr Asp  Ile Glu Gly
    4580                 4585                 4590

Glu Pro  Thr Ser Ser Leu Thr  Ala Gly Arg Lys Glu  Asn Ser Thr
    4595                 4600                 4605

Leu Gln  Glu Met Asn Ser Thr  Thr Glu Ser Asn Ile  Ile Leu Ser
    4610                 4615                 4620

Asn Val  Ser Val Gly Ala Ile  Thr Glu Ala Thr Lys  Met Glu Val
    4625                 4630                 4635

Pro Ser  Phe Asp Ala Thr Phe  Ile Pro Thr Pro Ala  Gln Ser Thr
    4640                 4645                 4650

Lys Phe  Pro Asp Ile Phe Ser  Val Ala Ser Ser Arg  Leu Ser Asn
    4655                 4660                 4665

Ser Pro  Pro Met Thr Ile Ser  Thr His Met Thr Thr  Thr Gln Thr
    4670                 4675                 4680
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```
Gly Ser  Ser Gly Ala Thr Ser  Lys Ile Pro Leu Ala  Leu Asp Thr
    4685                4690                4695

Ser Thr  Leu Glu Thr Ser Ala  Gly Thr Pro Ser Val  Val Thr Glu
    4700                4705                4710

Gly Phe  Ala His Ser Lys Ile  Thr Thr Ala Met Asn  Asn Asp Val
    4715                4720                4725

Lys Asp  Val Ser Gln Thr Asn  Pro Pro Phe Gln Asp  Glu Ala Ser
    4730                4735                4740

Ser Pro  Ser Ser Gln Ala Pro  Val Leu Val Thr Thr  Leu Pro Ser
    4745                4750                4755

Ser Val  Ala Phe Thr Pro Gln  Trp His Ser Thr Ser  Ser Pro Val
    4760                4765                4770

Ser Met  Ser Ser Val Leu Thr  Ser Ser Leu Val Lys  Thr Ala Gly
    4775                4780                4785

Lys Val  Asp Thr Ser Leu Glu  Thr Val Thr Ser Ser  Pro Gln Ser
    4790                4795                4800

Met Ser  Asn Thr Leu Asp Asp  Ile Ser Val Thr Ser  Ala Ala Thr
    4805                4810                4815

Thr Asp  Ile Glu Thr Thr His  Pro Ser Ile Asn Thr  Val Val Thr
    4820                4825                4830

Asn Val  Gly Thr Thr Gly Ser  Ala Phe Glu Ser His  Ser Thr Val
    4835                4840                4845

Ser Ala  Tyr Pro Glu Pro Ser  Lys Val Thr Ser Pro  Asn Val Thr
    4850                4855                4860

Thr Ser  Thr Met Glu Asp Thr  Thr Ile Ser Arg Ser  Ile Pro Lys
    4865                4870                4875

Ser Ser  Lys Thr Thr Arg Thr  Glu Thr Glu Thr Thr  Ser Ser Leu
    4880                4885                4890

Thr Pro  Lys Leu Arg Glu Thr  Ser Ile Ser Gln Glu  Ile Thr Ser
    4895                4900                4905

Ser Thr  Glu Thr Ser Thr Val  Pro Tyr Lys Glu Leu  Thr Gly Ala
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4910 4915 4920

Thr Thr Glu Val Ser Arg Thr Asp Val Thr Ser Ser Ser Ser Thr
4925 4930 4935

Ser Phe Pro Gly Pro Asp Gln Ser Thr Val Ser Leu Asp Ile Ser
4940 4945 4950

Thr Glu Thr Asn Thr Arg Leu Ser Thr Ser Pro Ile Met Thr Glu
4955 4960 4965

Ser Ala Glu Ile Thr Ile Thr Thr Gln Thr Gly Pro His Gly Ala
4970 4975 4980

Thr Ser Gln Asp Thr Phe Thr Met Asp Pro Ser Asn Thr Thr Pro
4985 4990 4995

Gln Ala Gly Ile His Ser Ala Met Thr His Gly Phe Ser Gln Leu
5000 5005 5010

Asp Val Thr Thr Leu Met Ser Arg Ile Pro Gln Asp Val Ser Trp
5015 5020 5025

Thr Ser Pro Pro Ser Val Asp Lys Thr Ser Ser Pro Ser Ser Phe
5030 5035 5040

Leu Ser Ser Pro Ala Met Thr Thr Pro Ser Leu Ile Ser Ser Thr
5045 5050 5055

Leu Pro Glu Asp Lys Leu Ser Ser Pro Met Thr Ser Leu Leu Thr
5060 5065 5070

Ser Gly Leu Val Lys Ile Thr Asp Ile Leu Arg Thr Arg Leu Glu
5075 5080 5085

Pro Val Thr Ser Ser Leu Pro Asn Phe Ser Ser Thr Ser Asp Lys
5090 5095 5100

Ile Leu Ala Thr Ser Lys Asp Ser Lys Asp Thr Lys Glu Ile Phe
5105 5110 5115

Pro Ser Ile Asn Thr Glu Glu Thr Asn Val Lys Ala Asn Asn Ser
5120 5125 5130

Gly His Glu Ser His Ser Pro Ala Leu Ala Asp Ser Glu Thr Pro
5135 5140 5145

Lys Ala Thr Thr Gln Met Val Ile Thr Thr Thr Val Gly Asp Pro
5150 5155 5160

Ala Pro Ser Thr Ser Met Pro Val His Gly Ser Ser Glu Thr Thr
5165 5170 5175

Asn Ile Lys Arg Glu Pro Thr Tyr Phe Leu Thr Pro Arg Leu Arg
5180 5185 5190

Glu Thr Ser Thr Ser Gln Glu Ser Ser Phe Pro Thr Asp Thr Ser
5195 5200 5205

Phe Leu Leu Ser Lys Val Pro Thr Gly Thr Ile Thr Glu Val Ser
5210 5215 5220

Ser Thr Gly Val Asn Ser Ser Ser Lys Ile Ser Thr Pro Asp His
5225 5230 5235

Asp Lys Ser Thr Val Pro Pro Asp Thr Phe Thr Gly Glu Ile Pro
5240 5245 5250

Arg Val Phe Thr Ser Ser Ile Lys Thr Lys Ser Ala Glu Met Thr
5255 5260 5265

Ile Thr Thr Gln Ala Ser Pro Pro Glu Ser Ala Ser His Ser Thr
5270 5275 5280

Leu Pro Leu Asp Thr Ser Thr Thr Leu Ser Gln Gly Gly Thr His
5285 5290 5295

Ser Thr Val Thr Gln Gly Phe Pro Tyr Ser Glu Val Thr Thr Leu
5300 5305 5310

Met Gly Met Gly Pro Gly Asn Val Ser Trp Met Thr Thr Pro Pro
5315 5320 5325

Val Glu Glu Thr Ser Ser Val Ser Ser Leu Met Ser Ser Pro Ala
5330 5335 5340

Met Thr Ser Pro Ser Pro Val Ser Ser Thr Ser Pro Gln Ser Ile
5345 5350 5355

Pro Ser Ser Pro Leu Pro Val Thr Ala Leu Pro Thr Ser Val Leu
5360 5365 5370

Val Thr Thr Thr Asp Val Leu Gly Thr Thr Ser Pro Glu Ser Val
5375 5380 5385

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Thr Ser  Ser Pro Pro Asn Leu  Ser Ser Ile Thr His  Glu Arg Pro
    5390                 5395                 5400

Ala Thr  Tyr Lys Asp Thr Ala  His Thr Glu Ala Ala  Met His His
    5405                 5410                 5415

Ser Thr  Asn Thr Ala Val Thr  Asn Val Gly Thr Ser  Gly Ser Gly
    5420                 5425                 5430

His Lys  Ser Gln Ser Ser Val  Leu Ala Asp Ser Glu  Thr Ser Lys
    5435                 5440                 5445

Ala Thr  Pro Leu Met Ser Thr  Thr Ser Thr Leu Gly  Asp Thr Ser
    5450                 5455                 5460

Val Ser  Thr Ser Thr Pro Asn  Ile Ser Gln Thr Asn  Gln Ile Gln
    5465                 5470                 5475

Thr Glu  Pro Thr Ala Ser Leu  Ser Pro Arg Leu Arg  Glu Ser Ser
    5480                 5485                 5490

Thr Ser  Glu Lys Thr Ser Ser  Thr Thr Glu Thr Asn  Thr Ala Phe
    5495                 5500                 5505

Ser Tyr  Val Pro Thr Gly Ala  Ile Thr Gln Ala Ser  Arg Thr Glu
    5510                 5515                 5520

Ile Ser  Ser Ser Arg Thr Ser  Ile Ser Asp Leu Asp  Arg Pro Thr
    5525                 5530                 5535

Ile Ala  Pro Asp Ile Ser Thr  Gly Met Ile Thr Arg  Leu Phe Thr
    5540                 5545                 5550

Ser Pro  Ile Met Thr Lys Ser  Ala Glu Met Thr Val  Thr Thr Gln
    5555                 5560                 5565

Thr Thr  Thr Pro Gly Ala Thr  Ser Gln Gly Ile Leu  Pro Trp Asp
    5570                 5575                 5580

Thr Ser  Thr Thr Leu Phe Gln  Gly Gly Thr His Ser  Thr Val Ser
    5585                 5590                 5595

Gln Gly  Phe Pro His Ser Glu  Ile Thr Thr Leu Arg  Ser Arg Thr
    5600                 5605                 5610

Pro Gly  Asp Val Ser Trp Met  Thr Thr Pro Pro Val  Glu Glu Thr
    5615                 5620                 5625
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Ser Ser  Gly Phe Ser Leu Met  Ser Pro Ser Met Thr  Ser Pro Ser
    5630                 5635                 5640

Pro Val  Ser Ser Thr Ser Pro  Glu Ser Ile Pro Ser  Ser Pro Leu
    5645                 5650                 5655

Pro Val  Thr Ala Leu Leu Thr  Ser Val Leu Val Thr  Thr Thr Asn
    5660                 5665                 5670

Val Leu  Gly Thr Thr Ser Pro  Glu Pro Val Thr Ser  Ser Pro Pro
    5675                 5680                 5685

Asn Leu  Ser Ser Pro Thr Gln  Glu Arg Leu Thr Thr  Tyr Lys Asp
    5690                 5695                 5700

Thr Ala  His Thr Glu Ala Met  His Ala Ser Met His  Thr Asn Thr
    5705                 5710                 5715

Ala Val  Ala Asn Val Gly Thr  Ser Ile Ser Gly His  Glu Ser Gln
    5720                 5725                 5730

Ser Ser  Val Pro Ala Asp Ser  His Thr Ser Lys Ala  Thr Ser Pro
    5735                 5740                 5745

Met Gly  Ile Thr Phe Ala Met  Gly Asp Thr Ser Val  Ser Thr Ser
    5750                 5755                 5760

Thr Pro  Ala Phe Phe Glu Thr  Arg Ile Gln Thr Glu  Ser Thr Ser
    5765                 5770                 5775

Ser Leu  Ile Pro Gly Leu Arg  Asp Thr Arg Thr Ser  Glu Glu Ile
    5780                 5785                 5790

Asn Thr  Val Thr Glu Thr Ser  Thr Val Leu Ser Glu  Val Pro Thr
    5795                 5800                 5805

Thr Thr  Thr Thr Glu Val Ser  Arg Thr Glu Val Ile  Thr Ser Ser
    5810                 5815                 5820

Arg Thr  Thr Ile Ser Gly Pro  Asp His Ser Lys Met  Ser Pro Tyr
    5825                 5830                 5835

Ile Ser  Thr Glu Thr Ile Thr  Arg Leu Ser Thr Phe  Pro Phe Val
    5840                 5845                 5850

Thr Gly  Ser Thr Glu Met Ala  Ile Thr Asn Gln Thr  Gly Pro Ile
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    5855                5860                5865

Gly Thr  Ile Ser Gln Ala Thr  Leu Thr Leu Asp Thr  Ser Ser Thr
    5870                5875                5880

Ala Ser  Trp Glu Gly Thr His  Ser Pro Val Thr Gln  Arg Phe Pro
    5885                5890                5895

His Ser  Glu Glu Thr Thr Thr  Met Ser Arg Ser Thr  Lys Gly Val
    5900                5905                5910

Ser Trp  Gln Ser Pro Pro Ser  Val Glu Glu Thr Ser  Ser Pro Ser
    5915                5920                5925

Ser Pro  Val Pro Leu Pro Ala  Ile Thr Ser His Ser  Ser Leu Tyr
    5930                5935                5940

Ser Ala  Val Ser Gly Ser Ser  Pro Thr Ser Ala Leu  Pro Val Thr
    5945                5950                5955

Ser Leu  Leu Thr Ser Gly Arg  Arg Lys Thr Ile Asp  Met Leu Asp
    5960                5965                5970

Thr His  Ser Glu Leu Val Thr  Ser Ser Leu Pro Ser  Ala Ser Ser
    5975                5980                5985

Phe Ser  Gly Glu Ile Leu Thr  Ser Glu Ala Ser Thr  Asn Thr Glu
    5990                5995                6000

Thr Ile  His Phe Ser Glu Asn  Thr Ala Glu Thr Asn  Met Gly Thr
    6005                6010                6015

Thr Asn  Ser Met His Lys Leu  His Ser Ser Val Ser  Ile His Ser
    6020                6025                6030

Gln Pro  Ser Gly His Thr Pro  Pro Lys Val Thr Gly  Ser Met Met
    6035                6040                6045

Glu Asp  Ala Ile Val Ser Thr  Ser Thr Pro Gly Ser  Pro Glu Thr
    6050                6055                6060

Lys Asn  Val Asp Arg Asp Ser  Thr Ser Pro Leu Thr  Pro Glu Leu
    6065                6070                6075

Lys Glu  Asp Ser Thr Ala Leu  Val Met Asn Ser Thr  Thr Glu Ser
    6080                6085                6090
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Asn Thr  Val Phe Ser Ser Val  Ser Leu Asp Ala Ala  Thr Glu Val
    6095                 6100                 6105

Ser Arg  Ala Glu Val Thr Tyr  Tyr Asp Pro Thr Phe  Met Pro Ala
    6110                 6115                 6120

Ser Ala  Gln Ser Thr Lys Ser  Pro Asp Ile Ser Pro  Glu Ala Ser
    6125                 6130                 6135

Ser Ser  His Ser Asn Ser Pro  Pro Leu Thr Ile Ser  Thr His Lys
    6140                 6145                 6150

Thr Ile  Ala Thr Gln Thr Gly  Pro Ser Gly Val Thr  Ser Leu Gly
    6155                 6160                 6165

Gln Leu  Thr Leu Asp Thr Ser  Thr Ile Ala Thr Ser  Ala Gly Thr
    6170                 6175                 6180

Pro Ser  Ala Arg Thr Gln Asp  Phe Val Asp Ser Glu  Thr Thr Ser
    6185                 6190                 6195

Val Met  Asn Asn Asp Leu Asn  Asp Val Leu Lys Thr  Ser Pro Phe
    6200                 6205                 6210

Ser Ala  Glu Glu Ala Asn Ser  Leu Ser Ser Gln Ala  Pro Leu Leu
    6215                 6220                 6225

Val Thr  Thr Ser Pro Ser Pro  Val Thr Ser Thr Leu  Gln Glu His
    6230                 6235                 6240

Ser Thr  Ser Ser Leu Val Ser  Val Thr Ser Val Pro  Thr Pro Thr
    6245                 6250                 6255

Leu Ala  Lys Ile Thr Asp Met  Asp Thr Asn Leu Glu  Pro Val Thr
    6260                 6265                 6270

Arg Ser  Pro Gln Asn Leu Arg  Asn Thr Leu Ala Thr  Ser Glu Ala
    6275                 6280                 6285

Thr Thr  Asp Thr His Thr Met  His Pro Ser Ile Asn  Thr Ala Val
    6290                 6295                 6300

Ala Asn  Val Gly Thr Thr Ser  Ser Pro Asn Glu Phe  Tyr Phe Thr
    6305                 6310                 6315

Val Ser  Pro Asp Ser Asp Pro  Tyr Lys Ala Thr Ser  Ala Val Val
    6320                 6325                 6330
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Ile Thr  Ser Thr Ser Gly Asp  Ser Ile Val Ser Thr  Ser Met Pro
    6335                 6340                 6345

Arg Ser  Ser Ala Met Lys Lys  Ile Glu Ser Glu Thr  Thr Phe Ser
    6350                 6355                 6360

Leu Ile  Phe Arg Leu Arg Glu  Thr Ser Thr Ser Gln  Lys Ile Gly
    6365                 6370                 6375

Ser Ser  Ser Asp Thr Ser Thr  Val Phe Asp Lys Ala  Phe Thr Ala
    6380                 6385                 6390

Ala Thr  Thr Glu Val Ser Arg  Thr Glu Leu Thr Ser  Ser Ser Arg
    6395                 6400                 6405

Thr Ser  Ile Gln Gly Thr Glu  Lys Pro Thr Met Ser  Pro Asp Thr
    6410                 6415                 6420

Ser Thr  Arg Ser Val Thr Met  Leu Ser Thr Phe Ala  Gly Leu Thr
    6425                 6430                 6435

Lys Ser  Glu Glu Arg Thr Ile  Ala Thr Gln Thr Gly  Pro His Arg
    6440                 6445                 6450

Ala Thr  Ser Gln Gly Thr Leu  Thr Trp Asp Thr Ser  Ile Thr Thr
    6455                 6460                 6465

Ser Gln  Ala Gly Thr His Ser  Ala Met Thr His Gly  Phe Ser Gln
    6470                 6475                 6480

Leu Asp  Leu Ser Thr Leu Thr  Ser Arg Val Pro Glu  Tyr Ile Ser
    6485                 6490                 6495

Gly Thr  Ser Pro Pro Ser Val  Glu Lys Thr Ser Ser  Ser Ser Ser
    6500                 6505                 6510

Leu Leu  Ser Leu Pro Ala Ile  Thr Ser Pro Ser Pro  Val Pro Thr
    6515                 6520                 6525

Thr Leu  Pro Glu Ser Arg Pro  Ser Ser Pro Val His  Leu Thr Ser
    6530                 6535                 6540

Leu Pro  Thr Ser Gly Leu Val  Lys Thr Thr Asp Met  Leu Ala Ser
    6545                 6550                 6555

Val Ala  Ser Leu Pro Pro Asn  Leu Gly Ser Thr Ser  His Lys Ile
    6560                 6565                 6570
```

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Pro Thr  Thr Ser Glu Asp Ile  Lys Asp Thr Glu Lys  Met Tyr Pro
    6575                 6580                 6585

Ser Thr  Asn Ile Ala Val Thr  Asn Val Gly Thr Thr  Thr Ser Glu
    6590                 6595                 6600

Lys Glu  Ser Tyr Ser Ser Val  Pro Ala Tyr Ser Glu  Pro Pro Lys
    6605                 6610                 6615

Val Thr  Ser Pro Met Val Thr  Ser Phe Asn Ile Arg  Asp Thr Ile
    6620                 6625                 6630

Val Ser  Thr Ser Met Pro Gly  Ser Ser Glu Ile Thr  Arg Ile Glu
    6635                 6640                 6645

Met Glu  Ser Thr Phe Ser Leu  Ala His Gly Leu Lys  Gly Thr Ser
    6650                 6655                 6660

Thr Ser  Gln Asp Pro Ile Val  Ser Thr Glu Lys Ser  Ala Val Leu
    6665                 6670                 6675

His Lys  Leu Thr Thr Gly Ala  Thr Glu Thr Ser Arg  Thr Glu Val
    6680                 6685                 6690

Ala Ser  Ser Arg Arg Thr Ser  Ile Pro Gly Pro Asp  His Ser Thr
    6695                 6700                 6705

Glu Ser  Pro Asp Ile Ser Thr  Glu Val Ile Pro Ser  Leu Pro Ile
    6710                 6715                 6720

Ser Leu  Gly Ile Thr Glu Ser  Ser Asn Met Thr Ile  Ile Thr Arg
    6725                 6730                 6735

Thr Gly  Pro Pro Leu Gly Ser  Thr Ser Gln Gly Thr  Phe Thr Leu
    6740                 6745                 6750

Asp Thr  Pro Thr Thr Ser Ser  Arg Ala Gly Thr His  Ser Met Ala
    6755                 6760                 6765

Thr Gln  Glu Phe Pro His Ser  Glu Met Thr Thr Val  Met Asn Lys
    6770                 6775                 6780

Asp Pro  Glu Ile Leu Ser Trp  Thr Ile Pro Pro Ser  Ile Glu Lys
    6785                 6790                 6795

Thr Ser  Phe Ser Ser Ser Leu  Met Pro Ser Pro Ala  Met Thr Ser
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     6800                6805                6810

Pro Pro  Val Ser Ser Thr Leu  Pro Lys Thr Ile His  Thr Thr Pro
     6815                6820                6825

Ser Pro  Met Thr Ser Leu Leu  Thr Pro Ser Leu Val  Met Thr Thr
     6830                6835                6840

Asp Thr  Leu Gly Thr Ser Pro  Glu Pro Thr Thr Ser  Ser Pro Pro
     6845                6850                6855

Asn Leu  Ser Ser Thr Ser His  Glu Ile Leu Thr Thr  Asp Glu Asp
     6860                6865                6870

Thr Thr  Ala Ile Glu Ala Met  His Pro Ser Thr Ser  Thr Ala Ala
     6875                6880                6885

Thr Asn  Val Glu Thr Thr Ser  Ser Gly His Gly Ser  Gln Ser Ser
     6890                6895                6900

Val Leu  Ala Asp Ser Glu Lys  Thr Lys Ala Thr Ala  Pro Met Asp
     6905                6910                6915

Thr Thr  Ser Thr Met Gly His  Thr Thr Val Ser Thr  Ser Met Ser
     6920                6925                6930

Val Ser  Ser Glu Thr Thr Lys  Ile Lys Arg Glu Ser  Thr Tyr Ser
     6935                6940                6945

Leu Thr  Pro Gly Leu Arg Glu  Thr Ser Ile Ser Gln  Asn Ala Ser
     6950                6955                6960

Phe Ser  Thr Asp Thr Ser Ile  Val Leu Ser Glu Val  Pro Thr Gly
     6965                6970                6975

Thr Thr  Ala Glu Val Ser Arg  Thr Glu Val Thr Ser  Ser Gly Arg
     6980                6985                6990

Thr Ser  Ile Pro Gly Pro Ser  Gln Ser Thr Val Leu  Pro Glu Ile
     6995                7000                7005

Ser Thr  Arg Thr Met Thr Arg  Leu Phe Ala Ser Pro  Thr Met Thr
     7010                7015                7020

Glu Ser  Ala Glu Met Thr Ile  Pro Thr Gln Thr Gly  Pro Ser Gly
     7025                7030                7035
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Ser Thr  Ser Gln Asp Thr Leu  Thr Leu Asp Thr Ser  Thr Thr Lys
    7040                 7045                 7050

Ser Gln  Ala Lys Thr His Ser  Thr Leu Thr Gln Arg  Phe Pro His
    7055                 7060                 7065

Ser Glu  Met Thr Thr Leu Met  Ser Arg Gly Pro Gly  Asp Met Ser
    7070                 7075                 7080

Trp Gln  Ser Ser Pro Ser Leu  Glu Asn Pro Ser Ser  Leu Pro Ser
    7085                 7090                 7095

Leu Leu  Ser Leu Pro Ala Thr  Thr Ser Pro Pro Pro  Ile Ser Ser
    7100                 7105                 7110

Thr Leu  Pro Val Thr Ile Ser  Ser Ser Pro Leu Pro  Val Thr Ser
    7115                 7120                 7125

Leu Leu  Thr Ser Ser Pro Val  Thr Thr Thr Asp Met  Leu His Thr
    7130                 7135                 7140

Ser Pro  Glu Leu Val Thr Ser  Ser Pro Pro Lys Leu  Ser His Thr
    7145                 7150                 7155

Ser Asp  Glu Arg Leu Thr Thr  Gly Lys Asp Thr Thr  Asn Thr Glu
    7160                 7165                 7170

Ala Val  His Pro Ser Thr Asn  Thr Ala Ala Ser Asn  Val Glu Ile
    7175                 7180                 7185

Pro Ser  Ser Gly His Glu Ser  Pro Ser Ser Ala Leu  Ala Asp Ser
    7190                 7195                 7200

Glu Thr  Ser Lys Ala Thr Ser  Pro Met Phe Ile Thr  Ser Thr Gln
    7205                 7210                 7215

Glu Asp  Thr Thr Val Ala Ile  Ser Thr Pro His Phe  Leu Glu Thr
    7220                 7225                 7230

Ser Arg  Ile Gln Lys Glu Ser  Ile Ser Ser Leu Ser  Pro Lys Leu
    7235                 7240                 7245

Arg Glu  Thr Gly Ser Ser Val  Glu Thr Ser Ser Ala  Ile Glu Thr
    7250                 7255                 7260

Ser Ala  Val Leu Ser Glu Val  Ser Ile Gly Ala Thr  Thr Glu Ile
    7265                 7270                 7275
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Ser Arg Thr Glu Val Thr Ser Ser Ser Arg Thr Ser Ile Ser Gly
7280 7285 7290

Ser Ala Glu Ser Thr Met Leu Pro Glu Ile Ser Thr Thr Arg Lys
7295 7300 7305

Ile Ile Lys Phe Pro Thr Ser Pro Ile Leu Ala Glu Ser Ser Glu
7310 7315 7320

Met Thr Ile Lys Thr Gln Thr Ser Pro Pro Gly Ser Thr Ser Glu
7325 7330 7335

Ser Thr Phe Thr Leu Asp Thr Ser Thr Thr Pro Ser Leu Val Ile
7340 7345 7350

Thr His Ser Thr Met Thr Gln Arg Leu Pro His Ser Glu Ile Thr
7355 7360 7365

Thr Leu Val Ser Arg Gly Ala Gly Asp Val Pro Arg Pro Ser Ser
7370 7375 7380

Leu Pro Val Glu Glu Thr Ser Pro Pro Ser Ser Gln Leu Ser Leu
7385 7390 7395

Ser Ala Met Ile Ser Pro Ser Pro Val Ser Ser Thr Leu Pro Ala
7400 7405 7410

Ser Ser His Ser Ser Ser Ala Ser Val Thr Ser Leu Leu Thr Pro
7415 7420 7425

Gly Gln Val Lys Thr Thr Glu Val Leu Asp Ala Ser Ala Glu Pro
7430 7435 7440

Glu Thr Ser Ser Pro Pro Ser Leu Ser Ser Thr Ser Val Glu Ile
7445 7450 7455

Leu Ala Thr Ser Glu Val Thr Thr Asp Thr Glu Lys Ile His Pro
7460 7465 7470

Phe Ser Asn Thr Ala Val Thr Lys Val Gly Thr Ser Ser Ser Gly
7475 7480 7485

His Glu Ser Pro Ser Ser Val Leu Pro Asp Ser Glu Thr Thr Lys
7490 7495 7500

Ala Thr Ser Ala Met Gly Thr Ile Ser Ile Met Gly Asp Thr Ser
7505 7510 7515

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Val Ser  Thr Leu Thr Pro Ala  Leu Ser Asn Thr Arg  Lys Ile Gln
    7520                 7525                 7530

Ser Glu  Pro Ala Ser Ser Leu  Thr Thr Arg Leu Arg  Glu Thr Ser
    7535                 7540                 7545

Thr Ser  Glu Glu Thr Ser Leu  Ala Thr Glu Ala Asn  Thr Val Leu
    7550                 7555                 7560

Ser Lys  Val Ser Thr Gly Ala  Thr Thr Glu Val Ser  Arg Thr Glu
    7565                 7570                 7575

Ala Ile  Ser Phe Ser Arg Thr  Ser Met Ser Gly Pro  Glu Gln Ser
    7580                 7585                 7590

Thr Met  Ser Gln Asp Ile Ser  Ile Gly Thr Ile Pro  Arg Ile Ser
    7595                 7600                 7605

Ala Ser  Ser Val Leu Thr Glu  Ser Ala Lys Met Thr  Ile Thr Thr
    7610                 7615                 7620

Gln Thr  Gly Pro Ser Glu Ser  Thr Leu Glu Ser Thr  Leu Asn Leu
    7625                 7630                 7635

Asn Thr  Ala Thr Thr Pro Ser  Trp Val Glu Thr His  Ser Ile Val
    7640                 7645                 7650

Ile Gln  Gly Phe Pro His Pro  Glu Met Thr Thr Ser  Met Gly Arg
    7655                 7660                 7665

Gly Pro  Gly Gly Val Ser Trp  Pro Ser Pro Pro Phe  Val Lys Glu
    7670                 7675                 7680

Thr Ser  Pro Pro Ser Ser Pro  Leu Ser Leu Pro Ala  Val Thr Ser
    7685                 7690                 7695

Pro His  Pro Val Ser Thr Thr  Phe Leu Ala His Ile  Pro Pro Ser
    7700                 7705                 7710

Pro Leu  Pro Val Thr Ser Leu  Leu Thr Ser Gly Pro  Ala Thr Thr
    7715                 7720                 7725

Thr Asp  Ile Leu Gly Thr Ser  Thr Glu Pro Gly Thr  Ser Ser Ser
    7730                 7735                 7740

Ser Ser  Leu Ser Thr Thr Ser  His Glu Arg Leu Thr  Thr Tyr Lys
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    7745                7750                7755

Asp Thr Ala His Thr Glu Ala Val His Pro Ser Thr Asn Thr Gly
    7760                7765                7770

Gly Thr Asn Val Ala Thr Thr Ser Ser Gly Tyr Lys Ser Gln Ser
    7775                7780                7785

Ser Val Leu Ala Asp Ser Ser Pro Met Cys Thr Thr Ser Thr Met
    7790                7795                7800

Gly Asp Thr Ser Val Leu Thr Ser Thr Pro Ala Phe Leu Glu Thr
    7805                7810                7815

Arg Arg Ile Gln Thr Glu Leu Ala Ser Ser Leu Thr Pro Gly Leu
    7820                7825                7830

Arg Glu Ser Ser Gly Ser Glu Gly Thr Ser Ser Gly Thr Lys Met
    7835                7840                7845

Ser Thr Val Leu Ser Lys Val Pro Thr Gly Ala Thr Thr Glu Ile
    7850                7855                7860

Ser Lys Glu Asp Val Thr Ser Ile Pro Gly Pro Ala Gln Ser Thr
    7865                7870                7875

Ile Ser Pro Asp Ile Ser Thr Arg Thr Val Ser Trp Phe Ser Thr
    7880                7885                7890

Ser Pro Val Met Thr Glu Ser Ala Glu Ile Thr Met Asn Thr His
    7895                7900                7905

Thr Ser Pro Leu Gly Ala Thr Thr Gln Gly Thr Ser Thr Leu Asp
    7910                7915                7920

Thr Ser Ser Thr Thr Ser Leu Thr Met Thr His Ser Thr Ile Ser
    7925                7930                7935

Gln Gly Phe Ser His Ser Gln Met Ser Thr Leu Met Arg Arg Gly
    7940                7945                7950

Pro Glu Asp Val Ser Trp Met Ser Pro Pro Leu Leu Glu Lys Thr
    7955                7960                7965

Arg Pro Ser Phe Ser Leu Met Ser Ser Pro Ala Thr Thr Ser Pro
    7970                7975                7980
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Ser Pro  Val Ser Ser Thr Leu  Pro Glu Ser Ile Ser  Ser Ser Pro
    7985                 7990                 7995

Leu Pro  Val Thr Ser Leu Leu  Thr Ser Gly Leu Ala  Lys Thr Thr
    8000                 8005                 8010

Asp Met  Leu His Lys Ser Ser  Glu Pro Val Thr Asn  Ser Pro Ala
    8015                 8020                 8025

Asn Leu  Ser Ser Thr Ser Val  Glu Ile Leu Ala Thr  Ser Glu Val
    8030                 8035                 8040

Thr Thr  Asp Thr Glu Lys Thr  His Pro Ser Ser Asn  Arg Thr Val
    8045                 8050                 8055

Thr Asp  Val Gly Thr Ser Ser  Ser Gly His Glu Ser  Thr Ser Phe
    8060                 8065                 8070

Val Leu  Ala Asp Ser Gln Thr  Ser Lys Val Thr Ser  Pro Met Val
    8075                 8080                 8085

Ile Thr  Ser Thr Met Glu Asp  Thr Ser Val Ser Thr  Ser Thr Pro
    8090                 8095                 8100

Gly Phe  Phe Glu Thr Ser Arg  Ile Gln Thr Glu Pro  Thr Ser Ser
    8105                 8110                 8115

Leu Thr  Leu Gly Leu Arg Lys  Thr Ser Ser Ser Glu  Gly Thr Ser
    8120                 8125                 8130

Leu Ala  Thr Glu Met Ser Thr  Val Leu Ser Gly Val  Pro Thr Gly
    8135                 8140                 8145

Ala Thr  Ala Glu Val Ser Arg  Thr Glu Val Thr Ser  Ser Ser Arg
    8150                 8155                 8160

Thr Ser  Ile Ser Gly Phe Ala  Gln Leu Thr Val Ser  Pro Glu Thr
    8165                 8170                 8175

Ser Thr  Glu Thr Ile Thr Arg  Leu Pro Thr Ser Ser  Ile Met Thr
    8180                 8185                 8190

Glu Ser  Ala Glu Met Met Ile  Lys Thr Gln Thr Asp  Pro Pro Gly
    8195                 8200                 8205

Ser Thr  Pro Glu Ser Thr His  Thr Val Asp Ile Ser  Thr Thr Pro
    8210                 8215                 8220
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Asn Trp  Val Glu Thr His Ser  Thr Val Thr Gln Arg  Phe Ser His
    8225                 8230                 8235

Ser Glu  Met Thr Thr Leu Val  Ser Arg Ser Pro Gly  Asp Met Leu
    8240                 8245                 8250

Trp Pro  Ser Gln Ser Ser Val  Glu Glu Thr Ser Ser  Ala Ser Ser
    8255                 8260                 8265

Leu Leu  Ser Leu Pro Ala Thr  Thr Ser Pro Ser Pro  Val Ser Ser
    8270                 8275                 8280

Thr Leu  Val Glu Asp Phe Pro  Ser Ala Ser Leu Pro  Val Thr Ser
    8285                 8290                 8295

Leu Leu  Asn Pro Gly Leu Val  Ile Thr Thr Asp Arg  Met Gly Ile
    8300                 8305                 8310

Ser Arg  Glu Pro Gly Thr Ser  Ser Thr Ser Asn Leu  Ser Ser Thr
    8315                 8320                 8325

Ser His  Glu Arg Leu Thr Thr  Leu Glu Asp Thr Val  Asp Thr Glu
    8330                 8335                 8340

Asp Met  Gln Pro Ser Thr His  Thr Ala Val Thr Asn  Val Arg Thr
    8345                 8350                 8355

Ser Ile  Ser Gly His Glu Ser  Gln Ser Ser Val Leu  Ser Asp Ser
    8360                 8365                 8370

Glu Thr  Pro Lys Ala Thr Ser  Pro Met Gly Thr Thr  Tyr Thr Met
    8375                 8380                 8385

Gly Glu  Thr Ser Val Ser Ile  Ser Thr Ser Asp Phe  Phe Glu Thr
    8390                 8395                 8400

Ser Arg  Ile Gln Ile Glu Pro  Thr Ser Ser Leu Thr  Ser Gly Leu
    8405                 8410                 8415

Arg Glu  Thr Ser Ser Ser Glu  Arg Ile Ser Ser Ala  Thr Glu Gly
    8420                 8425                 8430

Ser Thr  Val Leu Ser Glu Val  Pro Ser Gly Ala Thr  Thr Glu Val
    8435                 8440                 8445

Ser Arg  Thr Glu Val Ile Ser  Ser Arg Gly Thr Ser  Met Ser Gly
    8450                 8455                 8460
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Pro Asp Gln Phe Thr Ile Ser Pro Asp Ile Ser Thr Glu Ala Ile
8465 8470 8475

Thr Arg Leu Ser Thr Ser Pro Ile Met Thr Glu Ser Ala Glu Ser
8480 8485 8490

Ala Ile Thr Ile Glu Thr Gly Ser Pro Gly Ala Thr Ser Glu Gly
8495 8500 8505

Thr Leu Thr Leu Asp Thr Ser Thr Thr Thr Phe Trp Ser Gly Thr
8510 8515 8520

His Ser Thr Ala Ser Pro Gly Phe Ser His Ser Glu Met Thr Thr
8525 8530 8535

Leu Met Ser Arg Thr Pro Gly Asp Val Pro Trp Pro Ser Leu Pro
8540 8545 8550

Ser Val Glu Glu Ala Ser Ser Val Ser Ser Ser Leu Ser Ser Pro
8555 8560 8565

Ala Met Thr Ser Thr Ser Phe Phe Ser Thr Leu Pro Glu Ser Ile
8570 8575 8580

Ser Ser Ser Pro His Pro Val Thr Ala Leu Leu Thr Leu Gly Pro
8585 8590 8595

Val Lys Thr Thr Asp Met Leu Arg Thr Ser Ser Glu Pro Glu Thr
8600 8605 8610

Ser Ser Pro Pro Asn Leu Ser Ser Thr Ser Ala Glu Ile Leu Ala
8615 8620 8625

Thr Ser Glu Val Thr Lys Asp Arg Glu Lys Ile His Pro Ser Ser
8630 8635 8640

Asn Thr Pro Val Val Asn Val Gly Thr Val Ile Tyr Lys His Leu
8645 8650 8655

Ser Pro Ser Ser Val Leu Ala Asp Leu Val Thr Thr Lys Pro Thr
8660 8665 8670

Ser Pro Met Ala Thr Thr Ser Thr Leu Gly Asn Thr Ser Val Ser
8675 8680 8685

Thr Ser Thr Pro Ala Phe Pro Glu Thr Met Met Thr Gln Pro Thr

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    8690                8695                8700

Ser Ser  Leu Thr Ser Gly Leu  Arg Glu Ile Ser Thr  Ser Gln Glu
    8705                8710                8715

Thr Ser  Ser Ala Thr Glu Arg  Ser Ala Ser Leu Ser  Gly Met Pro
    8720                8725                8730

Thr Gly  Ala Thr Thr Lys Val  Ser Arg Thr Glu Ala  Leu Ser Leu
    8735                8740                8745

Gly Arg  Thr Ser Thr Pro Gly  Pro Ala Gln Ser Thr  Ile Ser Pro
    8750                8755                8760

Glu Ile  Ser Thr Glu Thr Ile  Thr Arg Ile Ser Thr  Pro Leu Thr
    8765                8770                8775

Thr Thr  Gly Ser Ala Glu Met  Thr Ile Thr Pro Lys  Thr Gly His
    8780                8785                8790

Ser Gly  Ala Ser Ser Gln Gly  Thr Phe Thr Leu Asp  Thr Ser Ser
    8795                8800                8805

Arg Ala  Ser Trp Pro Gly Thr  His Ser Ala Ala Thr  His Arg Ser
    8810                8815                8820

Pro His  Ser Gly Met Thr Thr  Pro Met Ser Arg Gly  Pro Glu Asp
    8825                8830                8835

Val Ser  Trp Pro Ser Arg Pro  Ser Val Glu Lys Thr  Ser Pro Pro
    8840                8845                8850

Ser Ser  Leu Val Ser Leu Ser  Ala Val Thr Ser Pro  Ser Pro Leu
    8855                8860                8865

Tyr Ser  Thr Pro Ser Glu Ser  Ser His Ser Ser Pro  Leu Arg Val
    8870                8875                8880

Thr Ser  Leu Phe Thr Pro Val  Met Met Lys Thr Thr  Asp Met Leu
    8885                8890                8895

Asp Thr  Ser Leu Glu Pro Val  Thr Thr Ser Pro Pro  Ser Met Asn
    8900                8905                8910

Ile Thr  Ser Asp Glu Ser Leu  Ala Thr Ser Lys Ala  Thr Met Glu
    8915                8920                8925
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Thr Glu  Ala Ile Gln Leu Ser  Glu Asn Thr Ala Val  Thr Gln Met
    8930                 8935                 8940

Gly Thr  Ile Ser Ala Arg Gln  Glu Phe Tyr Ser Ser  Tyr Pro Gly
    8945                 8950                 8955

Leu Pro  Glu Pro Ser Lys Val  Thr Ser Pro Val Val  Thr Ser Ser
    8960                 8965                 8970

Thr Ile  Lys Asp Ile Val Ser  Thr Thr Ile Pro Ala  Ser Ser Glu
    8975                 8980                 8985

Ile Thr  Arg Ile Glu Met Glu  Ser Thr Ser Thr Leu  Thr Pro Thr
    8990                 8995                 9000

Pro Arg  Glu Thr Ser Thr Ser  Gln Glu Ile His Ser  Ala Thr Lys
    9005                 9010                 9015

Pro Ser  Thr Val Pro Tyr Lys  Ala Leu Thr Ser Ala  Thr Ile Glu
    9020                 9025                 9030

Asp Ser  Met Thr Gln Val Met  Ser Ser Ser Arg Gly  Pro Ser Pro
    9035                 9040                 9045

Asp Gln  Ser Thr Met Ser Gln  Asp Ile Ser Thr Glu  Val Ile Thr
    9050                 9055                 9060

Arg Leu  Ser Thr Ser Pro Ile  Lys Thr Glu Ser Thr  Glu Met Thr
    9065                 9070                 9075

Ile Thr  Thr Gln Thr Gly Ser  Pro Gly Ala Thr Ser  Arg Gly Thr
    9080                 9085                 9090

Leu Thr  Leu Asp Thr Ser Thr  Thr Phe Met Ser Gly  Thr His Ser
    9095                 9100                 9105

Thr Ala  Ser Gln Gly Phe Ser  His Ser Gln Met Thr  Ala Leu Met
    9110                 9115                 9120

Ser Arg  Thr Pro Gly Asp Val  Pro Trp Leu Ser His  Pro Ser Val
    9125                 9130                 9135

Glu Glu  Ala Ser Ser Ala Ser  Phe Ser Leu Ser Ser  Pro Val Met
    9140                 9145                 9150

Thr Ser  Ser Ser Pro Val Ser  Ser Thr Leu Pro Asp  Ser Ile His
    9155                 9160                 9165
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Ser Ser  Ser Leu Pro Val Thr  Ser Leu Leu Thr Ser  Gly Leu Val
    9170                 9175                 9180

Lys Thr  Thr Glu Leu Leu Gly  Thr Ser Ser Glu Pro  Glu Thr Ser
    9185                 9190                 9195

Ser Pro  Pro Asn Leu Ser Ser  Thr Ser Ala Glu Ile  Leu Ala Ile
    9200                 9205                 9210

Thr Glu  Val Thr Thr Asp Thr  Glu Lys Leu Glu Met  Thr Asn Val
    9215                 9220                 9225

Val Thr  Ser Gly Tyr Thr His  Glu Ser Pro Ser Ser  Val Leu Ala
    9230                 9235                 9240

Asp Ser  Val Thr Thr Lys Ala  Thr Ser Ser Met Gly  Ile Thr Tyr
    9245                 9250                 9255

Pro Thr  Gly Asp Thr Asn Val  Leu Thr Ser Thr Pro  Ala Phe Ser
    9260                 9265                 9270

Asp Thr  Ser Arg Ile Gln Thr  Lys Ser Lys Leu Ser  Leu Thr Pro
    9275                 9280                 9285

Gly Leu  Met Glu Thr Ser Ile  Ser Glu Glu Thr Ser  Ser Ala Thr
    9290                 9295                 9300

Glu Lys  Ser Thr Val Leu Ser  Ser Val Pro Thr Gly  Ala Thr Thr
    9305                 9310                 9315

Glu Val  Ser Arg Thr Glu Ala  Ile Ser Ser Ser Arg  Thr Ser Ile
    9320                 9325                 9330

Pro Gly  Pro Ala Gln Ser Thr  Met Ser Ser Asp Thr  Ser Met Glu
    9335                 9340                 9345

Thr Ile  Thr Arg Ile Ser Thr  Pro Leu Thr Arg Lys  Glu Ser Thr
    9350                 9355                 9360

Asp Met  Ala Ile Thr Pro Lys  Thr Gly Pro Ser Gly  Ala Thr Ser
    9365                 9370                 9375

Gln Gly  Thr Phe Thr Leu Asp  Ser Ser Ser Thr Ala  Ser Trp Pro
    9380                 9385                 9390

Gly Thr  His Ser Ala Thr Thr  Gln Arg Phe Pro Gln  Ser Val Val
    9395                 9400                 9405
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Thr Thr  Pro Met Ser Arg Gly  Pro Glu Asp Val Ser  Trp Pro Ser
    9410                 9415                 9420

Pro Leu  Ser Val Glu Lys Asn  Ser Pro Pro Ser Ser  Leu Val Ser
    9425                 9430                 9435

Ser Ser  Ser Val Thr Ser Pro  Ser Pro Leu Tyr Ser  Thr Pro Ser
    9440                 9445                 9450

Gly Ser  Ser His Ser Ser Pro  Val Pro Val Thr Ser  Leu Phe Thr
    9455                 9460                 9465

Ser Ile  Met Met Lys Ala Thr  Asp Met Leu Asp Ala  Ser Leu Glu
    9470                 9475                 9480

Pro Glu  Thr Thr Ser Ala Pro  Asn Met Asn Ile Thr  Ser Asp Glu
    9485                 9490                 9495

Ser Leu  Ala Ala Ser Lys Ala  Thr Thr Glu Thr Glu  Ala Ile His
    9500                 9505                 9510

Val Phe  Glu Asn Thr Ala Ala  Ser His Val Glu Thr  Thr Ser Ala
    9515                 9520                 9525

Thr Glu  Glu Leu Tyr Ser Ser  Ser Pro Gly Phe Ser  Glu Pro Thr
    9530                 9535                 9540

Lys Val  Ile Ser Pro Val Val  Thr Ser Ser Ser Ile  Arg Asp Asn
    9545                 9550                 9555

Met Val  Ser Thr Thr Met Pro  Gly Ser Ser Gly Ile  Thr Arg Ile
    9560                 9565                 9570

Glu Ile  Glu Ser Met Ser Ser  Leu Thr Pro Gly Leu  Arg Glu Thr
    9575                 9580                 9585

Arg Thr  Ser Gln Asp Ile Thr  Ser Ser Thr Glu Thr  Ser Thr Val
    9590                 9595                 9600

Leu Tyr  Lys Met Pro Ser Gly  Ala Thr Pro Glu Val  Ser Arg Thr
    9605                 9610                 9615

Glu Val  Met Pro Ser Ser Arg  Thr Ser Ile Pro Gly  Pro Ala Gln
    9620                 9625                 9630

Ser Thr  Met Ser Leu Asp Ile  Ser Asp Glu Val Val  Thr Arg Leu
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     9635                9640                9645

Ser Thr  Ser Pro Ile Met Thr  Glu Ser Ala Glu Ile  Thr Ile Thr
     9650                9655                9660

Thr Gln  Thr Gly Tyr Ser Leu  Ala Thr Ser Gln Val  Thr Leu Pro
     9665                9670                9675

Leu Gly  Thr Ser Met Thr Phe  Leu Ser Gly Thr His  Ser Thr Met
     9680                9685                9690

Ser Gln  Gly Leu Ser His Ser  Glu Met Thr Asn Leu  Met Ser Arg
     9695                9700                9705

Gly Pro  Glu Ser Leu Ser Trp  Thr Ser Pro Arg Phe  Val Glu Thr
     9710                9715                9720

Thr Arg  Ser Ser Ser Ser Leu  Thr Ser Leu Pro Leu  Thr Thr Ser
     9725                9730                9735

Leu Ser  Pro Val Ser Ser Thr  Leu Leu Asp Ser Ser  Pro Ser Ser
     9740                9745                9750

Pro Leu  Pro Val Thr Ser Leu  Ile Leu Pro Gly Leu  Val Lys Thr
     9755                9760                9765

Thr Glu  Val Leu Asp Thr Ser  Ser Glu Pro Lys Thr  Ser Ser Ser
     9770                9775                9780

Pro Asn  Leu Ser Ser Thr Ser  Val Glu Ile Pro Ala  Thr Ser Glu
     9785                9790                9795

Ile Met  Thr Asp Thr Glu Lys  Ile His Pro Ser Ser  Asn Thr Ala
     9800                9805                9810

Val Ala  Lys Val Arg Thr Ser  Ser Ser Val His Glu  Ser His Ser
     9815                9820                9825

Ser Val  Leu Ala Asp Ser Glu  Thr Thr Ile Thr Ile  Pro Ser Met
     9830                9835                9840

Gly Ile  Thr Ser Ala Val Asp  Asp Thr Thr Val Phe  Thr Ser Asn
     9845                9850                9855

Pro Ala  Phe Ser Glu Thr Arg  Arg Ile Pro Thr Glu  Pro Thr Phe
     9860                9865                9870
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Ser Leu Thr Pro Gly Phe Arg Glu Thr Ser Thr Ser Glu Glu Thr
9875 9880 9885

Thr Ser Ile Thr Glu Thr Ser Ala Val Leu Tyr Gly Val Pro Thr
9890 9895 9900

Ser Ala Thr Thr Glu Val Ser Met Thr Glu Ile Met Ser Ser Asn
9905 9910 9915

Arg Ile His Ile Pro Asp Ser Asp Gln Ser Thr Met Ser Pro Asp
9920 9925 9930

Ile Ile Thr Glu Val Ile Thr Arg Leu Ser Ser Ser Ser Met Met
9935 9940 9945

Ser Glu Ser Thr Gln Met Thr Ile Thr Thr Gln Lys Ser Ser Pro
9950 9955 9960

Gly Ala Thr Ala Gln Ser Thr Leu Thr Leu Ala Thr Thr Thr Ala
9965 9970 9975

Pro Leu Ala Arg Thr His Ser Thr Val Pro Pro Arg Phe Leu His
9980 9985 9990

Ser Glu Met Thr Thr Leu Met Ser Arg Ser Pro Glu Asn Pro Ser
9995 10000 10005

Trp Lys Ser Ser Leu Phe Val Glu Lys Thr Ser Ser Ser Ser Ser
10010 10015 10020

Leu Leu Ser Leu Pro Val Thr Thr Ser Pro Ser Val Ser Ser Thr
10025 10030 10035

Leu Pro Gln Ser Ile Pro Ser Ser Ser Phe Ser Val Thr Ser Leu
10040 10045 10050

Leu Thr Pro Gly Met Val Lys Thr Thr Asp Thr Ser Thr Glu Pro
10055 10060 10065

Gly Thr Ser Leu Ser Pro Asn Leu Ser Gly Thr Ser Val Glu Ile
10070 10075 10080

Leu Ala Ala Ser Glu Val Thr Thr Asp Thr Glu Lys Ile His Pro
10085 10090 10095

Ser Ser Ser Met Ala Val Thr Asn Val Gly Thr Thr Ser Ser Gly
10100 10105 10110

His Glu Leu Tyr Ser Ser Val Ser Ile His Ser Glu Pro Ser Lys
10115 10120 10125

Ala Thr Tyr Pro Val Gly Thr Pro Ser Ser Met Ala Glu Thr Ser
10130 10135 10140

Ile Ser Thr Ser Met Pro Ala Asn Phe Glu Thr Thr Gly Phe Glu
10145 10150 10155

Ala Glu Pro Phe Ser His Leu Thr Ser Gly Phe Arg Lys Thr Asn
10160 10165 10170

Met Ser Leu Asp Thr Ser Ser Val Thr Pro Thr Asn Thr Pro Ser
10175 10180 10185

Ser Pro Gly Ser Thr His Leu Leu Gln Ser Ser Lys Thr Asp Phe
10190 10195 10200

Thr Ser Ser Ala Lys Thr Ser Ser Pro Asp Trp Pro Pro Ala Ser
10205 10210 10215

Gln Tyr Thr Glu Ile Pro Val Asp Ile Ile Thr Pro Phe Asn Ala
10220 10225 10230

Ser Pro Ser Ile Thr Glu Ser Thr Gly Ile Thr Ser Phe Pro Glu
10235 10240 10245

Ser Arg Phe Thr Met Ser Val Thr Glu Ser Thr His His Leu Ser
10250 10255 10260

Thr Asp Leu Leu Pro Ser Ala Glu Thr Ile Ser Thr Gly Thr Val
10265 10270 10275

Met Pro Ser Leu Ser Glu Ala Met Thr Ser Phe Ala Thr Thr Gly
10280 10285 10290

Val Pro Arg Ala Ile Ser Gly Ser Gly Ser Pro Phe Ser Arg Thr
10295 10300 10305

Glu Ser Gly Pro Gly Asp Ala Thr Leu Ser Thr Ile Ala Glu Ser
10310 10315 10320

Leu Pro Ser Ser Thr Pro Val Pro Phe Ser Ser Ser Thr Phe Thr
10325 10330 10335

Thr Thr Asp Ser Ser Thr Ile Pro Ala Leu His Glu Ile Thr Ser
10340 10345 10350

Ser Ser Ala Thr Pro Tyr Arg Val Asp Thr Ser Leu Gly Thr Glu
10355 10360 10365

Ser Ser Thr Thr Glu Gly Arg Leu Val Met Val Ser Thr Leu Asp
10370 10375 10380

Thr Ser Ser Gln Pro Gly Arg Thr Ser Ser Ser Pro Ile Leu Asp
10385 10390 10395

Thr Arg Met Thr Glu Ser Val Glu Leu Gly Thr Val Thr Ser Ala
10400 10405 10410

Tyr Gln Val Pro Ser Leu Ser Thr Arg Leu Thr Arg Thr Asp Gly
10415 10420 10425

Ile Met Glu His Ile Thr Lys Ile Pro Asn Glu Ala Ala His Arg
10430 10435 10440

Gly Thr Ile Arg Pro Val Lys Gly Pro Gln Thr Ser Thr Ser Pro
10445 10450 10455

Ala Ser Pro Lys Gly Leu His Thr Gly Gly Thr Lys Arg Met Glu
10460 10465 10470

Thr Thr Thr Thr Ala Leu Lys Thr Thr Thr Thr Ala Leu Lys Thr
10475 10480 10485

Thr Ser Arg Ala Thr Leu Thr Thr Ser Val Tyr Thr Pro Thr Leu
10490 10495 10500

Gly Thr Leu Thr Pro Leu Asn Ala Ser Met Gln Met Ala Ser Thr
10505 10510 10515

Ile Pro Thr Glu Met Met Ile Thr Thr Pro Tyr Val Phe Pro Asp
10520 10525 10530

Val Pro Glu Thr Thr Ser Ser Leu Ala Thr Ser Leu Gly Ala Glu
10535 10540 10545

Thr Ser Thr Ala Leu Pro Arg Thr Thr Pro Ser Val Phe Asn Arg
10550 10555 10560

Glu Ser Glu Thr Thr Ala Ser Leu Val Ser Arg Ser Gly Ala Glu
10565 10570 10575

Arg Ser Pro Val Ile Gln Thr Leu Asp Val Ser Ser Ser Glu Pro

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    10580                10585                10590

Asp Thr  Thr Ala Ser Trp Val  Ile His Pro Ala Glu  Thr Ile Pro
    10595                10600                10605

Thr Val  Ser Lys Thr Thr Pro  Asn Phe Phe His Ser  Glu Leu Asp
    10610                10615                10620

Thr Val  Ser Ser Thr Ala Thr  Ser His Gly Ala Asp  Val Ser Ser
    10625                10630                10635

Ala Ile  Pro Thr Asn Ile Ser  Pro Ser Glu Leu Asp  Ala Leu Thr
    10640                10645                10650

Pro Leu  Val Thr Ile Ser Gly  Thr Asp Thr Ser Thr  Thr Phe Pro
    10655                10660                10665

Thr Leu  Thr Lys Ser Pro His  Glu Thr Glu Thr Arg  Thr Thr Trp
    10670                10675                10680

Leu Thr  His Pro Ala Glu Thr  Ser Ser Thr Ile Pro  Arg Thr Ile
    10685                10690                10695

Pro Asn  Phe Ser His His Glu  Ser Asp Ala Thr Pro  Ser Ile Ala
    10700                10705                10710

Thr Ser  Pro Gly Ala Glu Thr  Ser Ser Ala Ile Pro  Ile Met Thr
    10715                10720                10725

Val Ser  Pro Gly Ala Glu Asp  Leu Val Thr Ser Gln  Val Thr Ser
    10730                10735                10740

Ser Gly  Thr Asp Arg Asn Met  Thr Ile Pro Thr Leu  Thr Leu Ser
    10745                10750                10755

Pro Gly  Glu Pro Lys Thr Ile  Ala Ser Leu Val Thr  His Pro Glu
    10760                10765                10770

Ala Gln  Thr Ser Ser Ala Ile  Pro Thr Ser Thr Ile  Ser Pro Ala
    10775                10780                10785

Val Ser  Arg Leu Val Thr Ser  Met Val Thr Ser Leu  Ala Ala Lys
    10790                10795                10800

Thr Ser  Thr Thr Asn Arg Ala  Leu Thr Asn Ser Pro  Gly Glu Pro
    10805                10810                10815
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Ala Thr   Thr Val Ser Leu Val   Thr His Pro Ala Gln   Thr Ser Pro
    10820                10825                10830

Thr Val   Pro Trp Thr Thr Ser   Ile Phe Phe His Ser   Lys Ser Asp
    10835                10840                10845

Thr Thr   Pro Ser Met Thr Thr   Ser His Gly Ala Glu   Ser Ser Ser
    10850                10855                10860

Ala Val   Pro Thr Pro Thr Val   Ser Thr Glu Val Pro   Gly Val Val
    10865                10870                10875

Thr Pro   Leu Val Thr Ser Ser   Arg Ala Val Ile Ser   Thr Thr Ile
    10880                10885                10890

Pro Ile   Leu Thr Leu Ser Pro   Gly Glu Pro Glu Thr   Thr Pro Ser
    10895                10900                10905

Met Ala   Thr Ser His Gly Glu   Glu Ala Ser Ser Ala   Ile Pro Thr
    10910                10915                10920

Pro Thr   Val Ser Pro Gly Val   Pro Gly Val Val Thr   Ser Leu Val
    10925                10930                10935

Thr Ser   Ser Arg Ala Val Thr   Ser Thr Thr Ile Pro   Ile Leu Thr
    10940                10945                10950

Phe Ser   Leu Gly Glu Pro Glu   Thr Thr Pro Ser Met   Ala Thr Ser
    10955                10960                10965

His Gly   Thr Glu Ala Gly Ser   Ala Val Pro Thr Val   Leu Pro Glu
    10970                10975                10980

Val Pro   Gly Met Val Thr Ser   Leu Val Ala Ser Ser   Arg Ala Val
    10985                10990                10995

Thr Ser   Thr Thr Leu Pro Thr   Leu Thr Leu Ser Pro   Gly Glu Pro
    11000                11005                11010

Glu Thr   Thr Pro Ser Met Ala   Thr Ser His Gly Ala   Glu Ala Ser
    11015                11020                11025

Ser Thr   Val Pro Thr Val Ser   Pro Glu Val Pro Gly   Val Val Thr
    11030                11035                11040

Ser Leu   Val Thr Ser Ser Ser   Gly Val Asn Ser Thr   Ser Ile Pro
    11045                11050                11055
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Thr Leu   Ile Leu Ser Pro Gly   Glu Leu Glu Thr Thr   Pro Ser Met
    11060                 11065                 11070

Ala Thr   Ser His Gly Ala Glu   Ala Ser Ser Ala Val   Pro Thr Pro
    11075                 11080                 11085

Thr Val   Ser Pro Gly Val Ser   Gly Val Val Thr Pro   Leu Val Thr
    11090                 11095                 11100

Ser Ser   Arg Ala Val Thr Ser   Thr Thr Ile Pro Ile   Leu Thr Leu
    11105                 11110                 11115

Ser Ser   Ser Glu Pro Glu Thr   Thr Pro Ser Met Ala   Thr Ser His
    11120                 11125                 11130

Gly Val   Glu Ala Ser Ser Ala   Val Leu Thr Val Ser   Pro Glu Val
    11135                 11140                 11145

Pro Gly   Met Val Thr Ser Leu   Val Thr Ser Ser Arg   Ala Val Thr
    11150                 11155                 11160

Ser Thr   Thr Ile Pro Thr Leu   Thr Ile Ser Ser Asp   Glu Pro Glu
    11165                 11170                 11175

Thr Thr   Thr Ser Leu Val Thr   His Ser Glu Ala Lys   Met Ile Ser
    11180                 11185                 11190

Ala Ile   Pro Thr Leu Ala Val   Ser Pro Thr Val Gln   Gly Leu Val
    11195                 11200                 11205

Thr Ser   Leu Val Thr Ser Ser   Gly Ser Glu Thr Ser   Ala Phe Ser
    11210                 11215                 11220

Asn Leu   Thr Val Ala Ser Ser   Gln Pro Glu Thr Ile   Asp Ser Trp
    11225                 11230                 11235

Val Ala   His Pro Gly Thr Glu   Ala Ser Ser Val Val   Pro Thr Leu
    11240                 11245                 11250

Thr Val   Ser Thr Gly Glu Pro   Phe Thr Asn Ile Ser   Leu Val Thr
    11255                 11260                 11265

His Pro   Ala Glu Ser Ser Ser   Thr Leu Pro Arg Thr   Thr Ser Arg
    11270                 11275                 11280

Phe Ser   His Ser Glu Leu Asp   Thr Met Pro Ser Thr   Val Thr Ser
    11285                 11290                 11295
```

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Pro Glu   Ala Glu Ser Ser Ser   Ala Ile Ser Thr Thr   Ile Ser Pro
    11300                 11305                 11310

Gly Ile   Pro Gly Val Leu Thr   Ser Leu Val Thr Ser   Ser Gly Arg
    11315                 11320                 11325

Asp Ile   Ser Ala Thr Phe Pro   Thr Val Pro Glu Ser   Pro His Glu
    11330                 11335                 11340

Ser Glu   Ala Thr Ala Ser Trp   Val Thr His Pro Ala   Val Thr Ser
    11345                 11350                 11355

Thr Thr   Val Pro Arg Thr Thr   Pro Asn Tyr Ser His   Ser Glu Pro
    11360                 11365                 11370

Asp Thr   Thr Pro Ser Ile Ala   Thr Ser Pro Gly Ala   Glu Ala Thr
    11375                 11380                 11385

Ser Asp   Phe Pro Thr Ile Thr   Val Ser Pro Asp Val   Pro Asp Met
    11390                 11395                 11400

Val Thr   Ser Gln Val Thr Ser   Ser Gly Thr Asp Thr   Ser Ile Thr
    11405                 11410                 11415

Ile Pro   Thr Leu Thr Leu Ser   Ser Gly Glu Pro Glu   Thr Thr Thr
    11420                 11425                 11430

Ser Phe   Ile Thr Tyr Ser Glu   Thr His Thr Ser Ser   Ala Ile Pro
    11435                 11440                 11445

Thr Leu   Pro Val Ser Pro Gly   Ala Ser Lys Met Leu   Thr Ser Leu
    11450                 11455                 11460

Val Ile   Ser Ser Gly Thr Asp   Ser Thr Thr Thr Phe   Pro Thr Leu
    11465                 11470                 11475

Thr Glu   Thr Pro Tyr Glu Pro   Glu Thr Thr Ala Ile   Gln Leu Ile
    11480                 11485                 11490

His Pro   Ala Glu Thr Asn Thr   Met Val Pro Arg Thr   Thr Pro Lys
    11495                 11500                 11505

Phe Ser   His Ser Lys Ser Asp   Thr Thr Leu Pro Val   Ala Ile Thr
    11510                 11515                 11520

Ser Pro   Gly Pro Glu Ala Ser   Ser Ala Val Ser Thr   Thr Thr Ile
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    11525                11530                11535

Ser Pro  Asp Met Ser Asp Leu  Val Thr Ser Leu Val  Pro Ser Ser
    11540                11545                11550

Gly Thr  Asp Thr Ser Thr Thr  Phe Pro Thr Leu Ser  Glu Thr Pro
    11555                11560                11565

Tyr Glu  Pro Glu Thr Thr Ala  Thr Trp Leu Thr His  Pro Ala Glu
    11570                11575                11580

Thr Ser  Thr Thr Val Ser Gly  Thr Ile Pro Asn Phe  Ser His Arg
    11585                11590                11595

Gly Ser  Asp Thr Ala Pro Ser  Met Val Thr Ser Pro  Gly Val Asp
    11600                11605                11610

Thr Arg  Ser Gly Val Pro Thr  Thr Thr Ile Pro Pro  Ser Ile Pro
    11615                11620                11625

Gly Val  Val Thr Ser Gln Val  Thr Ser Ser Ala Thr  Asp Thr Ser
    11630                11635                11640

Thr Ala  Ile Pro Thr Leu Thr  Pro Ser Pro Gly Glu  Pro Glu Thr
    11645                11650                11655

Thr Ala  Ser Ser Ala Thr His  Pro Gly Thr Gln Thr  Gly Phe Thr
    11660                11665                11670

Val Pro  Ile Arg Thr Val Pro  Ser Ser Glu Pro Asp  Thr Met Ala
    11675                11680                11685

Ser Trp  Val Thr His Pro Pro  Gln Thr Ser Thr Pro  Val Ser Arg
    11690                11695                11700

Thr Thr  Ser Ser Phe Ser His  Ser Ser Pro Asp Ala  Thr Pro Val
    11705                11710                11715

Met Ala  Thr Ser Pro Arg Thr  Glu Ala Ser Ser Ala  Val Leu Thr
    11720                11725                11730

Thr Ile  Ser Pro Gly Ala Pro  Glu Met Val Thr Ser  Gln Ile Thr
    11735                11740                11745

Ser Ser  Gly Ala Ala Thr Ser  Thr Thr Val Pro Thr  Leu Thr His
    11750                11755                11760
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Ser Pro   Gly Met Pro Glu Thr   Thr Ala Leu Leu Ser   Thr His Pro
    11765                 11770                 11775

Arg Thr   Glu Thr Ser Lys Thr   Phe Pro Ala Ser Thr   Val Phe Pro
    11780                 11785                 11790

Gln Val   Ser Glu Thr Thr Ala   Ser Leu Thr Ile Arg   Pro Gly Ala
    11795                 11800                 11805

Glu Thr   Ser Thr Ala Leu Pro   Thr Gln Thr Thr Ser   Ser Leu Phe
    11810                 11815                 11820

Thr Leu   Leu Val Thr Gly Thr   Ser Arg Val Asp Leu   Ser Pro Thr
    11825                 11830                 11835

Ala Ser   Pro Gly Val Ser Ala   Lys Thr Ala Pro Leu   Ser Thr His
    11840                 11845                 11850

Pro Gly   Thr Glu Thr Ser Thr   Met Ile Pro Thr Ser   Thr Leu Ser
    11855                 11860                 11865

Leu Gly   Leu Leu Glu Thr Thr   Gly Leu Leu Ala Thr   Ser Ser Ser
    11870                 11875                 11880

Ala Glu   Thr Ser Thr Ser Thr   Leu Thr Leu Thr Val   Ser Pro Ala
    11885                 11890                 11895

Val Ser   Gly Leu Ser Ser Ala   Ser Ile Thr Thr Asp   Lys Pro Gln
    11900                 11905                 11910

Thr Val   Thr Ser Trp Asn Thr   Glu Thr Ser Pro Ser   Val Thr Ser
    11915                 11920                 11925

Val Gly   Pro Pro Glu Phe Ser   Arg Thr Val Thr Gly   Thr Thr Met
    11930                 11935                 11940

Thr Leu   Ile Pro Ser Glu Met   Pro Thr Pro Pro Lys   Thr Ser His
    11945                 11950                 11955

Gly Glu   Gly Val Ser Pro Thr   Thr Ile Leu Arg Thr   Thr Met Val
    11960                 11965                 11970

Glu Ala   Thr Asn Leu Ala Thr   Thr Gly Ser Ser Pro   Thr Val Ala
    11975                 11980                 11985

Lys Thr   Thr Thr Thr Phe Asn   Thr Leu Ala Gly Ser   Leu Phe Thr
    11990                 11995                 12000
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Pro Leu   Thr Thr Pro Gly Met   Ser Thr Leu Ala Ser   Glu Ser Val
    12005                12010                12015

Thr Ser   Arg Thr Ser Tyr Asn   His Arg Ser Trp Ile   Ser Thr Thr
    12020                12025                12030

Ser Ser   Tyr Asn Arg Arg Tyr   Trp Thr Pro Ala Thr   Ser Thr Pro
    12035                12040                12045

Val Thr   Ser Thr Phe Ser Pro   Gly Ile Ser Thr Ser   Ser Ile Pro
    12050                12055                12060

Ser Ser   Thr Ala Ala Thr Val   Pro Phe Met Val Pro   Phe Thr Leu
    12065                12070                12075

Asn Phe   Thr Ile Thr Asn Leu   Gln Tyr Glu Glu Asp   Met Arg His
    12080                12085                12090

Pro Gly   Ser Arg Lys Phe Asn   Ala Thr Glu Arg Glu   Leu Gln Gly
    12095                12100                12105

Leu Leu   Lys Pro Leu Phe Arg   Asn Ser Ser Leu Glu   Tyr Leu Tyr
    12110                12115                12120

Ser Gly   Cys Arg Leu Ala Ser   Leu Arg Pro Glu Lys   Asp Ser Ser
    12125                12130                12135

Ala Thr   Ala Val Asp Ala Ile   Cys Thr His Arg Pro   Asp Pro Glu
    12140                12145                12150

Asp Leu   Gly Leu Asp Arg Glu   Arg Leu Tyr Trp Glu   Leu Ser Asn
    12155                12160                12165

Leu Thr   Asn Gly Ile Gln Glu   Leu Gly Pro Tyr Thr   Leu Asp Arg
    12170                12175                12180

Asn Ser   Leu Tyr Val Asn Gly   Phe Thr His Arg Ser   Ser Met Pro
    12185                12190                12195

Thr Thr   Ser Thr Pro Gly Thr   Ser Thr Val Asp Val   Gly Thr Ser
    12200                12205                12210

Gly Thr   Pro Ser Ser Ser Pro   Ser Pro Thr Thr Ala   Gly Pro Leu
    12215                12220                12225

Leu Met   Pro Phe Thr Leu Asn   Phe Thr Ile Thr Asn   Leu Gln Tyr
    12230                12235                12240
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Glu Glu Asp Met Arg Arg Thr Gly Ser Arg Lys Phe Asn Thr Met
12245 12250 12255

Glu Ser Val Leu Gln Gly Leu Leu Lys Pro Leu Phe Lys Asn Thr
12260 12265 12270

Ser Val Gly Pro Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu Arg
12275 12280 12285

Pro Glu Lys Asp Gly Ala Ala Thr Gly Val Asp Ala Ile Cys Thr
12290 12295 12300

His Arg Leu Asp Pro Lys Ser Pro Gly Leu Asn Arg Glu Gln Leu
12305 12310 12315

Tyr Trp Glu Leu Ser Lys Leu Thr Asn Asp Ile Glu Glu Leu Gly
12320 12325 12330

Pro Tyr Thr Leu Asp Arg Asn Ser Leu Tyr Val Asn Gly Phe Thr
12335 12340 12345

His Gln Ser Ser Val Ser Thr Thr Ser Thr Pro Gly Thr Ser Thr
12350 12355 12360

Val Asp Leu Arg Thr Ser Gly Thr Pro Ser Ser Leu Ser Ser Pro
12365 12370 12375

Thr Ile Met Ala Ala Gly Pro Leu Leu Val Pro Phe Thr Leu Asn
12380 12385 12390

Phe Thr Ile Thr Asn Leu Gln Tyr Gly Glu Asp Met Gly His Pro
12395 12400 12405

Gly Ser Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln Gly Leu
12410 12415 12420

Leu Gly Pro Ile Phe Lys Asn Thr Ser Val Gly Pro Leu Tyr Ser
12425 12430 12435

Gly Cys Arg Leu Thr Ser Leu Arg Ser Glu Lys Asp Gly Ala Ala
12440 12445 12450

Thr Gly Val Asp Ala Ile Cys Ile His His Leu Asp Pro Lys Ser
12455 12460 12465

Pro Gly Leu Asn Arg Glu Arg Leu Tyr Trp Glu Leu Ser Gln Leu

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    12470                12475                12480

Thr Asn  Gly Ile Lys Glu Leu  Gly Pro Tyr Thr Leu  Asp Arg Asn
    12485                12490                12495

Ser Leu  Tyr Val Asn Gly Phe  Thr His Arg Thr Ser  Val Pro Thr
    12500                12505                12510

Ser Ser  Thr Pro Gly Thr Ser  Thr Val Asp Leu Gly  Thr Ser Gly
    12515                12520                12525

Thr Pro  Phe Ser Leu Pro Ser  Pro Ala Thr Ala Gly  Pro Leu Leu
    12530                12535                12540

Val Leu  Phe Thr Leu Asn Phe  Thr Ile Thr Asn Leu  Lys Tyr Glu
    12545                12550                12555

Glu Asp  Met His Arg Pro Gly  Ser Arg Lys Phe Asn  Thr Thr Glu
    12560                12565                12570

Arg Val  Leu Gln Thr Leu Leu  Gly Pro Met Phe Lys  Asn Thr Ser
    12575                12580                12585

Val Gly  Leu Leu Tyr Ser Gly  Cys Arg Leu Thr Leu  Leu Arg Ser
    12590                12595                12600

Glu Lys  Asp Gly Ala Ala Thr  Gly Val Asp Ala Ile  Cys Thr His
    12605                12610                12615

Arg Leu  Asp Pro Lys Ser Pro  Gly Val Asp Arg Glu  Gln Leu Tyr
    12620                12625                12630

Trp Glu  Leu Ser Gln Leu Thr  Asn Gly Ile Lys Glu  Leu Gly Pro
    12635                12640                12645

Tyr Thr  Leu Asp Arg Asn Ser  Leu Tyr Val Asn Gly  Phe Thr His
    12650                12655                12660

Trp Ile  Pro Val Pro Thr Ser  Ser Thr Pro Gly Thr  Ser Thr Val
    12665                12670                12675

Asp Leu  Gly Ser Gly Thr Pro  Ser Ser Leu Pro Ser  Pro Thr Thr
    12680                12685                12690

Ala Gly  Pro Leu Leu Val Pro  Phe Thr Leu Asn Phe  Thr Ile Thr
    12695                12700                12705
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Asn Leu   Lys Tyr Glu Glu Asp   Met His Cys Pro Gly   Ser Arg Lys
    12710                 12715                 12720

Phe Asn   Thr Thr Glu Arg Val   Leu Gln Ser Leu Leu   Gly Pro Met
    12725                 12730                 12735

Phe Lys   Asn Thr Ser Val Gly   Pro Leu Tyr Ser Gly   Cys Arg Leu
    12740                 12745                 12750

Thr Leu   Leu Arg Ser Glu Lys   Asp Gly Ala Ala Thr   Gly Val Asp
    12755                 12760                 12765

Ala Ile   Cys Thr His Arg Leu   Asp Pro Lys Ser Pro   Gly Val Asp
    12770                 12775                 12780

Arg Glu   Gln Leu Tyr Trp Glu   Leu Ser Gln Leu Thr   Asn Gly Ile
    12785                 12790                 12795

Lys Glu   Leu Gly Pro Tyr Thr   Leu Asp Arg Asn Ser   Leu Tyr Val
    12800                 12805                 12810

Asn Gly   Phe Thr His Gln Thr   Ser Ala Pro Asn Thr   Ser Thr Pro
    12815                 12820                 12825

Gly Thr   Ser Thr Val Asp Leu   Gly Thr Ser Gly Thr   Pro Ser Ser
    12830                 12835                 12840

Leu Pro   Ser Pro Thr Ser Ala   Gly Pro Leu Leu Val   Pro Phe Thr
    12845                 12850                 12855

Leu Asn   Phe Thr Ile Thr Asn   Leu Gln Tyr Glu Glu   Asp Met His
    12860                 12865                 12870

His Pro   Gly Ser Arg Lys Phe   Asn Thr Thr Glu Arg   Val Leu Gln
    12875                 12880                 12885

Gly Leu   Leu Gly Pro Met Phe   Lys Asn Thr Ser Val   Gly Leu Leu
    12890                 12895                 12900

Tyr Ser   Gly Cys Arg Leu Thr   Leu Leu Arg Pro Glu   Lys Asn Gly
    12905                 12910                 12915

Ala Ala   Thr Gly Met Asp Ala   Ile Cys Ser His Arg   Leu Asp Pro
    12920                 12925                 12930

Lys Ser   Pro Gly Leu Asn Arg   Glu Gln Leu Tyr Trp   Glu Leu Ser
    12935                 12940                 12945
```

```
Gln Leu   Thr His Gly Ile Lys   Glu Leu Gly Pro Tyr   Thr Leu Asp
    12950                 12955                 12960

Arg Asn   Ser Leu Tyr Val Asn   Gly Phe Thr His Arg   Ser Ser Val
    12965                 12970                 12975

Ala Pro   Thr Ser Thr Pro Gly   Thr Ser Thr Val Asp   Leu Gly Thr
    12980                 12985                 12990

Ser Gly   Thr Pro Ser Ser Leu   Pro Ser Pro Thr Thr   Ala Val Pro
    12995                 13000                 13005

Leu Leu   Val Pro Phe Thr Leu   Asn Phe Thr Ile Thr   Asn Leu Gln
    13010                 13015                 13020

Tyr Gly   Glu Asp Met Arg His   Pro Gly Ser Arg Lys   Phe Asn Thr
    13025                 13030                 13035

Thr Glu   Arg Val Leu Gln Gly   Leu Leu Gly Pro Leu   Phe Lys Asn
    13040                 13045                 13050

Ser Ser   Val Gly Pro Leu Tyr   Ser Gly Cys Arg Leu   Ile Ser Leu
    13055                 13060                 13065

Arg Ser   Glu Lys Asp Gly Ala   Ala Thr Gly Val Asp   Ala Ile Cys
    13070                 13075                 13080

Thr His   His Leu Asn Pro Gln   Ser Pro Gly Leu Asp   Arg Glu Gln
    13085                 13090                 13095

Leu Tyr   Trp Gln Leu Ser Gln   Met Thr Asn Gly Ile   Lys Glu Leu
    13100                 13105                 13110

Gly Pro   Tyr Thr Leu Asp Arg   Asn Ser Leu Tyr Val   Asn Gly Phe
    13115                 13120                 13125

Thr His   Arg Ser Ser Gly Leu   Thr Thr Ser Thr Pro   Trp Thr Ser
    13130                 13135                 13140

Thr Val   Asp Leu Gly Thr Ser   Gly Thr Pro Ser Pro   Val Pro Ser
    13145                 13150                 13155

Pro Thr   Thr Thr Gly Pro Leu   Leu Val Pro Phe Thr   Leu Asn Phe
    13160                 13165                 13170

Thr Ile   Thr Asn Leu Gln Tyr   Glu Glu Asn Met Gly   His Pro Gly
    13175                 13180                 13185
```

Ser Arg Lys Phe Asn Ile Thr Glu Ser Val Leu Gln Gly Leu Leu
13190 13195 13200

Lys Pro Leu Phe Lys Ser Thr Ser Val Gly Pro Leu Tyr Ser Gly
13205 13210 13215

Cys Arg Leu Thr Leu Leu Arg Pro Glu Lys Asp Gly Val Ala Thr
13220 13225 13230

Arg Val Asp Ala Ile Cys Thr His Arg Pro Asp Pro Lys Ile Pro
13235 13240 13245

Gly Leu Asp Arg Gln Gln Leu Tyr Trp Glu Leu Ser Gln Leu Thr
13250 13255 13260

His Ser Ile Thr Glu Leu Gly Pro Tyr Thr Leu Asp Arg Asp Ser
13265 13270 13275

Leu Tyr Val Asn Gly Phe Thr Gln Arg Ser Ser Val Pro Thr Thr
13280 13285 13290

Ser Thr Pro Gly Thr Phe Thr Val Gln Pro Glu Thr Ser Glu Thr
13295 13300 13305

Pro Ser Ser Leu Pro Gly Pro Thr Ala Thr Gly Pro Val Leu Leu
13310 13315 13320

Pro Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu Gln Tyr Glu Glu
13325 13330 13335

Asp Met Arg Arg Pro Gly Ser Arg Lys Phe Asn Thr Thr Glu Arg
13340 13345 13350

Val Leu Gln Gly Leu Leu Met Pro Leu Phe Lys Asn Thr Ser Val
13355 13360 13365

Ser Ser Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu Arg Pro Glu
13370 13375 13380

Lys Asp Gly Ala Ala Thr Arg Val Asp Ala Val Cys Thr His Arg
13385 13390 13395

Pro Asp Pro Lys Ser Pro Gly Leu Asp Arg Glu Arg Leu Tyr Trp
13400 13405 13410

Lys Leu Ser Gln Leu Thr His Gly Ile Thr Glu Leu Gly Pro Tyr

13415 13420 13425

Thr Leu Asp Arg His Ser Leu Tyr Val Asn Gly Phe Thr His Gln
13430 13435 13440

Ser Ser Met Thr Thr Thr Arg Thr Pro Asp Thr Ser Thr Met His
13445 13450 13455

Leu Ala Thr Ser Arg Thr Pro Ala Ser Leu Ser Gly Pro Met Thr
13460 13465 13470

Ala Ser Pro Leu Leu Val Leu Phe Thr Ile Asn Phe Thr Ile Thr
13475 13480 13485

Asn Leu Arg Tyr Glu Glu Asn Met His His Pro Gly Ser Arg Lys
13490 13495 13500

Phe Asn Thr Thr Glu Arg Val Leu Gln Gly Leu Leu Arg Pro Val
13505 13510 13515

Phe Lys Asn Thr Ser Val Gly Pro Leu Tyr Ser Gly Cys Arg Leu
13520 13525 13530

Thr Leu Leu Arg Pro Lys Lys Asp Gly Ala Ala Thr Lys Val Asp
13535 13540 13545

Ala Ile Cys Thr Tyr Arg Pro Asp Pro Lys Ser Pro Gly Leu Asp
13550 13555 13560

Arg Glu Gln Leu Tyr Trp Glu Leu Ser Gln Leu Thr His Ser Ile
13565 13570 13575

Thr Glu Leu Gly Pro Tyr Thr Leu Asp Arg Asp Ser Leu Tyr Val
13580 13585 13590

Asn Gly Phe Thr Gln Arg Ser Ser Val Pro Thr Thr Ser Ile Pro
13595 13600 13605

Gly Thr Pro Thr Val Asp Leu Gly Thr Ser Gly Thr Pro Val Ser
13610 13615 13620

Lys Pro Gly Pro Ser Ala Ala Ser Pro Leu Leu Val Leu Phe Thr
13625 13630 13635

Leu Asn Phe Thr Ile Thr Asn Leu Arg Tyr Glu Glu Asn Met Gln
13640 13645 13650

```
His Pro   Gly Ser Arg Lys Phe   Asn Thr Thr Glu Arg   Val Leu Gln
    13655                13660                13665

Gly Leu   Leu Arg Ser Leu Phe   Lys Ser Thr Ser Val   Gly Pro Leu
    13670                13675                13680

Tyr Ser   Gly Cys Arg Leu Thr   Leu Leu Arg Pro Glu   Lys Asp Gly
    13685                13690                13695

Thr Ala   Thr Gly Val Asp Ala   Ile Cys Thr His His   Pro Asp Pro
    13700                13705                13710

Lys Ser   Pro Arg Leu Asp Arg   Glu Gln Leu Tyr Trp   Glu Leu Ser
    13715                13720                13725

Gln Leu   Thr His Asn Ile Thr   Glu Leu Gly Pro Tyr   Ala Leu Asp
    13730                13735                13740

Asn Asp   Ser Leu Phe Val Asn   Gly Phe Thr His Arg   Ser Ser Val
    13745                13750                13755

Ser Thr   Thr Ser Thr Pro Gly   Thr Pro Thr Val Tyr   Leu Gly Ala
    13760                13765                13770

Ser Lys   Thr Pro Ala Ser Ile   Phe Gly Pro Ser Ala   Ala Ser His
    13775                13780                13785

Leu Leu   Ile Leu Phe Thr Leu   Asn Phe Thr Ile Thr   Asn Leu Arg
    13790                13795                13800

Tyr Glu   Glu Asn Met Trp Pro   Gly Ser Arg Lys Phe   Asn Thr Thr
    13805                13810                13815

Glu Arg   Val Leu Gln Gly Leu   Leu Arg Pro Leu Phe   Lys Asn Thr
    13820                13825                13830

Ser Val   Gly Pro Leu Tyr Ser   Gly Cys Arg Leu Thr   Leu Leu Arg
    13835                13840                13845

Pro Glu   Lys Asp Gly Glu Ala   Thr Gly Val Asp Ala   Ile Cys Thr
    13850                13855                13860

His Arg   Pro Asp Pro Thr Gly   Pro Gly Leu Asp Arg   Glu Gln Leu
    13865                13870                13875

Tyr Leu   Glu Leu Ser Gln Leu   Thr His Ser Ile Thr   Glu Leu Gly
    13880                13885                13890
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Pro Tyr   Thr Leu Asp Arg Asp   Ser Leu Tyr Val Asn   Gly Phe Thr
    13895                13900                13905

His Arg   Ser Ser Val Pro Thr   Thr Ser Thr Gly Val   Val Ser Glu
    13910                13915                13920

Glu Pro   Phe Thr Leu Asn Phe   Thr Ile Asn Asn Leu   Arg Tyr Met
    13925                13930                13935

Ala Asp   Met Gly Gln Pro Gly   Ser Leu Lys Phe Asn   Ile Thr Asp
    13940                13945                13950

Asn Val   Met Gln His Leu Leu   Ser Pro Leu Phe Gln   Arg Ser Ser
    13955                13960                13965

Leu Gly   Ala Arg Tyr Thr Gly   Cys Arg Val Ile Ala   Leu Arg Ser
    13970                13975                13980

Val Lys   Asn Gly Ala Glu Thr   Arg Val Asp Leu Leu   Cys Thr Tyr
    13985                13990                13995

Leu Gln   Pro Leu Ser Gly Pro   Gly Leu Pro Ile Lys   Gln Val Phe
    14000                14005                14010

His Glu   Leu Ser Gln Gln Thr   His Gly Ile Thr Arg   Leu Gly Pro
    14015                14020                14025

Tyr Ser   Leu Asp Lys Asp Ser   Leu Tyr Leu Asn Gly   Tyr Asn Glu
    14030                14035                14040

Pro Gly   Pro Asp Glu Pro Pro   Thr Thr Pro Lys Pro   Ala Thr Thr
    14045                14050                14055

Phe Leu   Pro Pro Leu Ser Glu   Ala Thr Thr Ala Met   Gly Tyr His
    14060                14065                14070

Leu Lys   Thr Leu Thr Leu Asn   Phe Thr Ile Ser Asn   Leu Gln Tyr
    14075                14080                14085

Ser Pro   Asp Met Gly Lys Gly   Ser Ala Thr Phe Asn   Ser Thr Glu
    14090                14095                14100

Gly Val   Leu Gln His Leu Leu   Arg Pro Leu Phe Gln   Lys Ser Ser
    14105                14110                14115

Met Gly   Pro Phe Tyr Leu Gly   Cys Gln Leu Ile Ser   Leu Arg Pro
    14120                14125                14130
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```
Glu Lys   Asp Gly Ala Ala Thr   Gly Val Asp Thr Thr   Cys Thr Tyr
    14135                14140                14145

His Pro   Asp Pro Val Gly Pro   Gly Leu Asp Ile Gln   Gln Leu Tyr
    14150                14155                14160

Trp Glu   Leu Ser Gln Leu Thr   His Gly Val Thr Gln   Leu Gly Phe
    14165                14170                14175

Tyr Val   Leu Asp Arg Asp Ser   Leu Phe Ile Asn Gly   Tyr Ala Pro
    14180                14185                14190

Gln Asn   Leu Ser Ile Arg Gly   Glu Tyr Gln Ile Asn   Phe His Ile
    14195                14200                14205

Val Asn   Trp Asn Leu Ser Asn   Pro Asp Pro Thr Ser   Ser Glu Tyr
    14210                14215                14220

Ile Thr   Leu Leu Arg Asp Ile   Gln Asp Lys Val Thr   Thr Leu Tyr
    14225                14230                14235

Lys Gly   Ser Gln Leu His Asp   Thr Phe Arg Phe Cys   Leu Val Thr
    14240                14245                14250

Asn Leu   Thr Met Asp Ser Val   Leu Val Thr Val Lys   Ala Leu Phe
    14255                14260                14265

Ser Ser   Asn Leu Asp Pro Ser   Leu Val Glu Gln Val   Phe Leu Asp
    14270                14275                14280

Lys Thr   Leu Asn Ala Ser Phe   His Trp Leu Gly Ser   Thr Tyr Gln
    14285                14290                14295

Leu Val   Asp Ile His Val Thr   Glu Met Glu Ser Ser   Val Tyr Gln
    14300                14305                14310

Pro Thr   Ser Ser Ser Ser Thr   Gln His Phe Tyr Leu   Asn Phe Thr
    14315                14320                14325

Ile Thr   Asn Leu Pro Tyr Ser   Gln Asp Lys Ala Gln   Pro Gly Thr
    14330                14335                14340

Thr Asn   Tyr Gln Arg Asn Lys   Arg Asn Ile Glu Asp   Ala Leu Asn
    14345                14350                14355

Gln Leu   Phe Arg Asn Ser Ser   Ile Lys Ser Tyr Phe   Ser Asp Cys
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14360 14365 14370

Gln Val Ser Thr Phe Arg Ser Val Pro Asn Arg His His Thr Gly
14375 14380 14385

Val Asp Ser Leu Cys Asn Phe Ser Pro Leu Ala Arg Arg Val Asp
14390 14395 14400

Arg Val Ala Ile Tyr Glu Glu Phe Leu Arg Met Thr Arg Asn Gly
14405 14410 14415

Thr Gln Leu Gln Asn Phe Thr Leu Asp Arg Ser Ser Val Leu Val
14420 14425 14430

Asp Gly Tyr Ser Pro Asn Arg Asn Glu Pro Leu Thr Gly Asn Ser
14435 14440 14445

Asp Leu Pro Phe Trp Ala Val Ile Leu Ile Gly Leu Ala Gly Leu
14450 14455 14460

Leu Gly Val Ile Thr Cys Leu Ile Cys Gly Val Leu Val Thr Thr
14465 14470 14475

Arg Arg Arg Lys Lys Glu Gly Glu Tyr Asn Val Gln Gln Gln Cys
14480 14485 14490

Pro Gly Tyr Tyr Gln Ser His Leu Asp Leu Glu Asp Leu Gln
14495 14500 14505

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Thr Cys Leu Ile Cys Gly Val Leu
20

<210> 15
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<213> Artificial Sequence

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<223> Synthetic

<400> 15

Lys Ser Tyr Phe Ser Asp Cys Gln Val Ser Thr Phe Arg Ser Val Pro
1 5 10 15

Asn Arg His His Thr Gly Val Asp Ser Leu Cys Asn Phe Ser Pro Leu
20 25 30

<210> 16
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 16

Val Thr Thr Arg Arg Arg Lys Lys Glu Gly Glu Tyr Asn Val Gln Gln
1 5 10 15

Gln Cys Pro Gly Tyr Tyr Gln Ser His Leu Asp Leu Glu Asp Leu Gln
20 25 30

<210> 17
<211> 58
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 17

Asn Phe Ser Pro Leu Ala Arg Arg Val Asp Arg Val Ala Ile Tyr Glu
1 5 10 15

Glu Phe Leu Arg Met Thr Arg Asn Gly Thr Gln Leu Gln Asn Phe Thr
20 25 30

Leu Asp Arg Ser Ser Val Leu Val Asp Gly Tyr Ser Pro Asn Arg Asn
35 40 45

Glu Pro Leu Thr Gly Asn Ser Asp Leu Pro
50 55

<210> 18
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 18

Val Thr Thr Arg Arg Arg Lys Lys Glu Gly Glu Tyr Asn Val Gln Gln
1 5 10 15

Gln

<210> 19
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 19

Cys Gln Val Ser Thr Phe Arg Ser Val Pro Asn Arg His His Thr Gly
1 5 10 15

Val Asp Ser Leu Cys
20

<210> 20
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 20

Leu Val Thr Thr Arg Arg Arg Lys Lys Glu Gly Glu Tyr Asn Val Gln
1 5 10 15

Gln Gln

<210> 21
<211> 18
<212> PRT
<213> Mus musculus

<400> 21

Thr Leu Asp Arg Lys Ser Val Phe Val Asp Gly Tyr Ser Gln Asn Arg
1 5 10 15

Asp Asp

<210> 22

<211> 33
<212> PRT
<213> Mus musculus

<400> 22

Lys Ser Tyr Phe Ser Asp Cys Gln Val Leu Ala Phe Arg Ser Val Ser
1 5 10 15

Asn Asn Asn Asn His Thr Gly Val Asp Ser Leu Cys Asn Phe Ser Pro
20 25 30

Leu

<210> 23
<211> 31
<212> PRT
<213> Mus musculus

<400> 23

Ser Leu Tyr Ser Asn Cys Arg Leu Ala Ser Leu Arg Pro Lys Lys Asn
1 5 10 15

Gly Thr Ala Thr Gly Val Asn Ala Ile Cys Ser Tyr His Gln Asn
20 25 30

<210> 24
<211> 402
<212> PRT
<213> Mus musculus

<400> 24

His Leu Ile Arg Pro Leu Val Gln Asn Glu Ser Leu Tyr Ser Asn Cys
1 5 10 15

Arg Leu Ala Ser Leu Arg Pro Lys Lys Asn Gly Thr Ala Thr Gly Val
20 25 30

Asn Ala Ile Cys Ser Tyr His Gln Asn Pro Asp His Pro Glu Leu Asp
35 40 45

Thr Gln Glu Leu Tyr Thr Lys Leu Thr Gln Leu Thr Gln Gly Val Thr
50 55 60

Gln Leu Gly Ser Tyr Met Leu Asp Gln Asn Ser Ile Tyr Val Asn Gly
65 70 75 80

Tyr Val Pro Leu Asn Ile Thr Ile Gln Gly Lys Tyr Gln Leu Asn Phe
85 90 95

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Cys Ile Ile Asn Trp Asn Leu Asn Asn Thr Asp Pro Thr Ser Ser Glu
            100                 105                 110

Tyr Ile Thr Leu Glu Arg Asp Ile Glu Asp Lys Val Thr Thr Leu Tyr
        115                 120                 125

Thr Gly Ser Gln Leu Lys Glu Val Phe Gln Ser Cys Leu Val Thr Asn
    130                 135                 140

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

His Leu Asp Pro Asn Leu Val Lys Gln Val Phe Leu Asn Lys Thr Leu
                165                 170                 175

Asn Ala Ser Ser His Trp Leu Gly Ala Thr Tyr Gln Leu Lys Asp Leu
            180                 185                 190

His Val Ile Asp Met Lys Thr Ser Ile Leu Leu Pro Ala Glu Ile Pro
        195                 200                 205

Thr Thr Ser Ser Ser Ser Gln His Phe Asn Leu Asn Phe Thr Ile Thr
    210                 215                 220

Asn Leu Pro Tyr Ser Gln Asp Ile Ala Gln Pro Ser Thr Thr Lys Tyr
225                 230                 235                 240

Gln Gln Thr Lys Arg Ser Ile Glu Asn Ala Leu Asn Gln Leu Phe Arg
                245                 250                 255

Asn Ser Ser Ile Lys Ser Tyr Phe Ser Asp Cys Gln Val Leu Ala Phe
            260                 265                 270

Arg Ser Val Ser Asn Asn Asn Asn His Thr Gly Val Asp Ser Leu Cys
        275                 280                 285

Asn Phe Ser Pro Leu Ala Arg Arg Val Asp Arg Val Ala Ile Tyr Glu
    290                 295                 300

Glu Phe Leu Arg Met Thr His Asn Gly Thr Gln Leu Leu Asn Phe Thr
305                 310                 315                 320

Leu Asp Arg Lys Ser Val Phe Val Asp Gly Tyr Ser Gln Asn Arg Asp
                325                 330                 335

Asp Asp Val Met Lys Asn Ser Gly Leu Pro Phe Trp Ala Ile Ile Leu
            340                 345                 350
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Ile Cys Leu Ala Val Leu Leu Val Leu Ile Thr Cys Leu Met Cys Cys
355 360 365

Phe Leu Val Thr Val Cys Arg Arg Lys Lys Glu Gly Asp Tyr Gln Val
370 375 380

Gln Arg His Arg Leu Ala Tyr Tyr Leu Ser His Leu Asp Leu Arg Lys
385 390 395 400

Leu Gln

<210> 25
<211> 400
<212> PRT
<213> Homo sapiens

<400> 25

His Leu Leu Arg Pro Leu Phe Gln Lys Ser Ser Met Gly Pro Phe Tyr
1 5 10 15

Leu Gly Cys Gln Leu Ile Ser Leu Arg Pro Glu Lys Asp Gly Ala Ala
20 25 30

Thr Gly Val Asp Thr Thr Cys Thr Tyr His Pro Asp Pro Val Gly Pro
35 40 45

Gly Leu Asp Ile Gln Gln Leu Tyr Trp Glu Leu Ser Gln Leu Thr His
50 55 60

Gly Val Thr Gln Leu Gly Phe Tyr Val Leu Asp Arg Asp Ser Leu Phe
65 70 75 80

Ile Asn Gly Tyr Ala Pro Gln Asn Leu Ser Ile Arg Gly Glu Tyr Gln
85 90 95

Ile Asn Phe His Ile Val Asn Trp Asn Leu Ser Asn Pro Asp Pro Thr
100 105 110

Ser Ser Glu Tyr Ile Thr Leu Leu Arg Asp Ile Gln Asp Lys Val Thr
115 120 125

Thr Leu Tyr Lys Gly Ser Gln Leu His Asp Thr Phe Arg Phe Cys Leu
130 135 140

Val Thr Asn Leu Thr Met Asp Ser Val Leu Val Thr Val Lys Ala Leu
145 150 155 160

Phe Ser Ser Asn Leu Asp Pro Ser Leu Val Glu Gln Val Phe Leu Asp
165 170 175

Lys Thr Leu Asn Ala Ser Phe His Trp Leu Gly Ser Thr Tyr Gln Leu
180 185 190

Val Asp Ile His Val Thr Glu Met Glu Ser Ser Val Tyr Gln Pro Thr
195 200 205

Ser Ser Ser Ser Thr Gln His Phe Tyr Leu Asn Phe Thr Ile Thr Asn
210 215 220

Leu Pro Tyr Ser Gln Asp Lys Ala Gln Pro Gly Thr Thr Asn Tyr Gln
225 230 235 240

Arg Asn Lys Arg Asn Ile Glu Asp Ala Leu Asn Gln Leu Phe Arg Asn
245 250 255

Ser Ser Ile Lys Ser Tyr Phe Ser Asp Cys Gln Val Ser Thr Phe Arg
260 265 270

Ser Val Pro Asn Arg His His Thr Gly Val Asp Ser Leu Cys Asn Phe
275 280 285

Ser Pro Leu Ala Arg Arg Val Asp Arg Val Ala Ile Tyr Glu Glu Phe
290 295 300

Leu Arg Met Thr Arg Asn Gly Thr Gln Leu Gln Asn Phe Thr Leu Asp
305 310 315 320

Arg Ser Ser Val Leu Val Asp Gly Tyr Ser Pro Asn Arg Asn Glu Pro
325 330 335

Leu Thr Gly Asn Ser Asp Leu Pro Phe Trp Ala Val Ile Leu Ile Gly
340 345 350

Leu Ala Gly Leu Leu Gly Val Ile Thr Cys Leu Ile Cys Gly Val Leu
355 360 365

Val Thr Thr Arg Arg Arg Lys Lys Glu Gly Glu Tyr Asn Val Gln Gln
370 375 380

Gln Cys Pro Gly Tyr Tyr Gln Ser His Leu Asp Leu Glu Asp Leu Gln
385 390 395 400

<210> 26

<211> 360
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 26
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ccaggaaagg gtatggaatg ggttgctcgc ataagaagta aaagtggaaa ttatgcaaca 180

tattatgccg attcagtgaa agacagattc accatctcca gaaatgattc acagagcatg 240

ctctatctgc aaatgaacaa cctgaaaact gaggacacag ccatatatta ctgtgtgaga 300

gcgggtaaca acggggcctt tccttactgg ggccaaggga ccacggtcac cgtctcctca 360

<210> 27
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 27

Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Lys Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30

Ala Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Met Glu Trp Val
35 40 45

Ala Arg Ile Arg Ser Lys Ser Gly Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asn Asp Ser Gln Ser Met
65 70 75 80

Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Ile Tyr
85 90 95

Tyr Cys Val Arg Ala Gly Asn Asn Gly Ala Phe Pro Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 28
<211> 330
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 28
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atcacttgca aggctagcca agatattaag aagtatatag cttggtacca acacaagcct 120

ggaaaaactc ctcgactact catacatttc acatctacat tacagacagg catcccatca 180

aggttcagtg gacgtgggtc tgggagagac tattccttca gcatcagcaa cctggagtct 240

gaagatattg caacttatta ttgtctacag tatgatagtc tgtacacgtt cggagggggg 300

accaagctgg agatcaaacg ggcggccgca 330

<210> 29
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 29

Asp Ile Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Leu Gly
1 5 10 15

Gly Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Lys Lys Tyr
20 25 30

Ile Ala Trp Tyr Gln His Lys Pro Gly Lys Thr Pro Arg Leu Leu Ile
35 40 45

His Phe Thr Ser Thr Leu Gln Thr Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Arg Gly Ser Gly Arg Asp Tyr Ser Phe Ser Ile Ser Asn Leu Glu Ser
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln Tyr Asp Ser Leu Tyr Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala
100 105 110

<210> 30
<211> 14
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic

<400> 30

Lys Ser Tyr Phe Ser Asp Cys Gln Val Asn Asn Phe Arg Ser
1 5 10

<210> 31
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 31

Thr Leu Asp Arg Ser Ser Val Leu Val Asp Gly Tyr Ser Gln Asn Arg
1 5 10 15

Asp Asp

<210> 32
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> CD8 leader sequence

<400> 32
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<210> 33
<211> 335
<212> DNA
<213> Artificial Sequence

<220>
<223> CD3 zeta chain intracellular domain

<400> 33
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tataacgagc tcaatctagg acgaagagag gagtacgatg ttttggacaa gagacgtggc 120

cgggaccctg agatgggggg aaagccgaga aggaagaacc ctcaggaagg cctgtacaat 180

gaactgcaga aagataagat ggcggaggcc tacagtgaga ttgggatgaa aggcgagcgc 240

cggaggggca aggggcacga tggcctttac cagggtctca gtacagccac caaggacacc 300

tacgacgccc ttcacatgca ggccctgccc cctcg 335

<210> 34
<211> 45

<212> DNA
<213> Artificial Sequence

<220>
<223> (G4S)3 serine-glycine linker

<400> 34
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<210> 35
<211> 225
<212> DNA
<213> Artificial Sequence

<220>
<223> CD8 transmembrane domain

<400> 35
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tcgcagcccc tgtccctgcg cccagaggcg tgccggccag cggcgggggg cgcagtgcac 120

acgagggggc tggacttcgc ctgtgatatc tacatctggg cgcccttggc cgggacttgt 180

ggggtccttc tcctgtcact ggttatcacc ctttactgca accac 225

<210> 36
<211> 223
<212> DNA
<213> Artificial Sequence

<220>
<223> CD28 transmembrane + intracellular domains (-STOP)

<400> 36
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tccatgtgaa agggaaacac ctttgtccaa gtcccctatt tcccggacct tctaagccct 120

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cctttattat tttctgggtg aggagtaaga ggagcaggct cct 223

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<211> 7726
<212> DNA
<213> Artificial Sequence

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<223> SFG_4H11z forward sequence

<400> 37
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ttatttagtc tccagaaaaa ggggggaatg aaagacccca cctgtaggtt tggcaagcta 180

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agcagttcct gccccggctc agggccaaga acagatggaa cagctgaata tgggccaaac 360

aggatatctg tggtaagcag ttcctgcccc ggctcagggc caagaacaga tggtccccag 420

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Claims

1. A single chain variable fragment (scFv) comprising the CDRs of a VH chain sequence encoded by SEQ ID NO:06 and of a VL chain sequence encoded by SEQ ID NO:07.

2. A scFv comprising a variable heavy ("VH") chain and a variable light ("VL") chain, wherein

(a) all or substantially all of the hypervariable loops of the VH chain and VL chain correspond to those of a nonhuman immunoglobulin, wherein the nonhuman immunoglobulin comprises a VH chain sequence encoded by SEQ ID NO: 06 and a VL chain sequence encoded by SEQ ID NO: 07; and
(b) all or substantially all of the framework region residues of the VH chain and VL chain are those of a human immunoglobulin sequence.

3. A scFv comprising a VH chain sequence encoded by SEQ ID NO:06 and a VL chain sequence encoded by SEQ ID NO:07.

4. The scFv of claim 2, wherein the VH chain and the VL chain are of a humanized antibody or antigen-binding fragment thereof, wherein the humanized antibody or antigen-binding fragment thereof is made by substituting the complementarity determining regions of an antibody comprising a VH chain sequence encoded by SEQ ID NO:06 and a VL chain sequence encoded by SEQ ID NO:07 into a human framework domain, wherein the humanized antibody or antigen-binding fragment thereof specifically binds to the MUC16 polypeptide of SEQ ID NO:02 or to an antigenic portion thereof.

5. The scFv of claim 1, further comprising human framework domain residues.

6. The scFv of claim 5, wherein the scFv comprises a framework domain in which human framework domain residues are replaced by corresponding nonhuman residues.

7. The scFv of any one of claims 1 to 6, wherein the scFv specifically binds to the MUC16 polypeptide of SEQ ID NO:02 or to an antigenic portion thereof.

8. A chimeric antigen receptor (CAR) comprising the scFv of any one of claims 1 to 7.

9. The CAR of claim 8, further comprising a transmembrane domain and a T cell receptor ζ chain cytoplasmic signaling domain.

10. The CAR of claim 9, wherein the scFv is fused to the transmembrane domain, and wherein the transmembrane domain is fused to the T cell receptor ζ chain cytoplasmic signaling domain.

11. The CAR of claim 9 or 10, further comprising a cytoplasmic signaling domain of a co-stimulatory receptor, wherein the co-stimulatory receptor comprises CD28, 4-1BB or OX40.

12. The CAR of any one of claims 8 to 11, comprising in amino- to carboxy-terminal order, the scFv, a human CD28 transmembrane domain and cytoplasmic signaling domain, and a CD3-zeta signaling domain.

13. The CAR of any one of claims 8 to 12, comprising in amino- to carboxy-terminal order: a human CD8 leader peptide, the scFv comprising the VH chain sequence encoded by SEQ ID NO:06 and the VL chain sequence encoded by SEQ ID NO:07, a human CD8 hinge domain, a human CD8 transmembrane domain, and a CD3-zeta signaling domain.

14. The CAR of any one of claims 8 to 12, consisting essentially of, in amino- to carboxy-terminal order: a human CD8 leader peptide, the scFv comprising the VH chain sequence encoded by SEQ ID NO:06 and the VL chain sequence encoded by SEQ ID NO:07, a human CD8 hinge domain, a human CD8 transmembrane domain, and a CD3-zeta signaling domain.

15. The CAR of any one of claims 8 to 12, comprising in amino- to carboxy-terminal order: a human CD8 leader peptide, the scFv comprising the VH chain sequence encoded by SEQ ID NO:06 and the VL chain sequence encoded by SEQ ID NO:07, a human CD28 transmembrane domain, a human CD28 intracellular domain, and a CD3-zeta signaling domain.

16. The CAR of any one of claims 8 to 12, consisting essentially of, in amino- to carboxy-terminal order: a human CD8 leader peptide, the scFv comprising the VH chain sequence encoded by SEQ ID NO:06 and the VL chain sequence encoded by SEQ ID NO:07, a human CD28 transmembrane domain, a human CD28 intracellular domain, and a CD3-zeta signaling domain.

17. The CAR of any one of claims 8 to 12, comprising in amino- to carboxy-terminal order: a human CD8 leader peptide, a VH chain sequence encoded by SEQ ID NO:06, a spacer encoded by SEQ ID NO:34, a VL chain sequence encoded by SEQ ID NO:07, a human CD8 hinge domain, a human CD8 transmembrane domain, and a CD3-zeta signaling domain.

18. The CAR of any one of claims 8 to 12, comprising in amino- to carboxy-terminal order: a human CD8 leader peptide, a VH chain sequence encoded by SEQ ID NO:06, a spacer encoded by SEQ ID NO:34, a VL chain sequence encoded by SEQ ID NO:07, a human CD28 transmembrane domain, a human CD28 intracellular domain, and a CD3-zeta signaling domain.

19. A T cell expressing the CAR of any one of claims 8 to 18.

20. The T cell of claim 19 for use in treating a cancer in which MUC16 is expressed.

21. The T cell for use of claim 20, wherein the T cell is for administration to the subject intraperitoneally or intravenously.

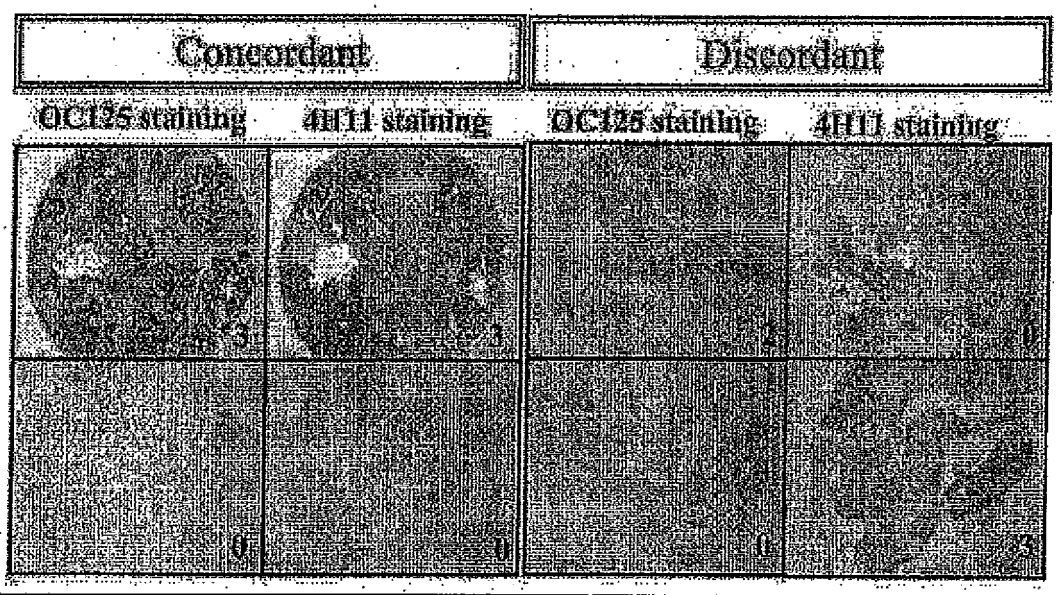
22. The T cell for use of claim 20 or 21, wherein the cancer is ovarian cancer.

Peptide 1 near Cleavage Site:
NFSPLARRVDRVAIYEE (SEQ ID NO:01)

Peptide 2 before Transmembrane:
TLDRSSVLVDGYSPNRNE (SEQ ID NO:02)

Peptide 3 inside Transmembrane:
CGVLVTTRRRKKEGEYNVQQQ (SEQ ID NO:03)

FIGURE 1



Tissue microarrays were scored from 0 to 3 in one-half (+) increments for cytoplasmic and/or membranous staining

0 – no staining
1 – strong or weak <5%
1+ - strong or weak 5-50%
2 – strong 51-75% or weak 51-100%
2+ - strong (76-99%)
3 – strong (100%)

	Stain score		
n=518	0	1-1+	2-3
OC125	60 (11%)	122 (24%)	336 (65%)
4H11	129 (25%)	116 (22%)	273 (53%)

Concordance between OC125 and 4H11 staining was based on the amount of difference between the scores

Concordant = 0 - ½ difference
Partially concordant = 1-1 ½ difference
Discordant = 2-3 difference

OC125/4H11 (n=518)	
Concordant	286 (55%)
Partially concordant	193 (37%)
Discordant	39 (8%)

Of the 39 discordant cases,
OC125 > 4H11 in 59%
4H11 > OC125 in 41%

OC125>4H11	23 (59%)
4H11>OC125	16 (41%)

Overall 3-4% of all ovarian serous carcinomas expressed 4H11 but not OC125

FIGURE 2

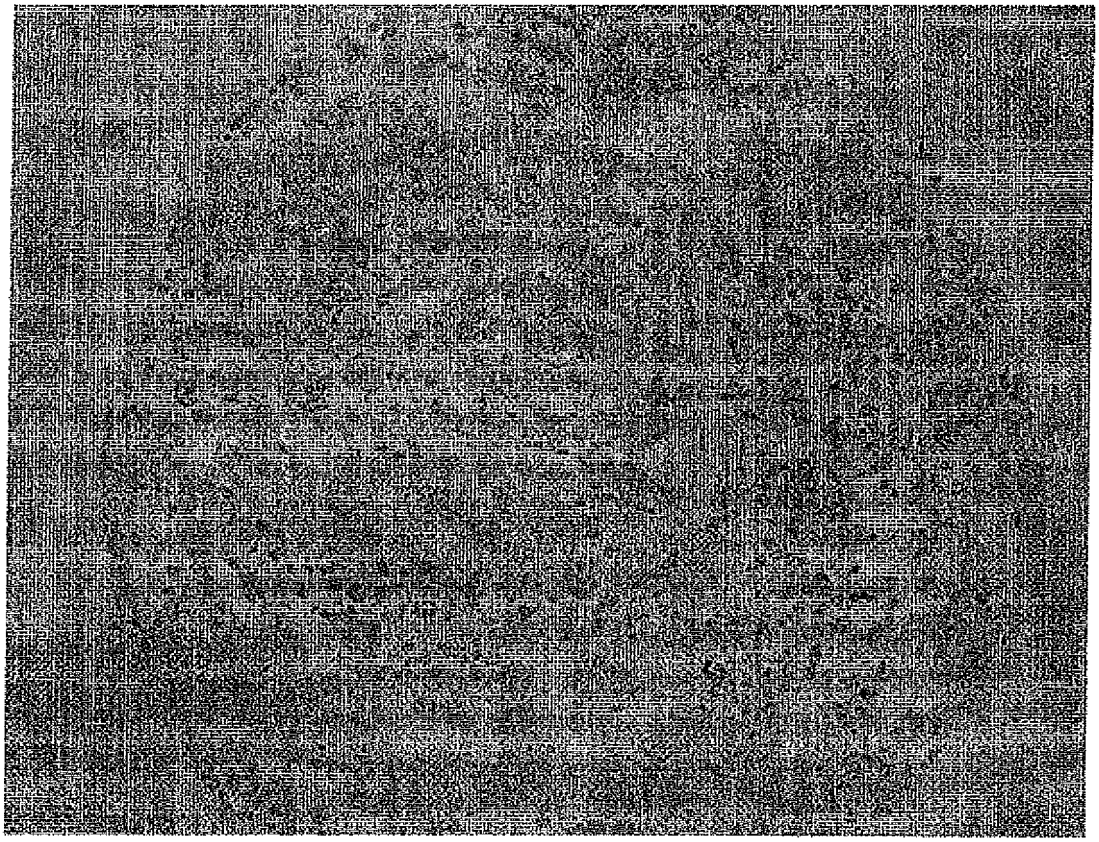


FIGURE 3A

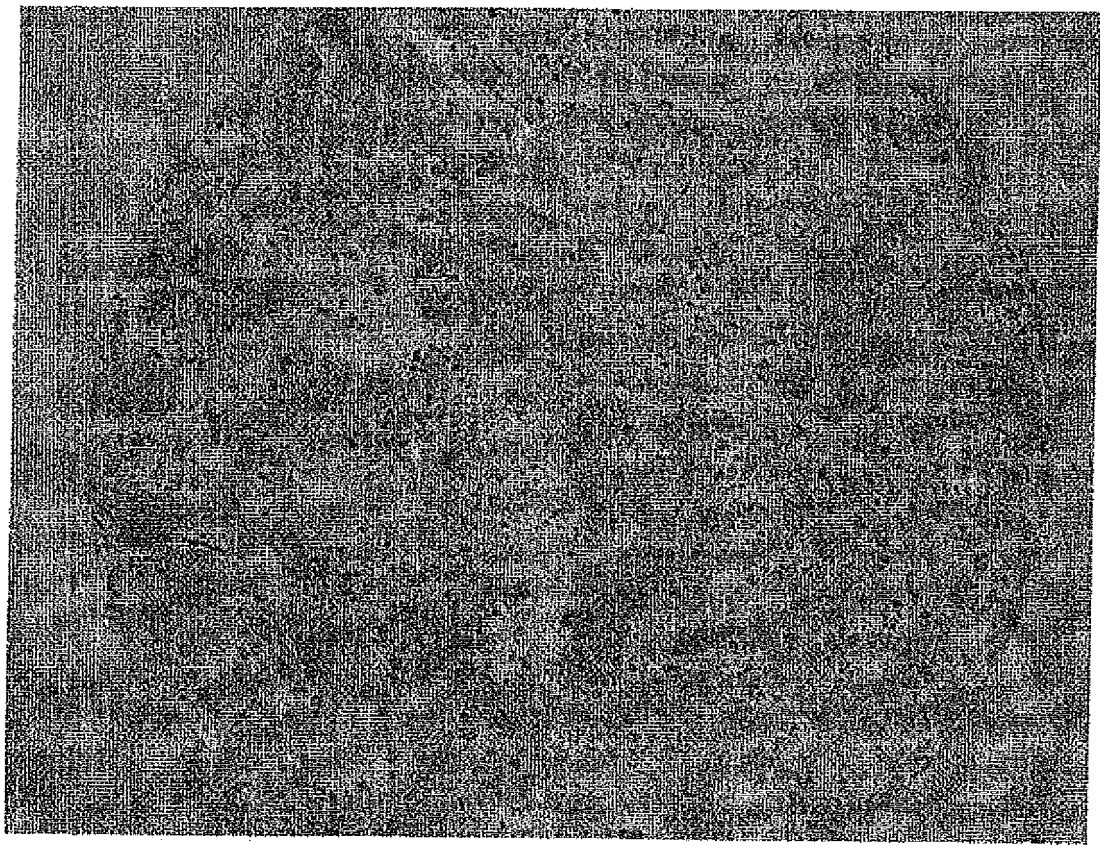


FIGURE 3B

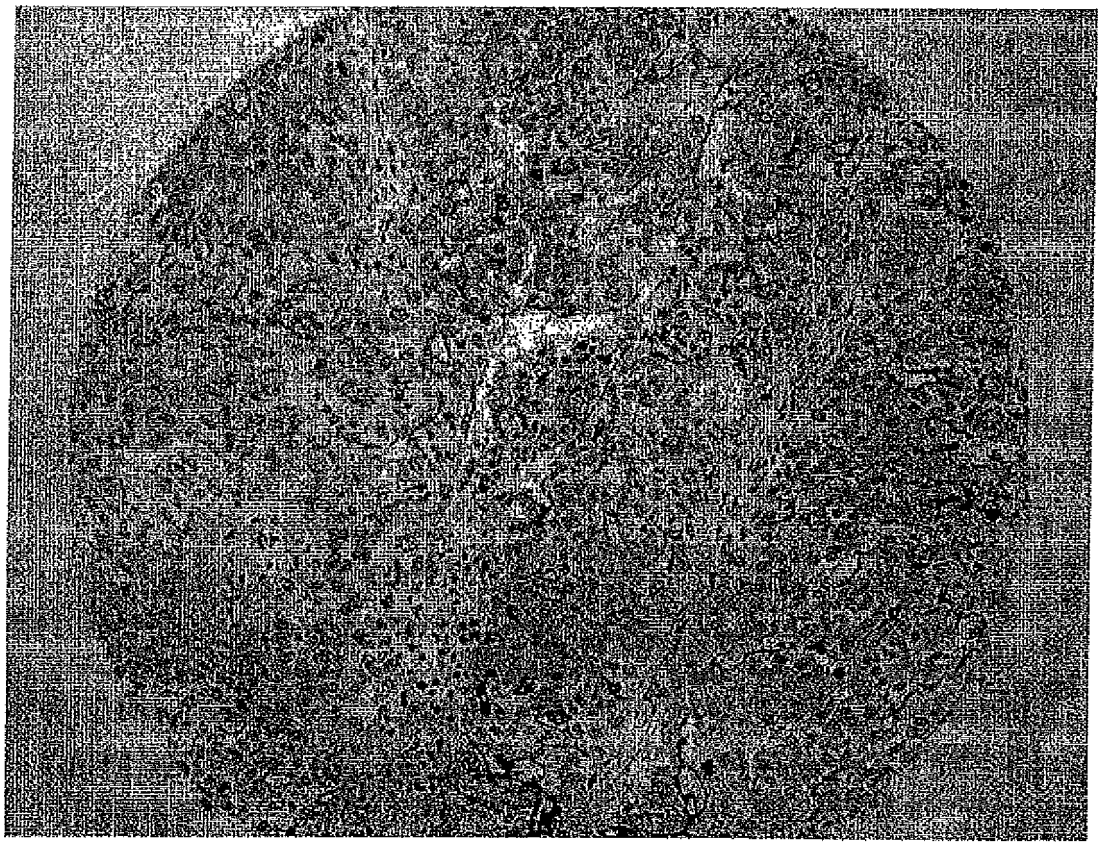


FIGURE 3C

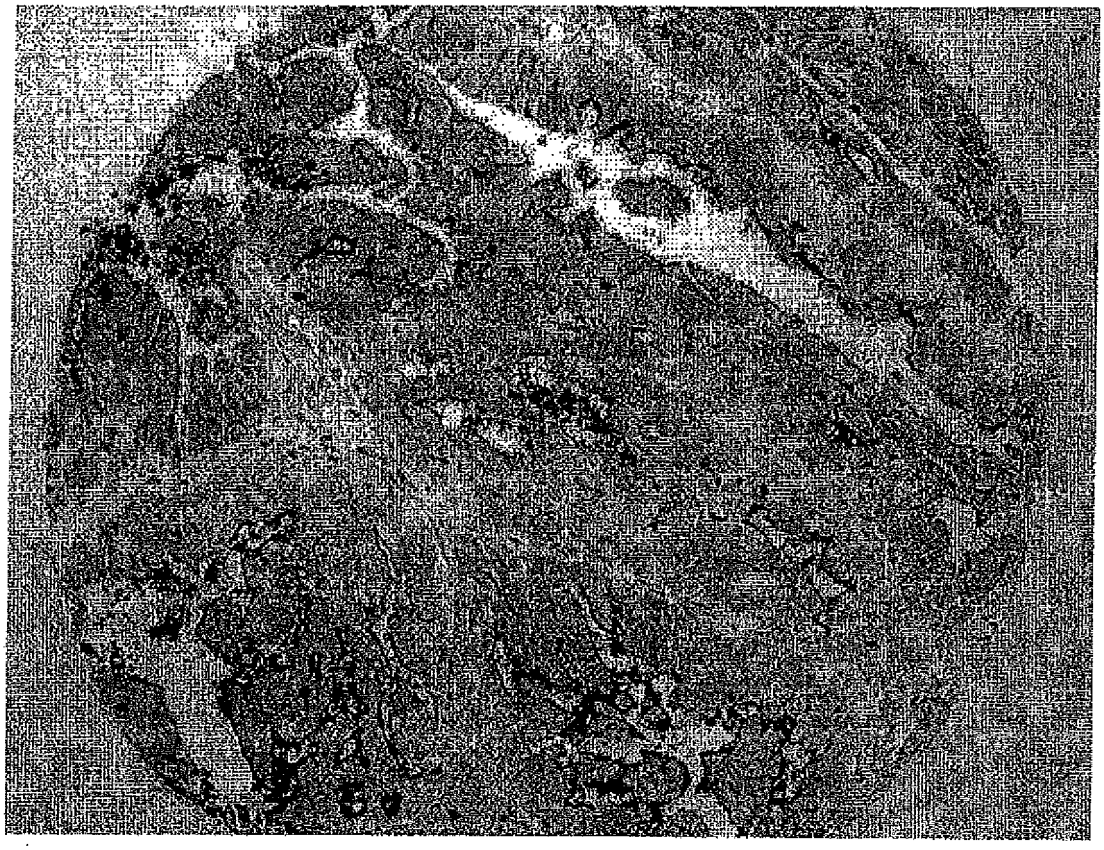


FIGURE 3D

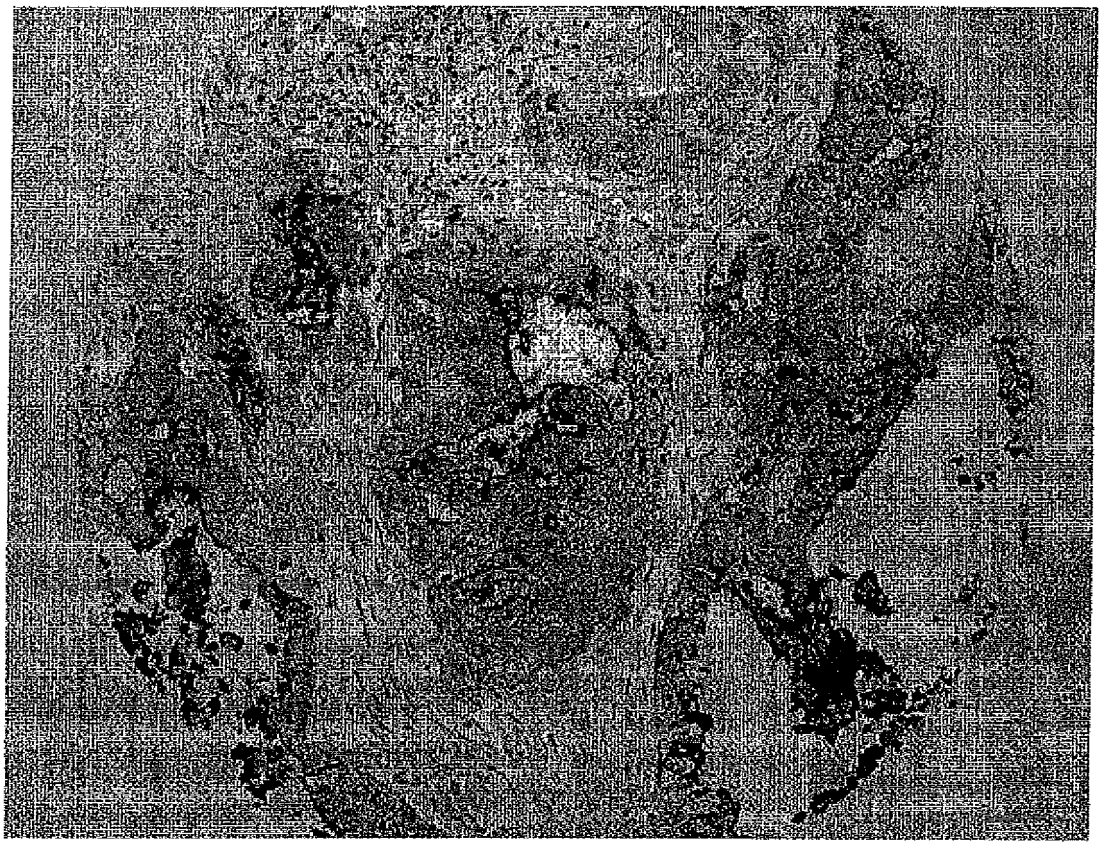


FIGURE 3E

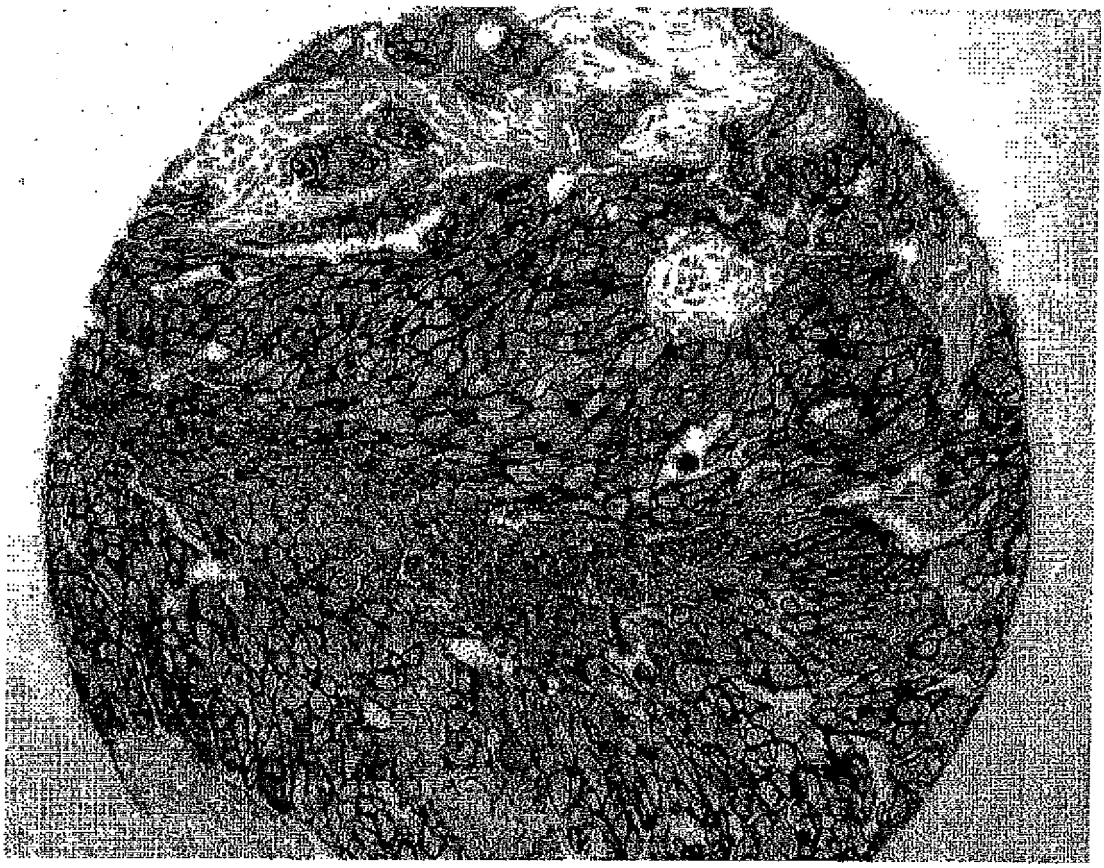


FIGURE 3F

FIGURE 3G

FIGURE 3H

FIGURE 3I

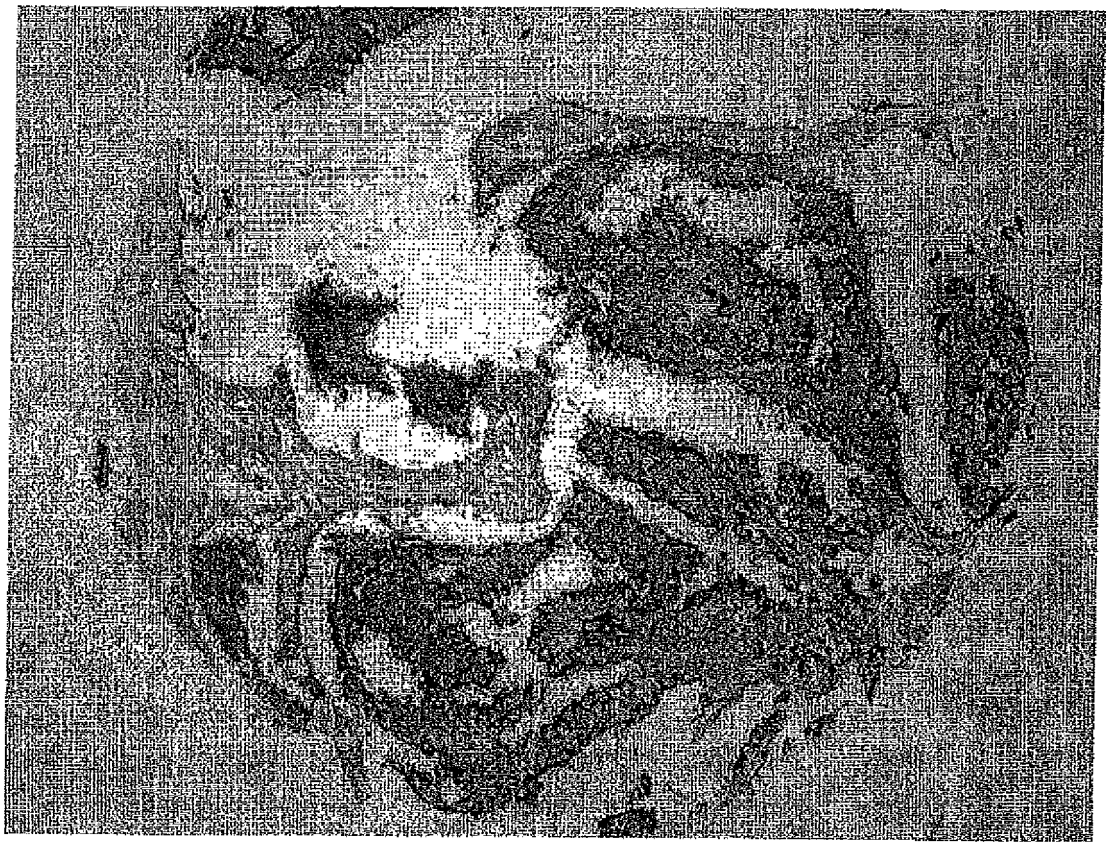


FIGURE 3J

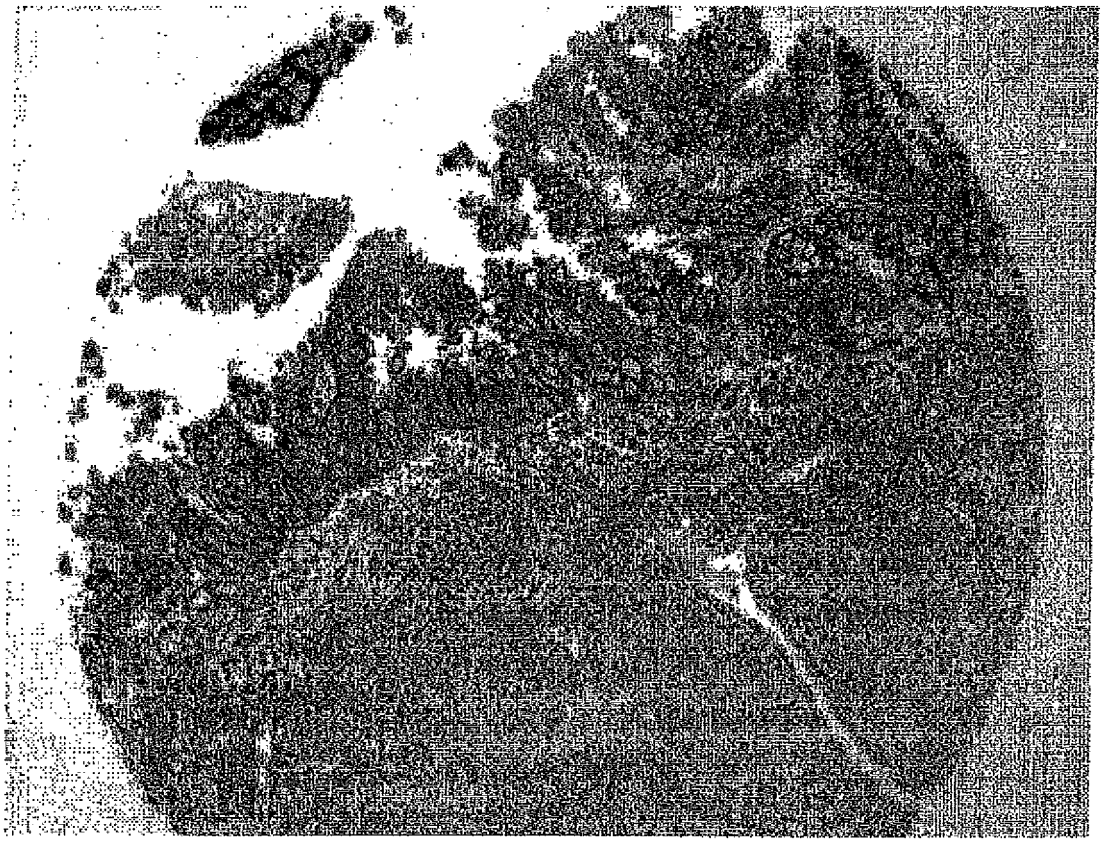


FIGURE 3K

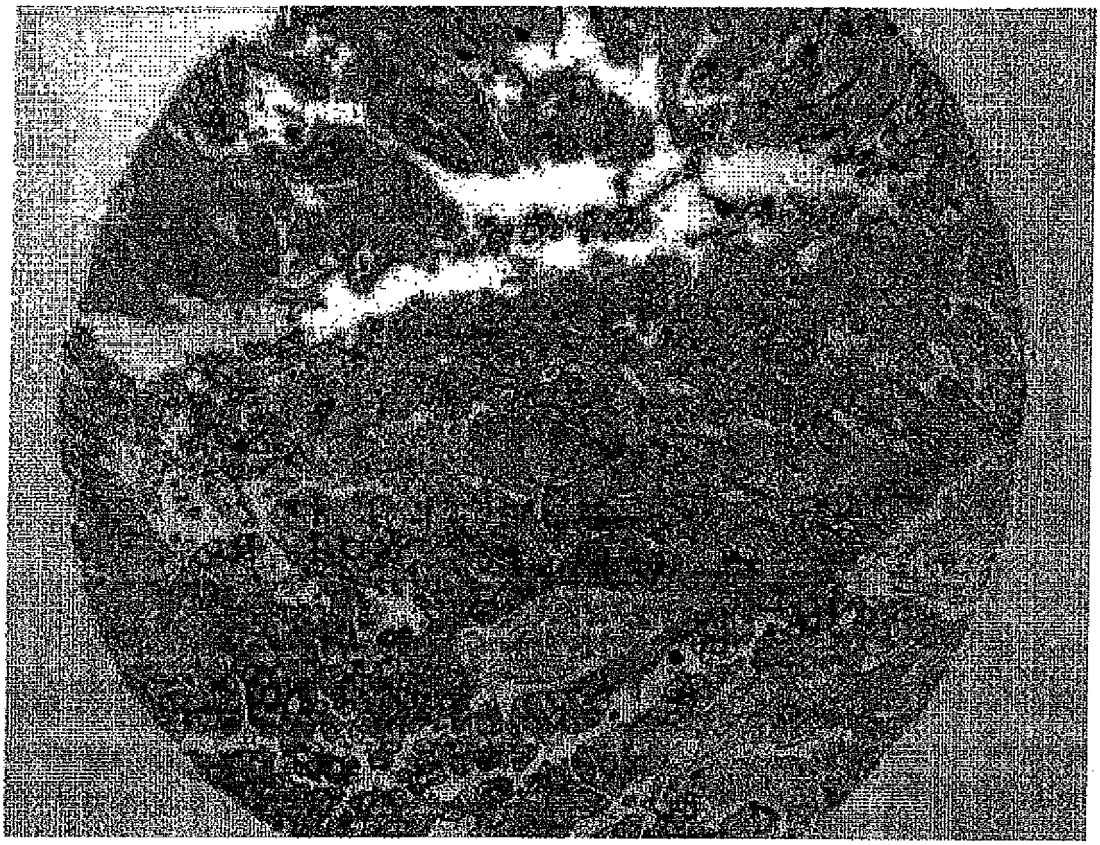


FIGURE 3L

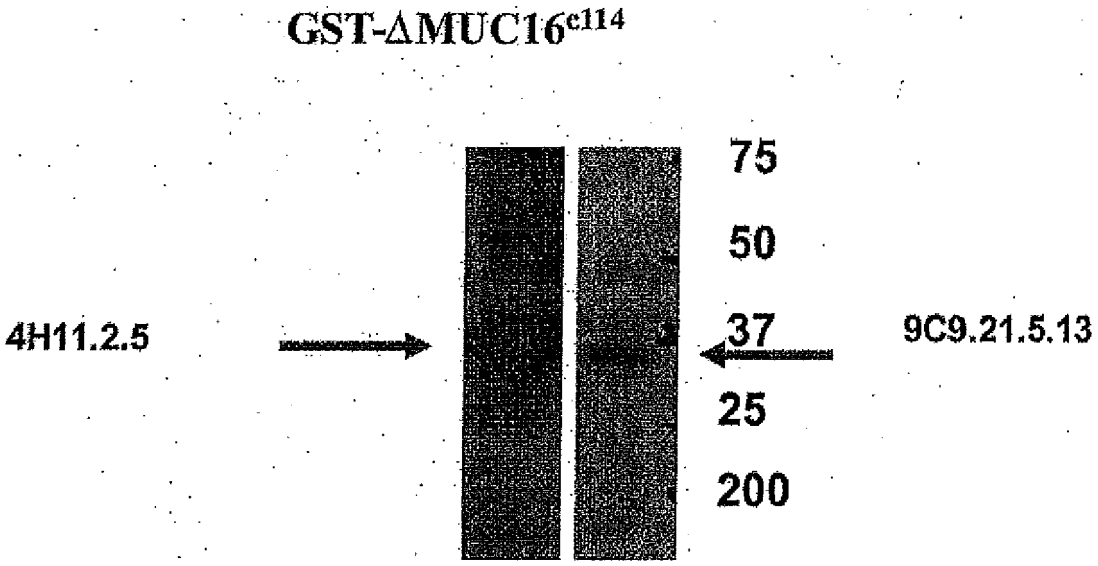


FIGURE 4A

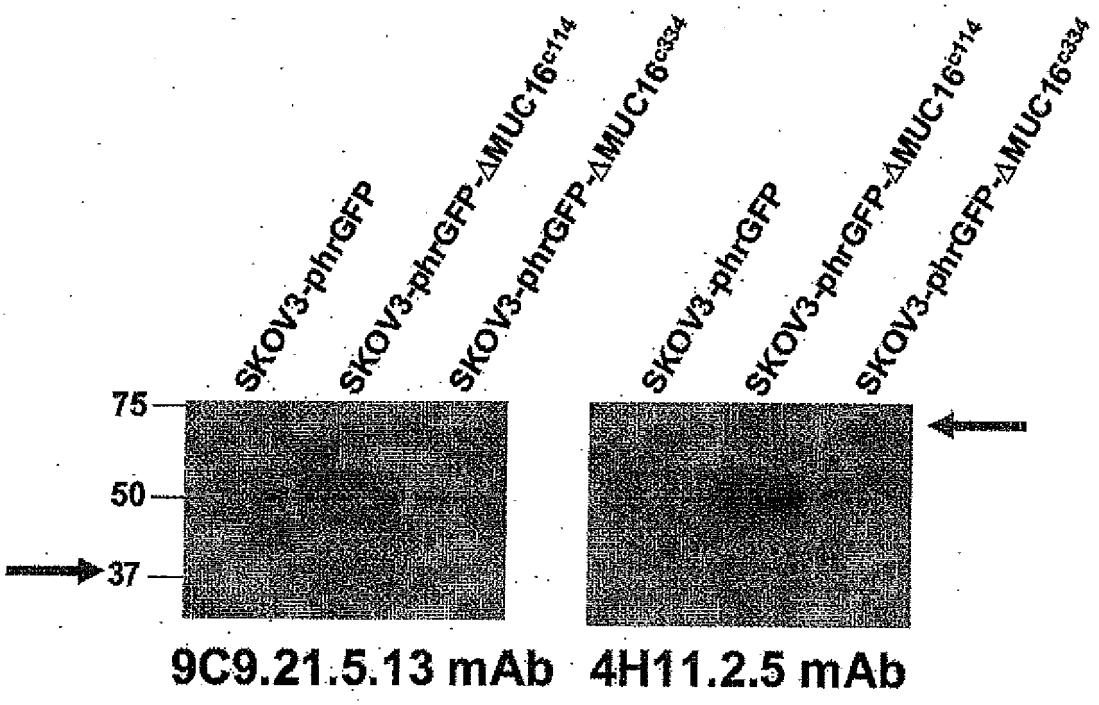

SKOV3-phrGFP
SKOV3-phrGFP-ΔMUC16c-114
SKOV3-phrGFP-ΔMUC16c-334
SKOV3-phrGFP
SKOV3-phrGFP-ΔMUC16c-114
SKOV3-phrGFP-ΔMUC16c-334
75
50
37
9C9.21.5.13 mAb
4H11.2.5 mAb

FIGURE 4B

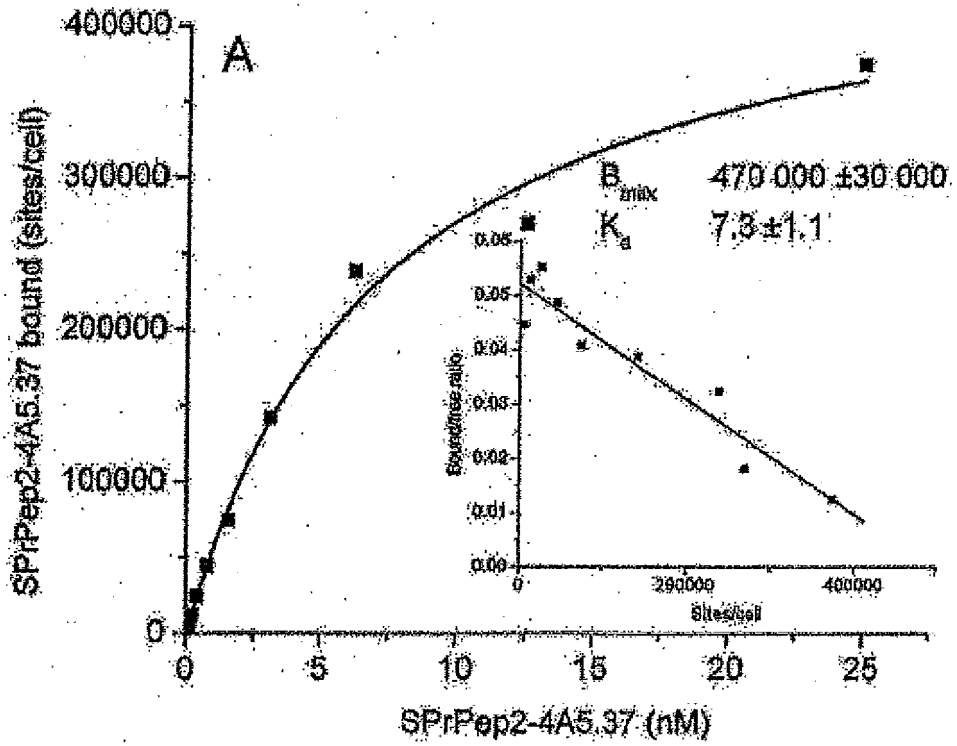


FIGURE 5A, PANEL A

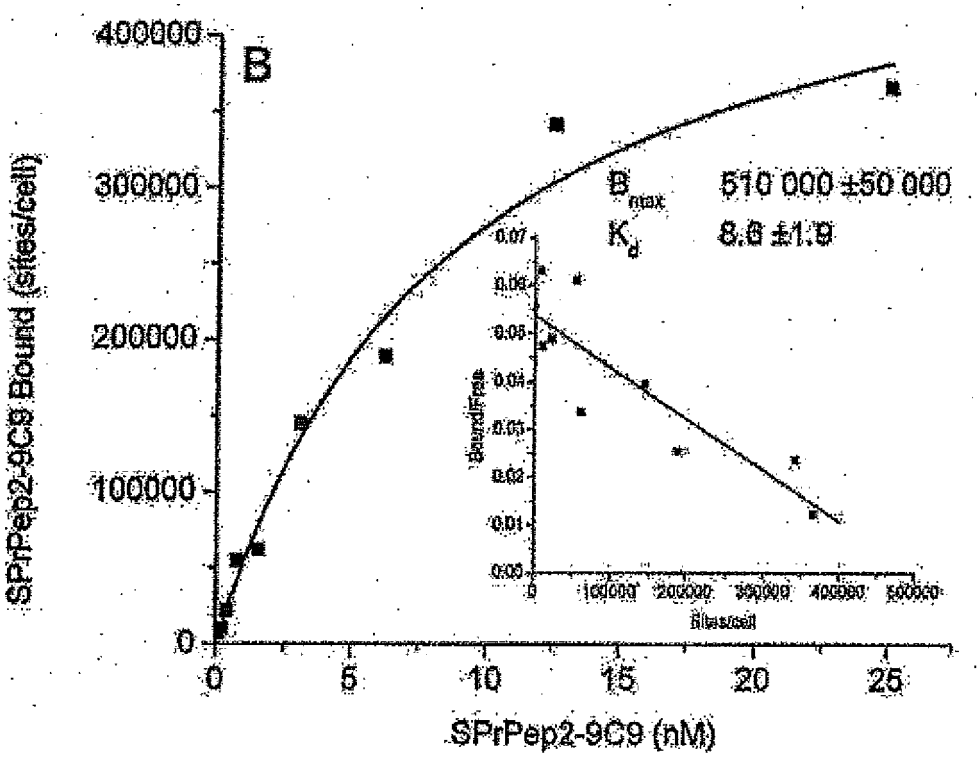


FIGURE 5A, PANEL B

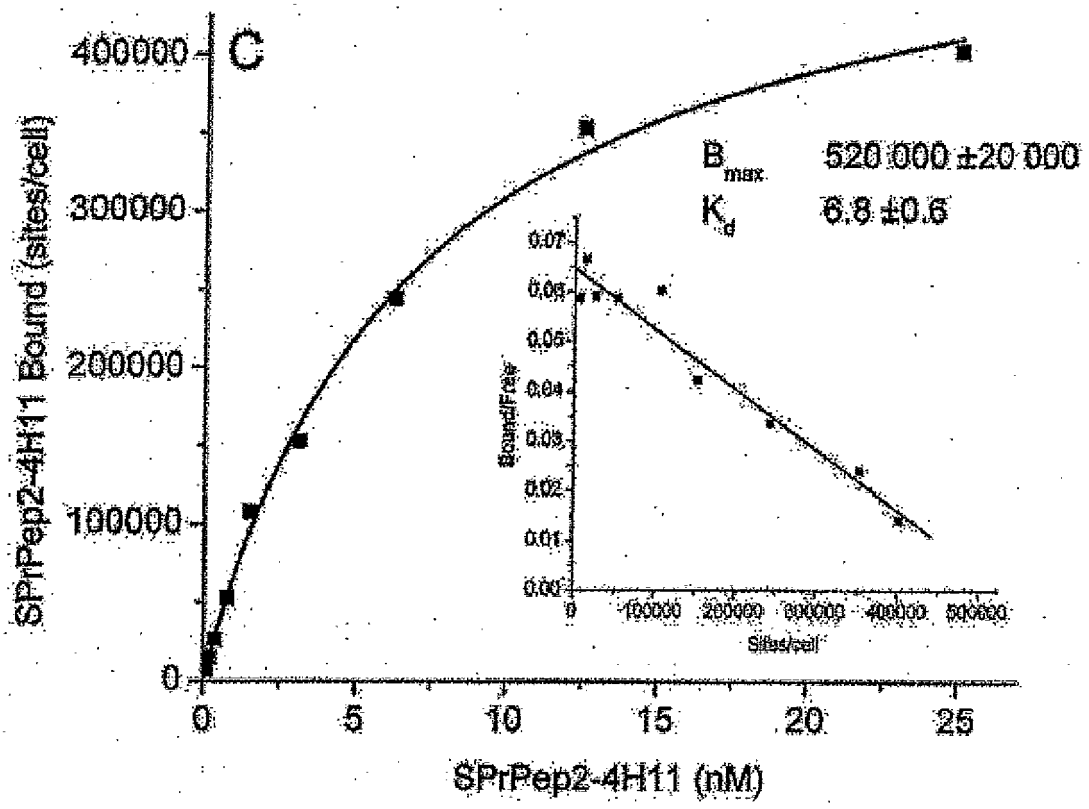


FIGURE 5A, PANEL C

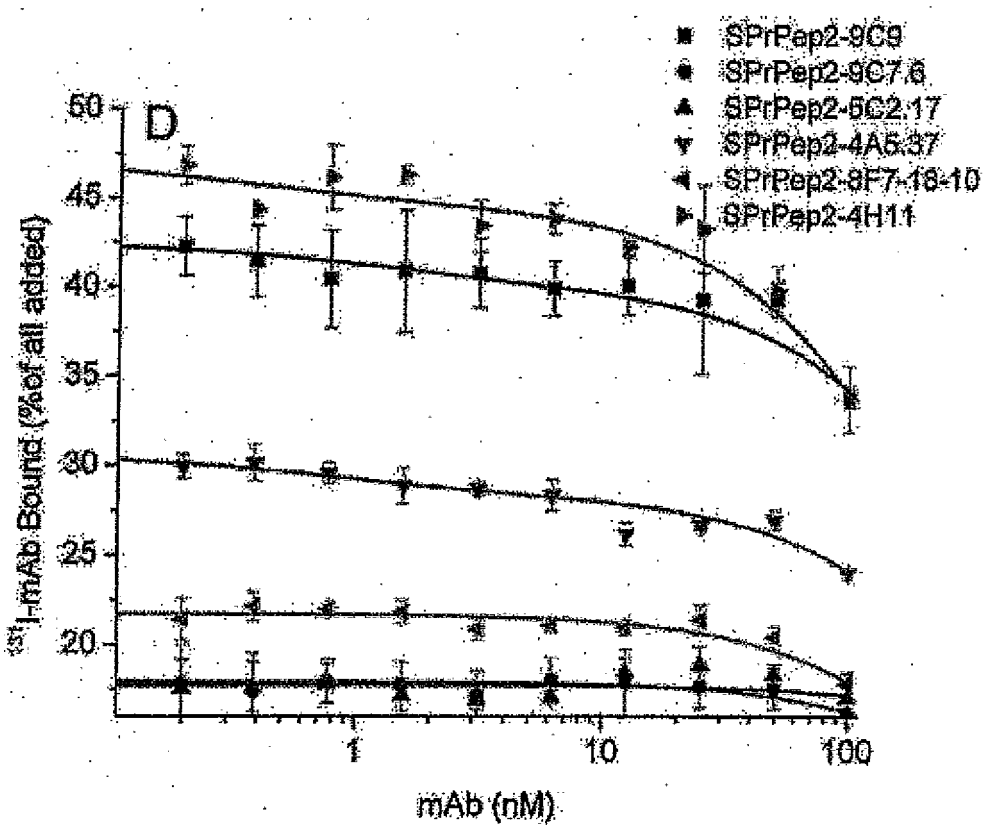


FIGURE 5A, PANEL D

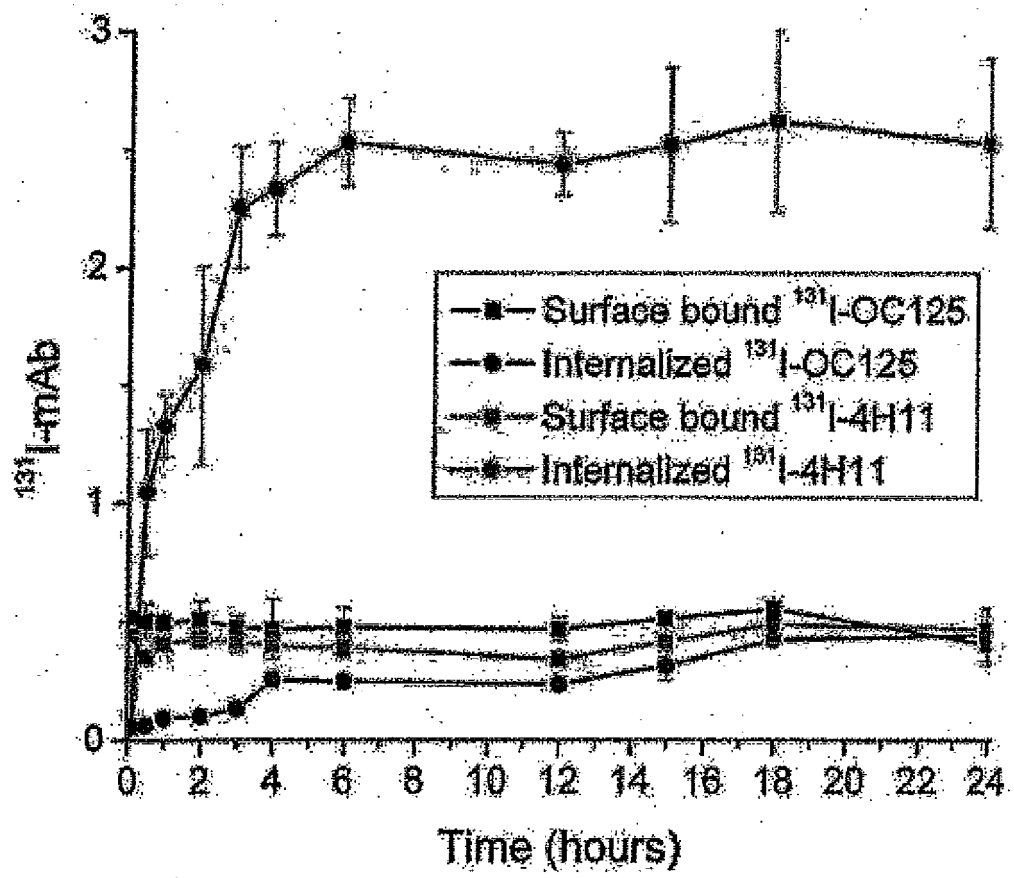

Surface bound 131I-OC125
Internalized 131I-OC125
Surface bound 131I-4H11
Internalized 131I-4H11
131I-mAb
Time (hours)
0
1
2
3
0 2 4 6 8 10 12 14 16 18 20 22 24

FIGURE 5B

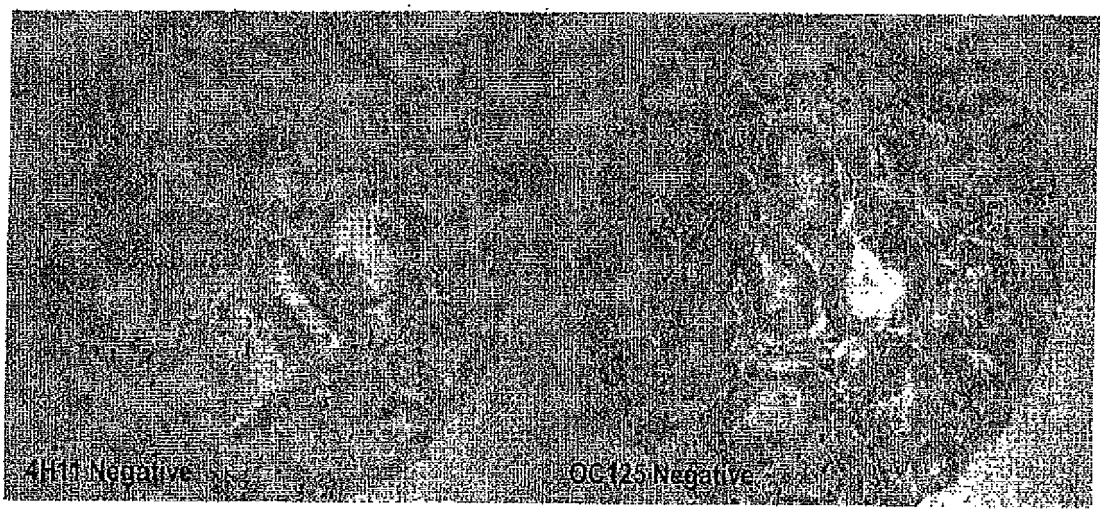


FIGURE 6A

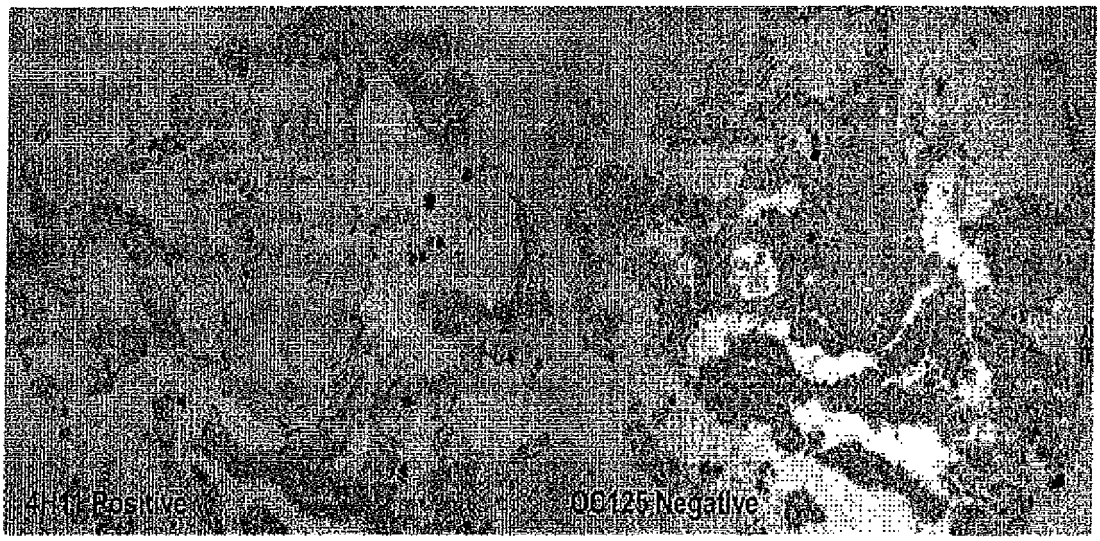


FIGURE 6B

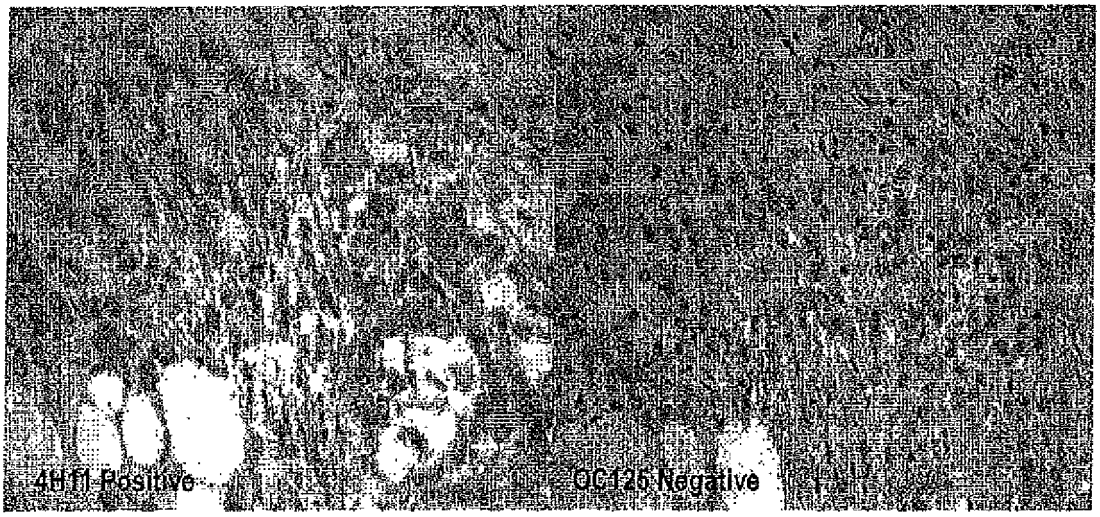


FIGURE 6C

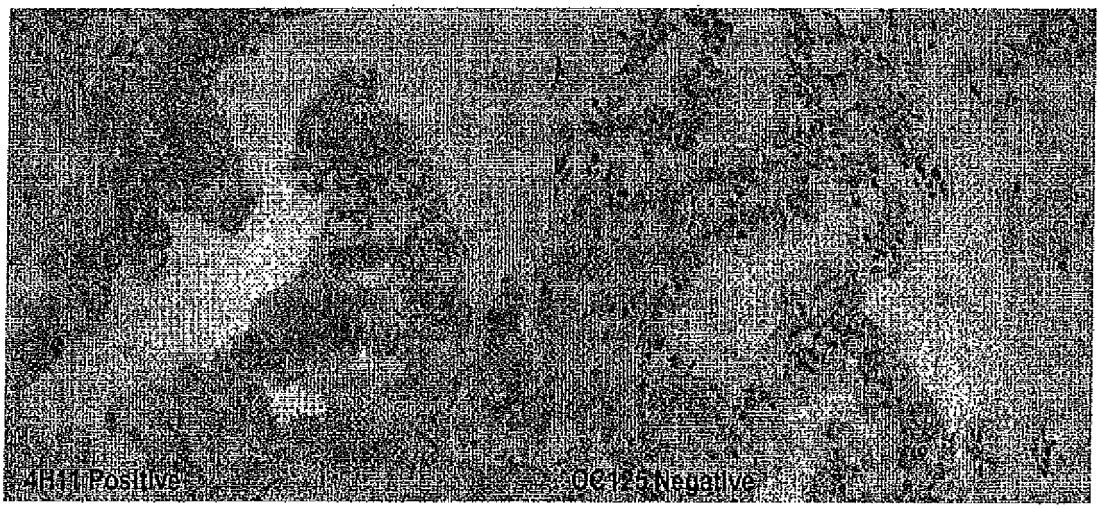


FIGURE 6D

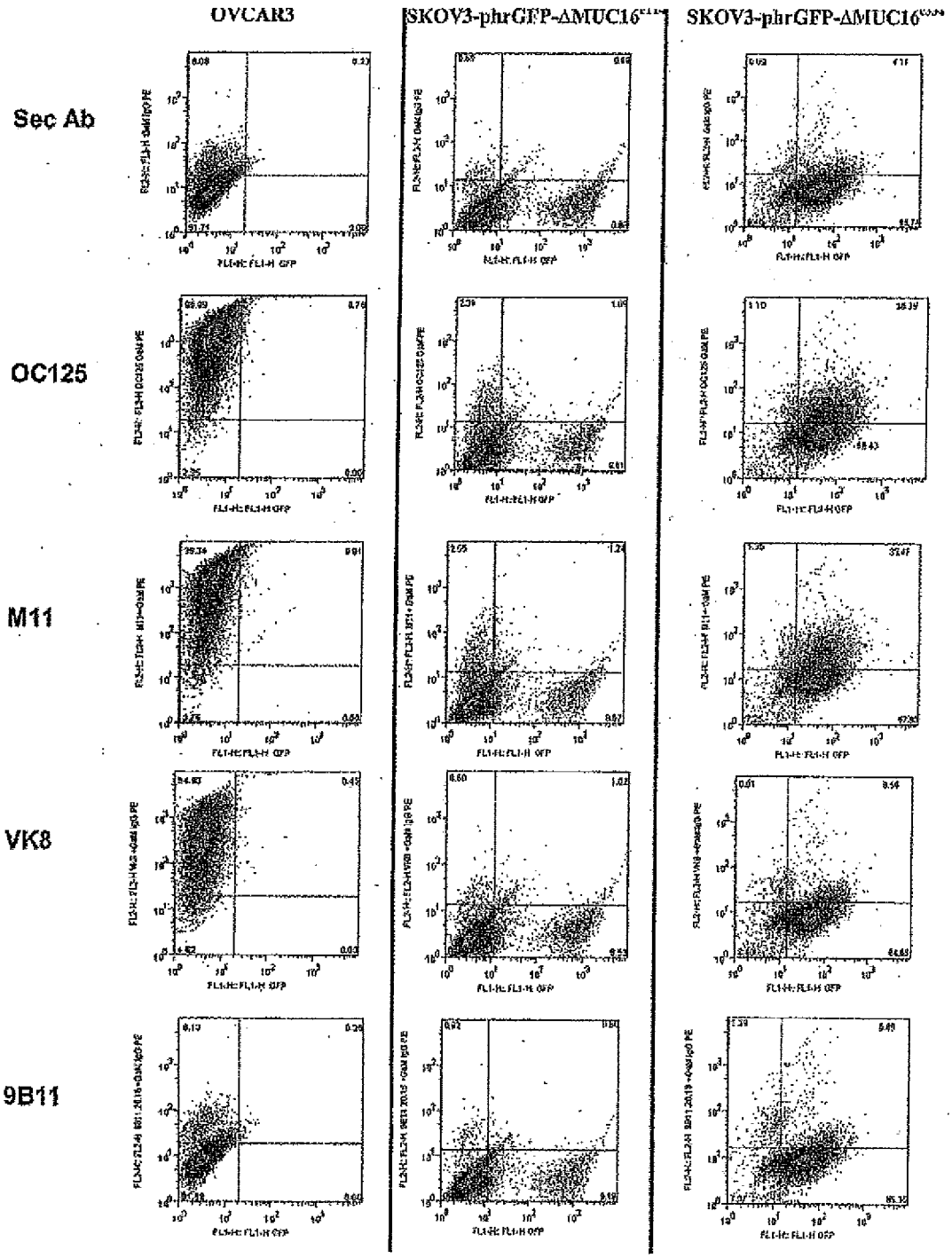


FIGURE 7, PAGE 1 OF 2

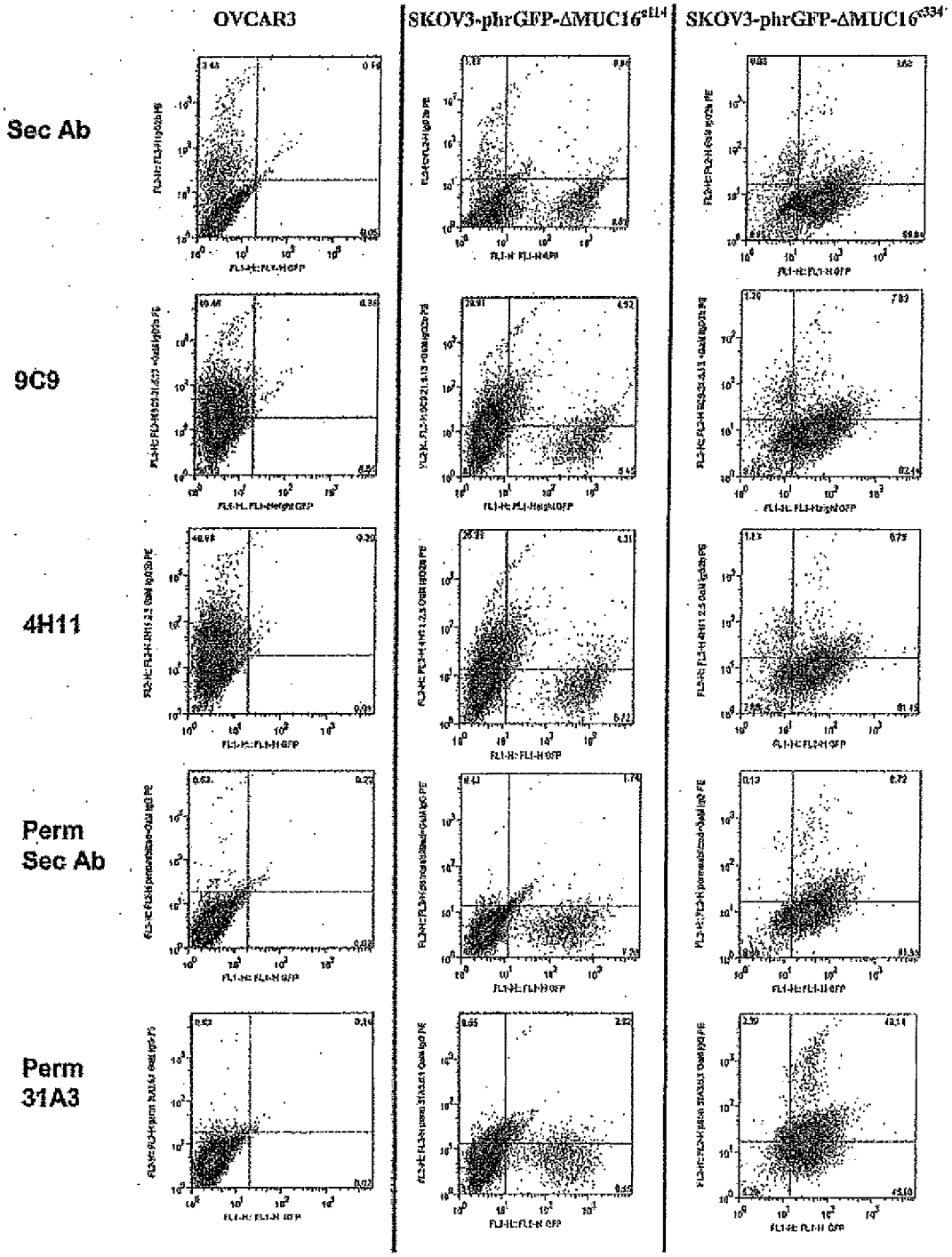


FIGURE 7, PAGE 2 OF 2

(A) 4A5 VH (SEQ ID NO:04)
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tgctatggactactggggccaagggaccacggtcaccgtctcctca

(B) 4A5 VL (SEQ ID NO:05)
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(C) 4H11 VH (SEQ ID NO:06)
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caagctggaggtcaaacgg

(E) 9B11 VH (SEQ ID NO:08)
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aaggttcacatgttccgtggacgttcggtggagggaccaagctggagatcaaacgg

FIGURE 8 (1 of 2)

(H) 24B3-VH (SEQ ID NO:11)
GAGGTGAAGCTGGAGGAGTCAGGACCTGAACTGGTGAAGCCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGTTA
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ACAATGGTGCTACTTTCTACAATCAGAAGTTCACGGGCAAGGCCACAATGACTGTAGACAAATCCTCTACCACAGCCCAC
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CTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA

(I) 24B3-VL (SEQ ID NO:12)
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TGCAATCTGGAATTCCATCAAGGTTCAGTGGCAGTGGATCTGGTACAGATTTCACTCTCACCATCAGTAGCCTGGAGCCT
GAAGATTTTGCAATGTATTACTGTCAACAGCATAATGAATACCCGTGGACGTTCGGTGGAGGGACCAAGCTGGAGATCAA
ACGGGCGGCCGCA

FIGURE 8 (2 of 2)

(A) Homo sapiens MUCIN-16 (GenBank NP_078966) (SEQ ID NO:13)

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   1 mlkpsglpgs ssptrslmtg srstkatpem dsgltgatls pktstgaivv tehtlpfts
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 121 smvsglsspr trtsstegnf tkeastytlt vettsgpvte kytvptetst tegdstetp
 181 dtryipvkit spmktfadst askenapvsm tpaettvtds htpgrtnpsf gtlyssfld
 241 spkgtpnsrg etslelilst tgypfsspep gsaghsrist saplsssasv ldnkisets
 301 fsgqsltspl spgvpearas tmpnsaipfs mtlsnaetsa ervrstissl gtpsistkq
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 421 hhstsgkete gtlntsmtpl etsapgeese mtatlvptlg fttldskirs psqvssshp
 481 relrttgsts grqssstaah gssdilratt sstskasswt sestaqqfse pqhtqwvet
 541 psmkterppa stsvaapitt svpsvvsgft tlktsstkgi wleetsadtl igestagpt
 601 hqfavptgis mtggsstrgs qgtthlltra tassetsadl tlatngvpvs vspavskta
 661 gssppggtkp sytmvssvip etsslqssaf regtslgltp lntrhpfssp epdsaghtk
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 781 spervrnats plthpspsge etagsvltls tsaettdspn ihptgtltse ssespstls
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 901 mdtwptrsaq fssshlvsel ratsstsvtn stgsalpkis hltgtatmsq tnrdtfnds
 961 apqsttwpet sprfktglps atttvstsat slsatvmvsk ftspatssme atsirepst
1021 ilttettngp gsmavastni pigkgyiteg rldtshlpig ttassetsmd ftmakesvs
1081 svspsqsmda agsstpgrts qfvdtfsddv yhltsreiti prdgtssalt pqmtathpp
1141 pdpgsarstw lgilssspss ptpkvtmsst fstqrvttsm imdtvetsrw nmpnlpstt
1201 ltpsniptsg aigkstlvpl dtpspatsle asegglptls typestntps ihlgahass
1261 spstikltma svvkpgsytp ltfpsiethi hvstarmays sgsspemtap getntgstw
1321 pttyitttdp kdtssaqvst phsvrtlrtt enhpktesat paaysgspki ssspnltsp
1381 tkawtitdtt ehstqlhytk laekssgfet qsapgpvsvv iptsptigss tleltsdvp
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1501 kseadtsair ntdsttldqh lgirslgrtg dlttvpitpl tttwtsvieh stqaqdtls
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1621 gptniqstpp mdntttgsss sgvtlgiahl pigtsspaet stnmalerrs statvsmag
1681 mgllvtsapg rsisqslgrv ssvlsestte gvtdsskgss prlntqgnta lsslepsy
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1801 gstentgkek lrtasmdlps ptpsmevtpw isltlsnapn ttdsldlshg vhtssagtl
1861 tdrslntgvt rasrlengsd tsskslsmgn sthtsmtyte ksevsssihp rpetsapga
1921 ttltstpgnr aisltlpfss ipveevistg itsgpdinsa pmthspitpp tivwtstgt
1981 eqstqplhav ssekvsvqtq stpyvnsvav saspthensv ssgsstsspy ssaslesld
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2221 vnsagppgls tvasdvidtp sdgaesiptv sfspspdtev ttishfpekt thsfrtiss
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2341 vsldnettvk tsdildarkt nelpsdssss sdlintsias stmdvtktas isptsisgm
2401 assspslfss drpqvptstt etntatspsv ssntysldgg snvggtpstl ppftithpv
2461 tssallawsr pvrtfstmvs tdtasgenpt ssnsvvtsvp apgtwtsvgs ttdlpamgf
2521 ktspageahs llastiepat aftphlsaav vtgssatsea sllttseska ihsspqtpt
```

FIGURE 9 (page 1 of 6)

2581 ptsganwets atpesllvvt etsdttltsk ilvtdtilfs tvstppskfp stgtlsgas
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2701 tvsnpdrsip gitiqgvtes plhpsstsps kivaprntty egsitvalst lpagttgsl
2761 fsqssenset talvdssagl erasvmpltt gsqgmassgg irsgsthstg tktfsslpl
2821 mnpgevtams eittnrltat qstapkgipv kptsaesgll tpvsasssps kafasltta
2881 ptwgipqstl tfefsevpsl dtksaslptp gqslntipds dastasssls kspeknpra
2941 mmtstkaisa ssfqstgfte tpegsaspsm agheprvpts gtgdpryase smsypdpsk
3001 ssamtstsla sklttlfstg qaarsgssss pislsteket sflsptasts rktslflgp
3061 marqpnilvh lqtsaltlsp tstlnmsqee ppeltssqti aeeegttaet qtltftpse
3121 ptsllpvssp teptarrkss petwassisv paktslvett dgtlvttikm ssqaaqgns
3181 wpapaeetgs spagtspgsp emsttlkims skepsispei rstvrnspwk tpettvpme
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3301 pggtrqslat mssvslespt arsitgtgqq sspelvsktt gmefsmwhgs tggttgdth
3361 slstssnile dpvtspnsvs sltdkskhkt etwvsttaip stvlnnkima aeqqtsrsv
3421 eaysstssws dqtsgsditl gaspdvtntl yitstaqtts lvslpsgdqg itsltnpsg
3481 ktssassvts psigletlra nvsavksdia ptaghlsqts spaevsildv ttaptpgis
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3661 rtlspsdtta stpistfsrv qrmsisvpdi lstswtpsst eaedvpvsmv stdhastkt
3721 pntplstflf dslstldwdt grslssatat tsapqgattp qeltletmis patsqlpfs
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3841 htaktpdatf qrqgqtaltt earatsdswn ekekstpsap witemmnsvs edtikevts
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4321 atetstvith vssgdatthv tktqatfssg tsissphqfi tstntftdvs tnpstslim
4381 essgvtittq tgptgaatqg pylldtstmp yltetplavt pdfmqsektt liskgpkdv
4441 wtsppsvaet sypssltpfl vttippatst lqgqhtsspv satsvltsgl vkttdmlnt
4501 mepvtnspqn lnnpsneila tlaattdiet ihpsinkavt nmgtassahv lhstlpvss
4561 pstatspmvp assmgdalas isipgsettd iegeptsslt agrkenstlq emnsttesn
4621 ilsnvsvgai teatkmevps fdatfiptpa qstkfpdifs vassrlsnsp pmtisthmt
4681 tqtgssgats kiplaldtst letsagtpsv vtegfahski ttamnndvkd vsqtnppfq
4741 easspssqap vlvttlpssv aftpqwhsts spvsmssvlt sslvktagkv dtsletvts
4801 pqsmsntldd isvtsaattd ietthpsint vvtnvgttgs afeshstvsa ypepskvts
4861 nvttstmedt tisrsipkss kttrtetett ssltpklret sisqeitsst etstvpyke
4921 tgattevsrt dvtsssstsf pgpdqstvsl distetntrl stspimtesa eitittqtg
4981 hgatsqdtft mdpsnttpqa gihsamthgf sqldvttlms ripqdvswts ppsvdktss
5041 ssflsspamt tpslisstlp edklsspmts lltsglvkit dilrtrlepv tsslpnfss
5101 sdkilatskd skdtkeifps inteetnvka nnsgheshsp aladsetpka ttqmvittt
5161 gdpapstsmp vhgssettni kreptyfltp rlretstsqe ssfptdtsfl lskvptgti

FIGURE 9 (page 2 of 6)

```
5221 evsstgvnss skistpdhdk stvppdtftg eiprvftssi ktksaemtit tqasppesa
5281 hstlpldtst tlsqggthst vtqgfpysev ttlmgmgpgn vswmttppve etssvsslm
5341 spamtspspv sstspqsips splpvtalpt svlvtttdvl gttspesvts sppnlssit
5401 erpatykdta hteaamhhst ntavtnvgts gsghksqssv ladsetskat plmsttstl
5461 dtsvststpn isqtnqiqte ptaslsprlr esstsektss ttetntafsy vptgaitqa
5521 rteisssrts isdldrptia pdistgmitr lftspimtks aemtvttqtt tpgatsqgi
5581 pwdtsttlfq ggthstvsqg fphseittlr srtpgdvswm ttppveetss gfslmspsm
5641 spspvsstsp esipssplpv talltsvlvt ttnvlgttsp epvtssppnl ssptqerlt
5701 ykdtahteam hasmhtntav anvgtsisgh esqssvpads htskatspmg itfamgdts
5761 ststpaffet riqtestssl ipglrdtrts eeintvtets tvlsevpttt ttevsrtev
5821 tssrttisgp dhskmspyis tetitrlstf pfvtgstema itnqtgpigt isqatltld
5881 sstaswegth spvtqrfphs eetttmsrst kgvswqspps veetsspssp vplpaitsh
5941 slysavsgss ptsalpvtsl ltsgrrktid mldthselvt sslpsassfs geiltseas
6001 ntetihfsen taetnmgttn smhklhssvs ihsqpsghtp pkvtgsmmed aivststpg
6061 petknvdrds tspltpelke dstalvmnst tesntvfssv sldaatevsr aevtyydpt
6121 mpasaqstks pdispeasss hsnsppltis thktiatqtg psgvtslgql tldtstiat
6181 agtpsartqd fvdsettsvm nndlndvlkt spfsaeeans lssqapllvt tspspvtst
6241 qehstsslvs vtsvptptla kitdmdtnle pvtrspqnlr ntlatseatt dthtmhpsi
6301 tavanvgtts spnefyftvs pdsdpykats avvitstsgd sivstsmprs samkkiese
6361 tfslifrlre tstsqkigss sdtstvfdka ftaattevsr teltsssrts iqgtekptm
6421 pdtstrsvtm lstfagltks eertiatqtg phratsqgtl twdtsittsq agthsamth
6481 fsqldlstlt srvpeyisgt sppsvektss sssllslpai tspspvpttl pesrpsspv
6541 ltslptsglv kttdmlasva slppnlgsts hkipttsedi kdtekmypst niavtnvgt
6601 tsekesyssv payseppkvt spmvtsfnir dtivstsmpg sseitrieme stfslahgl
6661 gtstsqdpiv steksavlhk lttgatetsr tevassrrts ipgpdhstes pdistevip
6721 lpislgites snmtiitrtg pplgstsqgt ftldtpttss ragthsmatq efphsemtt
6781 mnkdpeilsw tippsiekts fsssimpspa mtsppvsstl pktihttpsp mtsllтpsl
6841 mttdtlgtsp epttssppnl sstsheiltt dedttaieam hpststaatn vettssghg
6901 qssvladsek tkatapmdtt stmghttvst smsvssettk ikrestyslt pglretsis
6961 nasfstdtsi vlsevptgtt aevsrtevts sgrtsipgps qstvlpeist rtmtrlfas
7021 tmtesaemti ptqtgpsgst sqdtltldts ttksqakths tltqrfphse mttlmsrgp
7081 dmswqsspsl enpsslpsll slpattsppp isstlpvtis ssplpvtsll tsspvtttd
7141 lhtspelvts sppklshtsd erlttgkdtt nteavhpstn taasnveips sghespssa
7201 adsetskats pmfitstqed ttvaistphf letsriqkes isslspklre tgssvetss
7261 ietsavlsev sigatteisr tevtsssrts isgsaestml peisttrkii kfptspila
7321 ssemtiktqt sppgstsest ftldtsttps lvithstmtq rlphseittl vsrgagdvp
7381 psslpveets ppssqlslsa mispspvsst lpasshsssa svtslltpgq vkttevlda
7441 aepetsspps lsstsveila tsevttdtek ihpfsntavt kvgtsssghe spssvlpds
7501 ttkatsamgt isimgdtsvs tltpalsntr kiqsepassl ttrlretsts eetslatea
7561 tvlskvstga ttevsrteai sfsrtsmsgp eqstmsqdis igtiprisas svltesakm
7621 ittqtgpses tlestlnlnt attpswveth siviqgfphp emttsmgrgp ggvswpspp
7681 vketsppssp lslpavtsph pvsttflahi ppsplpvtsl ltsgpatttd ilgtstepg
7741 sssssslstts herlttykdt ahteavhpst ntggtnvatt ssgyksqssv ladsspmct
7801 stmgdtsvlt stpafletrr iqtelasslt pglressgse gtssgtkmst vlskvptga
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FIGURE 9 (page 3 of 6)

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 7861 teiskedvts ipgpaqstis pdistrtvsw fstspvmtes aeitmnthts plgattqgt
 7921 tldtssttsl tmthstisqg fshsqmstlm rrgpedvswm sppllektrp sfslmsspa
 7981 tspspvsstl pesissplp vtslltsgla kttdmlhkss epvtnspanl sstsveila
 8041 sevttdtekt hpssnrtvtd vgtsssghes tsfvladsqt skvtspmvit stmedtsvs
 8101 stpgffetsr iqteptsslt lglrktssse gtslatemst vlsgvptgat aevsrtevt
 8161 ssrtsisgfa qltvspetst etitrlptss imtesaemmi ktqtdppgst pesthtvdi
 8221 ttpnwveths tvtqrfshse mttlvsrspg dmlwpsqssv eetssassll slpattsps
 8281 vsstlvedfp saslpvtsll npglvittdr mgisrepgts stsnlsstsh erlttledt
 8341 dtedmqpsth tavtnvrtsi sghesqssvl sdsetpkats pmgttytmge tsvsistsd
 8401 fetsriqiep tssltsglre tssserissa tegstvlsev psgattevsr tevissrgt
 8461 msgpdqftis pdisteaitr lstspimtes aesaitietg spgatsegtl tldtstttf
 8521 sgthstaspg fshsemttlm srtpgdvpwp slpsveeass vssslsspam tstsffstl
 8581 esissphpv talltlgpvk ttdmlrtsse pctssppnls stsaeilats evtkdreki
 8641 pssntpvvnv gtviykhlsp ssvladlvtt kptspmatts tlgntsvsts tpafpetmm
 8701 qptssltsgl reistsqets satersasls gmptgattkv srtealslgr tstpgpaqs
 8761 ispeisteti tristplttt gsaemtitpk tghsgassqg tftldtssra swpgthsaa
 8821 hrsphsgmtt pmsrgpedvs wpsrpsvekt sppsslvsls avtspsplys tpsesshss
 8881 lrvtslftpv mmkttdmldt slepvttspp smnitsdesl atskatmete aiqlsenta
 8941 tqmgtisarq efyssypglp epskvtspvv tsstikdivs ttipasseit riemestst
 9001 tptpretsts qeihsatkps tvpykaltsa tiedsmtqvm sssrgpspdq stmsqdist
 9061 vitrlstspi ktestemtit tqtgspgats rgtltldtst tfmsgthsta sqgfshsqm
 9121 almsrtpgdv pwlshpsvee assasfslss pvmtssspvs stlpdsihss slpvtsllt
 9181 glvkttellg tssepetssp pnlsstsaei laitevttdt eklemtnvvt sgythespsi
 9241 vladsvttka tssmgitypt gdtnvltstp afsdtsriqt kskslsltpgl metsiseet
 9301 satekstvls svptgattev srteaisssr tsipgpaqst mssdtsmeti tristpltr
 9361 estdmaitpk tgpsgatsqg tftldsssta swpgthsatt qrfpqsvvtt pmsrgpedv
 9421 wpsplsvekn sppsslvsss svtspsplys tpsgsshssp vpvtslftsi mmkatdmld
 9481 slepettsap nmnitsdesl aaskattete aihvfentaa shvettsate elysssgpgf
 9541 eptkvispvv tsssirdnmv sttmpgssgi trieiesmss ltpglretrt sqditsste
 9601 stvlykmpsg atpevsrtev mpssrtsipg paqstmsldi sdevvtrlst spimtesae
 9661 tittqtgysl atsqvtlplg tsmtflsgth stmsqglshs emtnlmsrgp eslswtspr
 9721 vettrssssl tslplttsls pvsstlldss pssplpvtsl ilpglvktte vldtssepkt
 9781 ssspnlssts veipatseim tdtekihpss ntavakvrts ssvheshssv ladsettit
 9841 psmgitsavd dttvftsnpa fsetrripte ptfsltpgfr etstseetts itetsavly
 9901 vptsattevs mteimssnri hipdsdqstm spdiitevit rlssssmmse stqmtittql
 9961 sspgataqst ltlatttapl arthstvppr flhsemttlm srspenpswk sslfvekts
10021 sssllslpvt tspsvsstlp qsipsssfsv tslltpgmvk ttdtstepgt slspnlsgt
10081 veilaasevt tdtekihpss smavtnvgtt ssghelyssv sihsepskat ypvgtpssm
10141 etsistsmpa nfettgfeae pfshltsgfr ktnmsldtss vtptntpssp gsthllqss
10201 tdftssakts spdwppasqy teipvdiitp fnaspsites tgitsfpesr ftmsvtestl
10261 hlstdllpsa etistgtvmp slseamtsfa ttgvpraisg sgspfsrtes gpgdatlst
10321 aeslpsstpv pfssstfttt dsstipalhe itsssatpyr vdtslgtess ttegrlvmv
10381 tldtssqpgr tssspildtr mtesvelgtv tsayqvpsls trltrtdgim ehitkipne
10441 ahrgtirpvk gpqtstspas pkglhtggtk rmettttalk ttttalktts ratlttsvy
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FIGURE 9 (page 4 of 6)

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10501 ptlgtltpln asmqmastip temmittpyv fpdvpettss latslgaets talprttps
10561 fnresettas lvsrsgaers pviqtldvss sepdttaswv ihpaetiptv skttpnffk
10621 eldtvsstat shgadvssai ptnispseld altplvtisg tdtsttfptl tksphetet
10681 ttwlthpaet sstiprtipn fshhesdatp siatspgaet ssaipimtvs pgaedlvts
10741 vtssgtdrnm tiptltlspg epktiaslvt hpeaqtssai ptstispavs rlvtsmvts
10801 aaktsttnra ltnspgepat tvslvthpaq tsptvpwtts iffhsksdtt psmttshga
10861 sssavptptv stevpgvvtp lvtssravis ttipiltlsp gepettpsma tshgeeass
10921 iptptvspgv pgvvtslvts sravtsttip iltfslgepe ttpsmatshg teagsavpt
10981 lpevpgmvts lvassravts ttlptltlsp gepettpsma tshgaeasst vptvspevp
11041 vvtslvtsss gvnstsiptl ilspgelett psmatshgae assavptptv spgvsgvvt
11101 lvtssravts ttipiltlss sepettpsma tshgveassa vltvspevpg mvtslvtss
11161 avtsttiptl tissdepett tslvthseak misaiptlav sptvqglvts lvtssgset
11221 afsnltvass qpetidswva hpgteassvv ptltvstgep ftnislvthp aessstlpr
11281 tsrfshseld tmpstvtspe aesssaistt ispgipgvlt slvtssgrdi satfptvpe
11341 pheseatasw vthpavtstt vprttpnysh sepdttpsia tspgaeatsd fptitvspd
11401 pdmvtsqvts sgtdtsitip tltlssgepe tttsfityse thtssaiptl pvspgaskm
11461 tslvissgtd stttfptlte tpyepettai qlihpaetnt mvprttpkfs hsksdttlp
11521 aitspgpeas savstttisp dmsdlvtslv pssgtdtstt fptlsetpye pettatwlt
11581 paetsttvsg tipnfshrgs dtapsmvtsp gvdtrsgvpt ttippsipgv vtsqvtssa
11641 dtstaiptlt pspgepetta ssathpgtqt gftvpirtvp ssepdtmasw vthppqtst
11701 vsrttssfsh sspdatpvma tsprteassa vlttispgap emvtsqitss gaatsttvp
11761 lthspgmpet tallsthprt etsktfpast vfpqvsetta sltirpgaet stalptqtt
11821 slftllvtgt srvdlsptas pgvsaktapl sthpgtetst miptstlslg llettglla
11881 sssaetstst ltltvspavs glssasittd kpqtvtswnt etspsvtsvg ppefsrtvt
11941 ttmtlipsem ptppktshge gvspttilrt tmveatnlat tgssptvakt tttfntlag
12001 lftplttpgm stlasesvts rtsynhrswi sttssynrry wtpatstpvt stfspgist
12061 sipsstaatv pfmvpftlnf titnlqyeed mrhpgsrkfn aterelqgll kplfrnssl
12121 ylysgcrlas lrpekdssat avdaicthrp dpedlgldre rlywelsnlt ngiqelgpy
12181 ldrnslyvng fthrssmptt stpgtstvdv gtsgtpsssp spttagpllm pftlnftit
12241 lqyeedmrrt gsrkfntmes vlqgllkplf kntsvgplys gcrltllrpe kdgaatgvd
12301 icthrldpks pglnreqlyw elskltndie elgpytldrn slyvngfthq ssvsttstp
12361 tstvdlrtsg tpsslsspti maagpllvpf tlnftitnlq ygedmghpgs rkfntterv
12421 qgllgpifkn tsvgplysgc rltslrsekd gaatgvdaic ihhldpkspg lnrerlywe
12481 sqltngikel gpytldrnsl yvngfthrts vptsstpgts tvdlgtsgtp fslpspata
12541 pllvlftlnf titnlkyeed mhrpgsrkfn ttervlqtll gpmfkntsvg llysgcrlt
12601 lrsekdgaat gvdaicthrl dpkspgvdre qlywelsqlt ngikelgpyt ldrnslyvn
12661 fthwipvpts stpgtstvdl gsgtpsslps pttagpllvp ftlnftitnl kyeedmhcp
12721 srkfntterv lqsllgpmfk ntsvgplysg crltllrsek dgaatgvdai cthrldpks
12781 gvdreqlywe lsqltngike lgpytldrns lyvngfthqt sapntstpgt stvdlgtsg
12841 psslpsptsa gpllvpftln ftitnlqyee dmhhpgsrkf nttervlqgl lgpmfknts
12901 gllysgcrlt llrpekngaa tgmdaicshr ldpkspglnr eqlywelsql thgikelgp
12961 tldrnslyvn gfthrssvap tstpgtstvd lgtsgtpssl pspttavpll vpftlnfti
13021 nlqygedmrh pgsrkfntte rvlqgllgpl fknssvgply sgcrlislrs ekdgaatgv
13081 aicthhlnpq spgldreqly wqlsqmtngi kelgpytldr nslyvngfth rssglttst
```

FIGURE 9 (page 5 of 6)

13141 wtstvdlgts gtpspvpspt ttgpllvpft lnftitnlqy eenmghpgsr kfnitesvl
13201 gllkplfkst svgplysgcr ltllrpekdg vatrvdaict hrpdpkipgl drqqlywel
13261 qlthsitelg pytldrdsly vngftqrssv pttstpgtft vqpetsetps slpgptatg
13321 vllpftlnft itnlqyeedm rrpgsrkfnt tervlqgllm plfkntsvss lysgcrltl
13381 rpekdgaatr vdavcthrpd pkspgldrer lywklsqlth gitelgpytl drhslyvng
13441 thqssmtttr tpdtstmhla tsrtpaslsg pmtaspllvl ftinftitnl ryeenmhhp
13501 srkfntterv lqgllrpvfk ntsvgplysg crltllrpkk dgaatkvdai ctyrpdpks
13561 gldreqlywe lsqlthsite lgpytldrds lyvngftqrs svpttsipgt ptvdlgtsg
13621 pvskpgpsaa spllvlftln ftitnlryee nmqhpgsrkf nttervlqgl lrslfksts
13681 gplysgcrlt llrpekdgta tgvdaicthh pdpkspr1dr eqlywelsql thnitelgp
13741 aldndslfvn gfthrssvst tstpgtptvy lgasktpasi fgpsaashll ilftlnfti
13801 nlryeenmwp gsrkfntter vlqgllrplf kntsvgplys gcrltllrpe kdgeatgvd
13861 icthrpdptg pgldreqlyl elsqlthsit elgpytldrd slyvngfthr ssvpttstg
13921 vseepftlnf tinnlrymad mgqpgslkfn itdnvmqhll splfqrsslg arytgcrvi
13981 lrsvkngaet rvdllctylq plsgpglpik qvfhelsqqt hgitrlgpys ldkdslyln
14041 ynepgpdepp ttpkpattfl pplseattam gyhlktltln ftisnlqysp dmgkgsatf
14101 stegvlqhll rplfqkssmg pfylgcqlis lrpekdgaat gvdttctyhp dpvgpgldi
14161 qlywelsqlt hgvtqlgfyv ldrdslfing yapqnlsirg eyqinfhivn wnlsnpdpt
14221 seyitllrdi qdkvttlykg sqlhdtfrfc lvtnltmdsv lvtvkalfss nldpslveq
14281 fldktlnasf hwlgstyqlv dihvtemess vyqptsssst qhfylnftit nlpysqdka
14341 pgttnyqrnk rniedalnql frnssiksyf sdcqvstfrs vpnrhhtgvd slcnfspla
14401 rvdrvaiyee flrmtrngtq lqnftldrss vlvdgyspnr nepltgnsdl pfwavilig
14461 agllgvitcl icgvlvttrr rkkegeynvq qqcpgyyqsh ldledlq

(B) Peptide 1
14394 14410
nfsplar rvdrvaiyee (SEQ ID NO:01)

(C) Peptide 2
14425 14442
tldrss vlvdgyspnr ne (SEQ ID NO:02)

(D) Peptide 3
14472 14492
cgvlvttrr rkkegeynvq qq (SEQ ID NO:03)

(E) Transmembrane Region:
14452 14475
fwaviligl agllgvitcl icgvl (SEQ ID NO:14)

(F) Peptide containing the cysteine loop peptide:
14367 14398
ksyf sdcqvstfrs vpnrhhtgvd slcnfspl (SEQ ID NO:15)

FIGURE 9 (page 6 of 6)

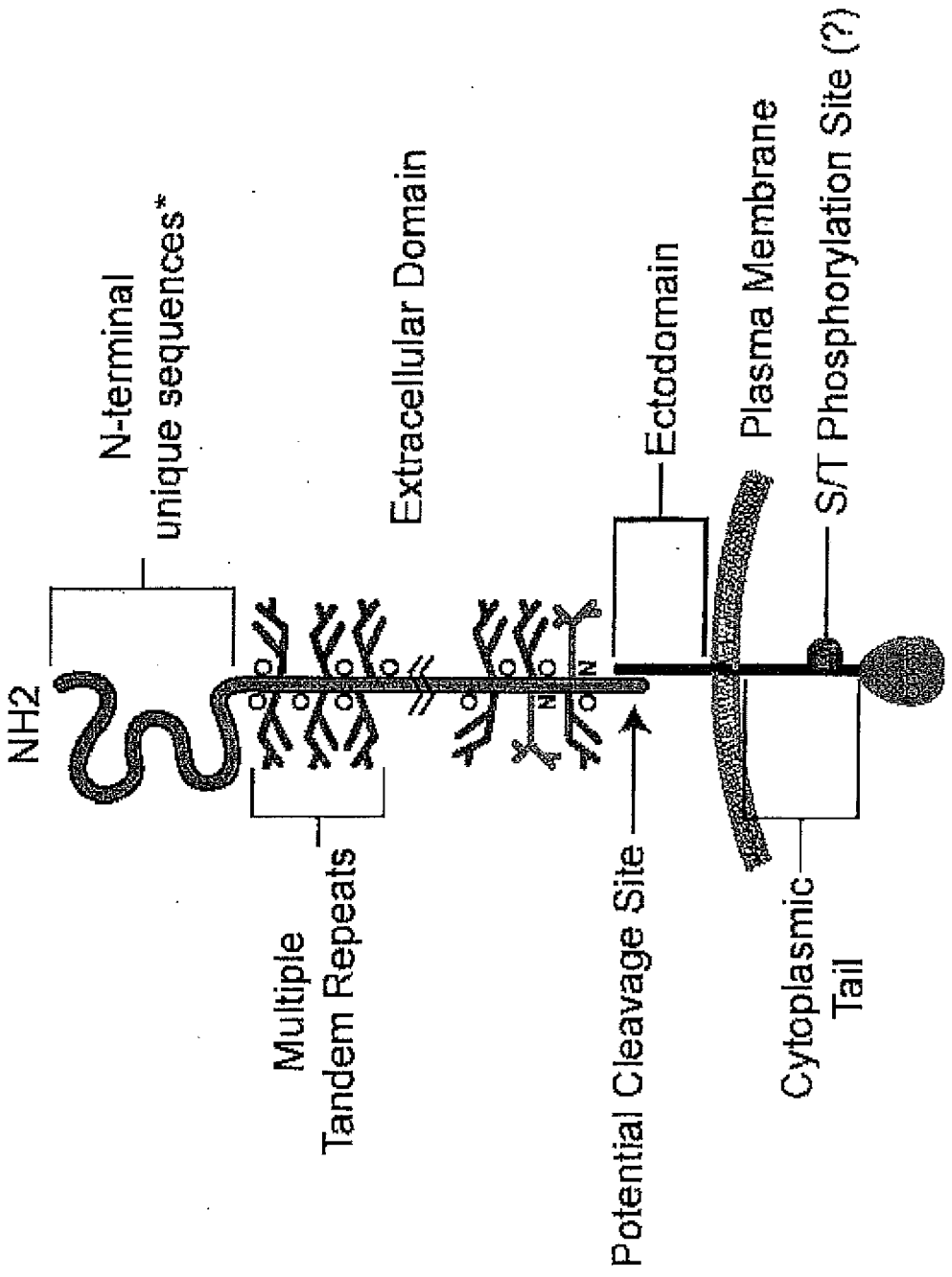
NH2
N-terminal
unique sequences*
Extracellular Domain
Ectodomain
Plasma Membrane
S/T Phosphorylation Site (?)
Multiple
Tandem Repeats
Potential Cleavage Site
Cytoplasmic
Tail

Figure 10

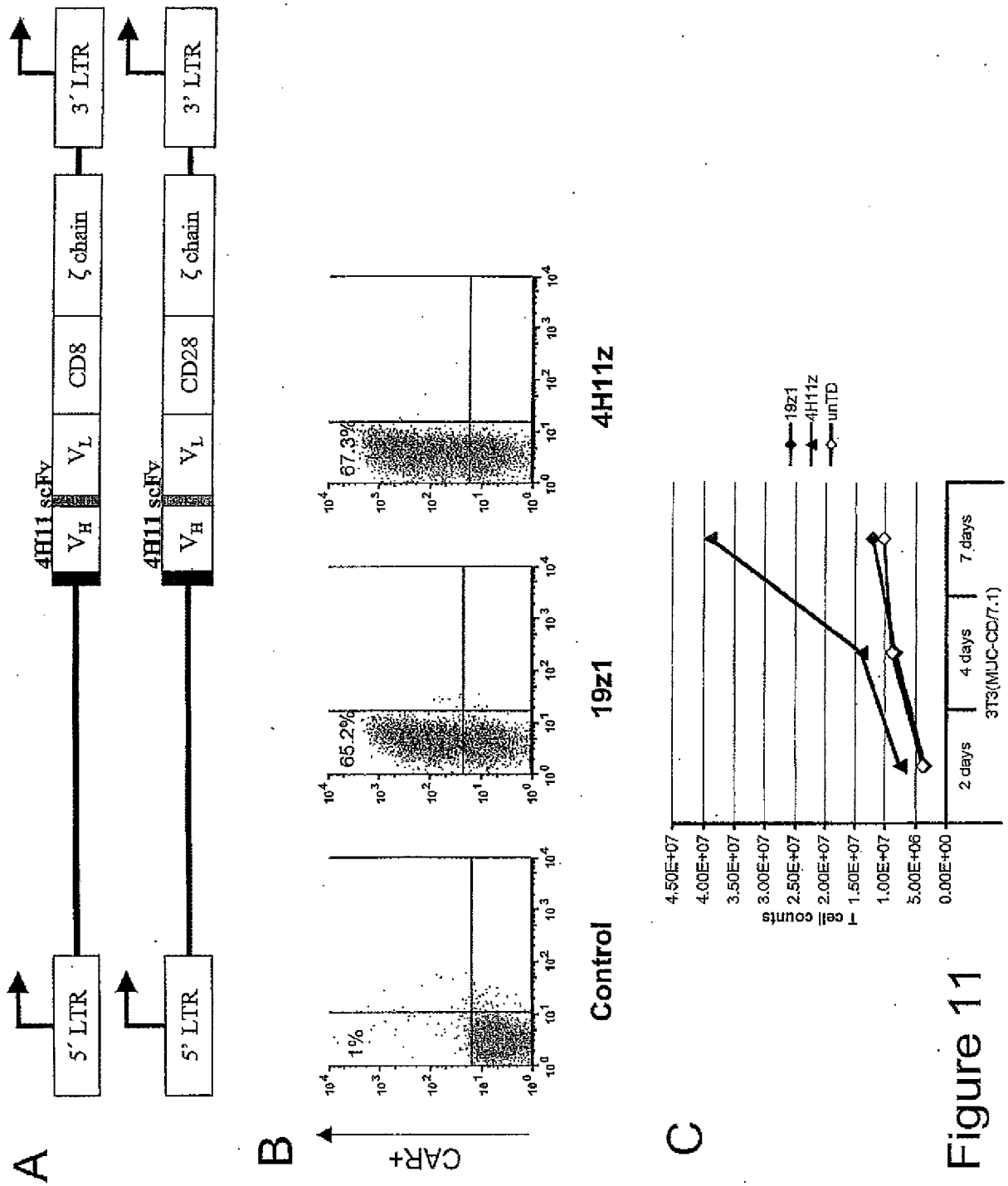
A
5' LTR
3' LTR
4H11 scFv
V_H
V_L
CD8
ζ chain
CD28
B
CAR+
1%
65.2%
67.3%
Control
19z1
4H11z
C
19z1
4H11z
unTD
T cell counts
4.50E+07
4.00E+07
3.50E+07
3.00E+07
2.50E+07
2.00E+07
1.50E+07
1.00E+07
5.00E+06
0.00E+00
2 days
4 days
7 days
3T3(MUC-CD/7.1)

Figure 11

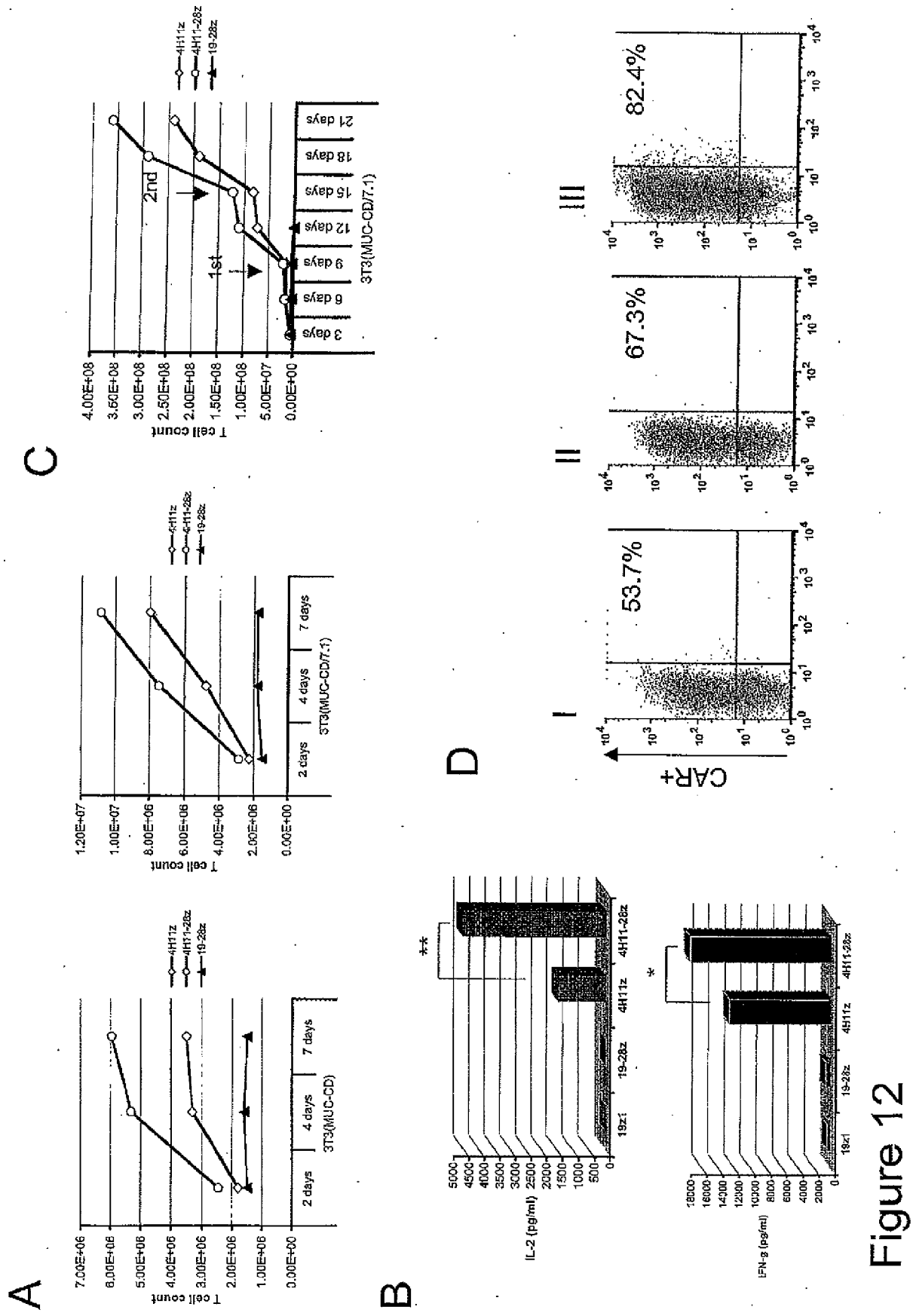
A
T cell count
4H11z
4H11-28z
19-28z
2 days
4 days
7 days
3T3(MUC-CD)
B
IL-2 (pg/ml)
IFN-g (pg/ml)
**
*
19z1
19-28z
4H11z
4H11-28z
C
T cell count
1st
2nd
3 days
6 days
9 days
12 days
15 days
18 days
21 days
3T3(MUC-CD/7.1)
D
I
II
III
CAR+
53.7%
67.3%
82.4%

Figure 12

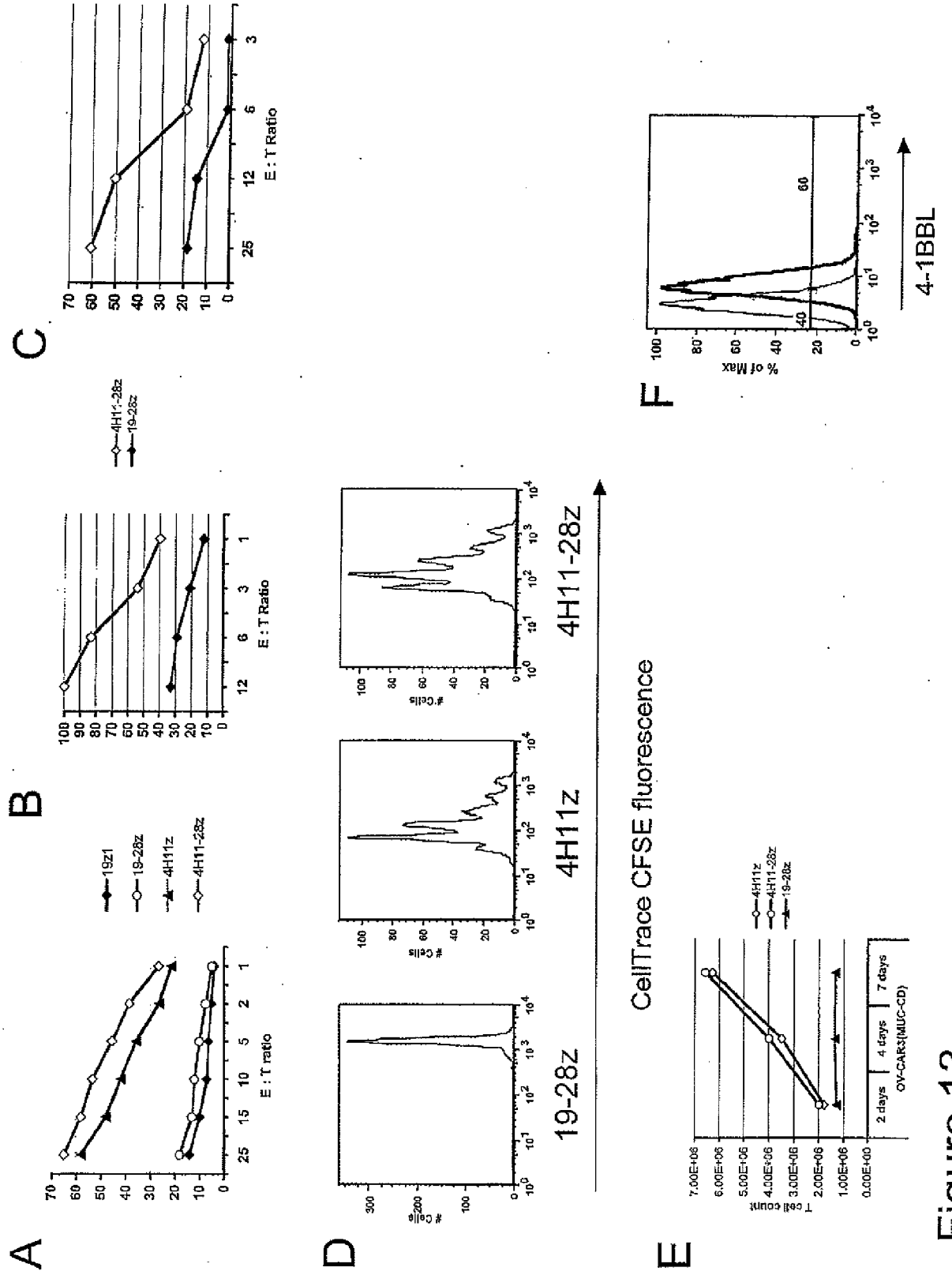
A
B
C
D
E
F
19z1
19-28z
4H11z
4H11-28z
E : T ratio
E : T Ratio
CellTrace CFSE fluorescence
19-28z
4H11z
4H11-28z
cells
T cell count
2 days
4 days
7 days
OV-CAR3(MUC-CD)
% of Max
4-1BBL

Figure 13

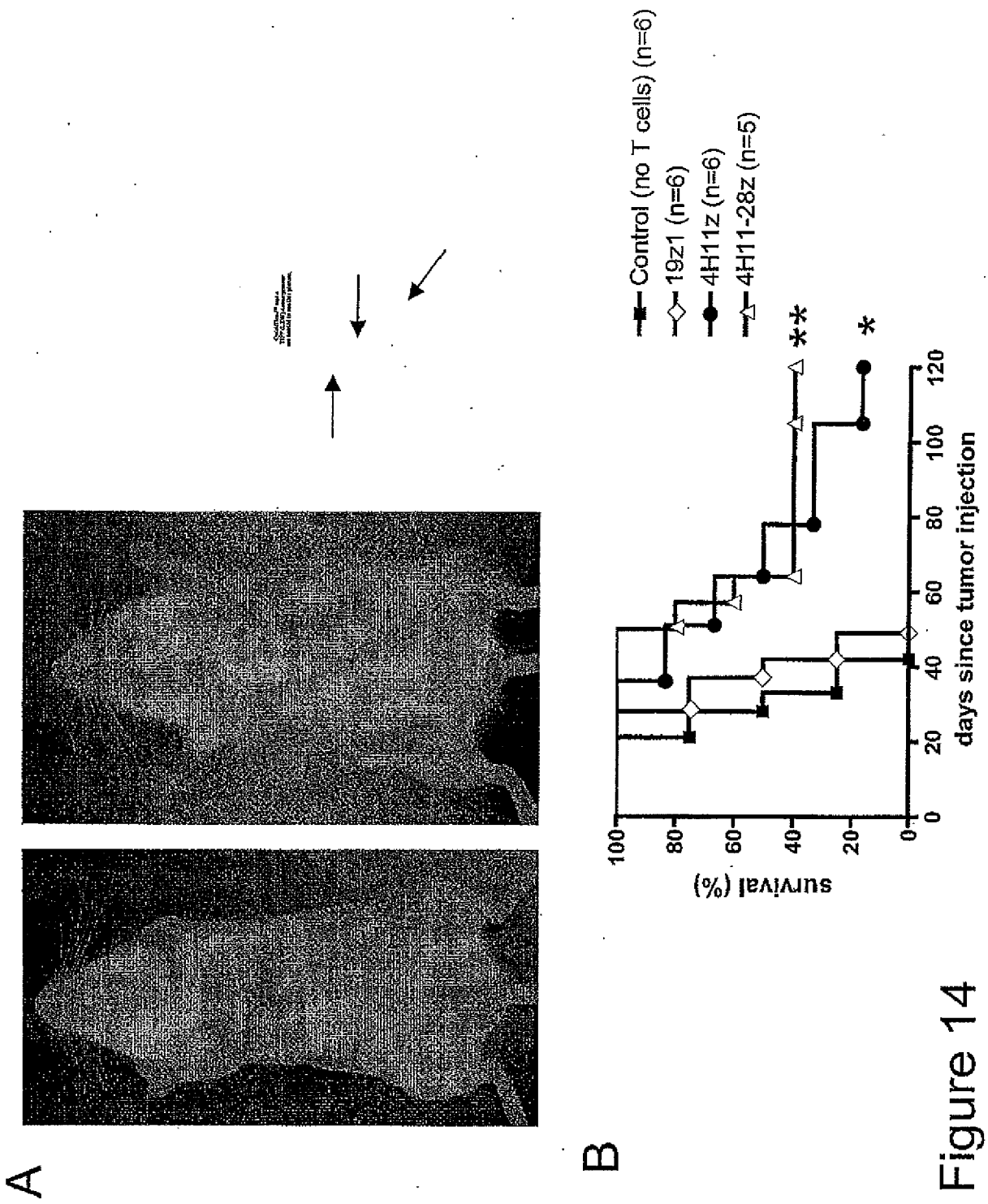
A
B
Control (no T cells) (n=6)
19z1 (n=6)
4H11z (n=6)
4H11-28z (n=5)
**
*
survival (%)
days since tumor injection
0
20
40
60
80
100
120

Figure 14

A

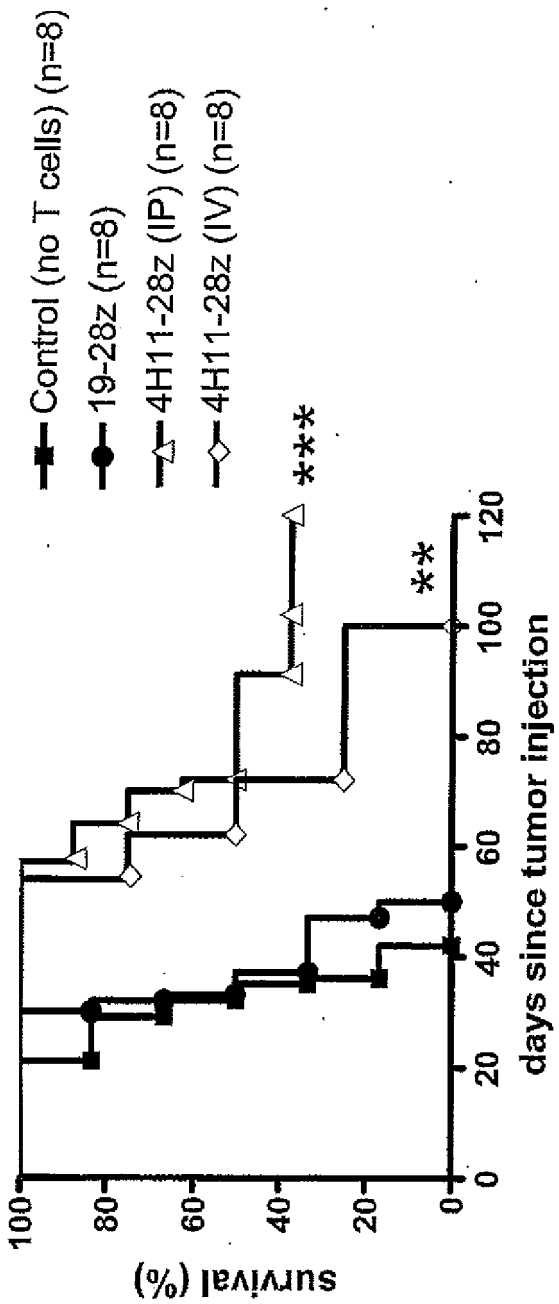

Figure 15 (1 of 3)

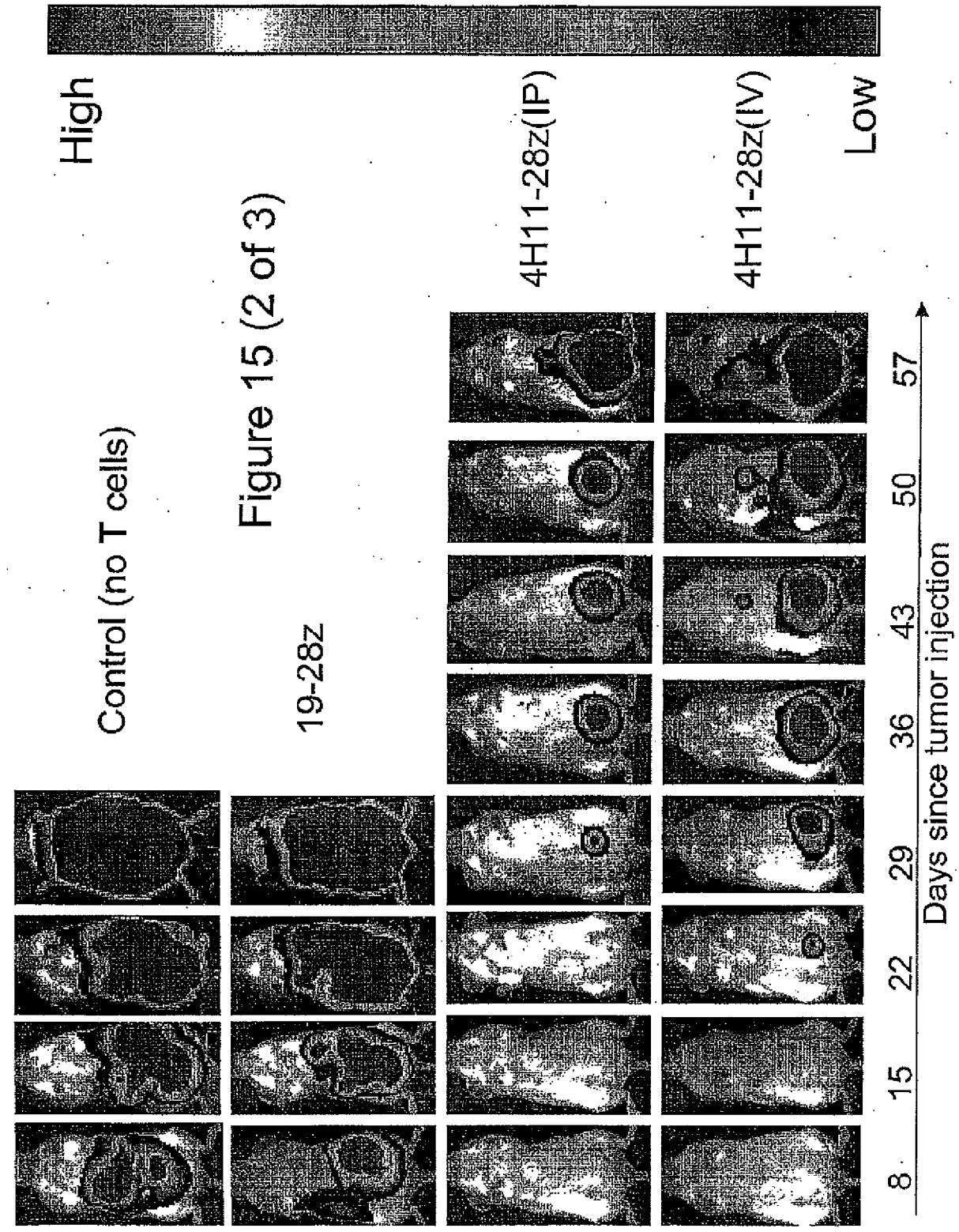

Figure 15 (2 of 3)

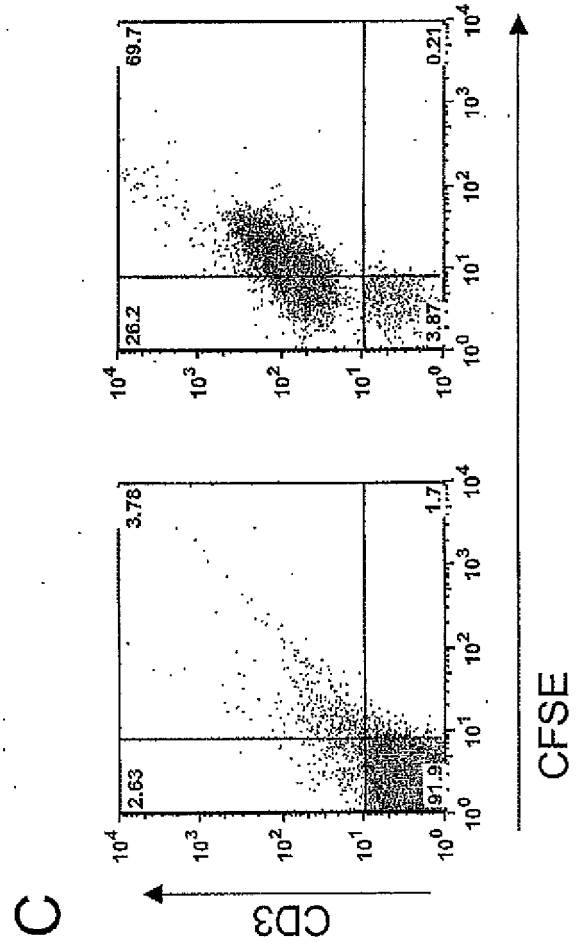

Figure 15 (3 of 3)

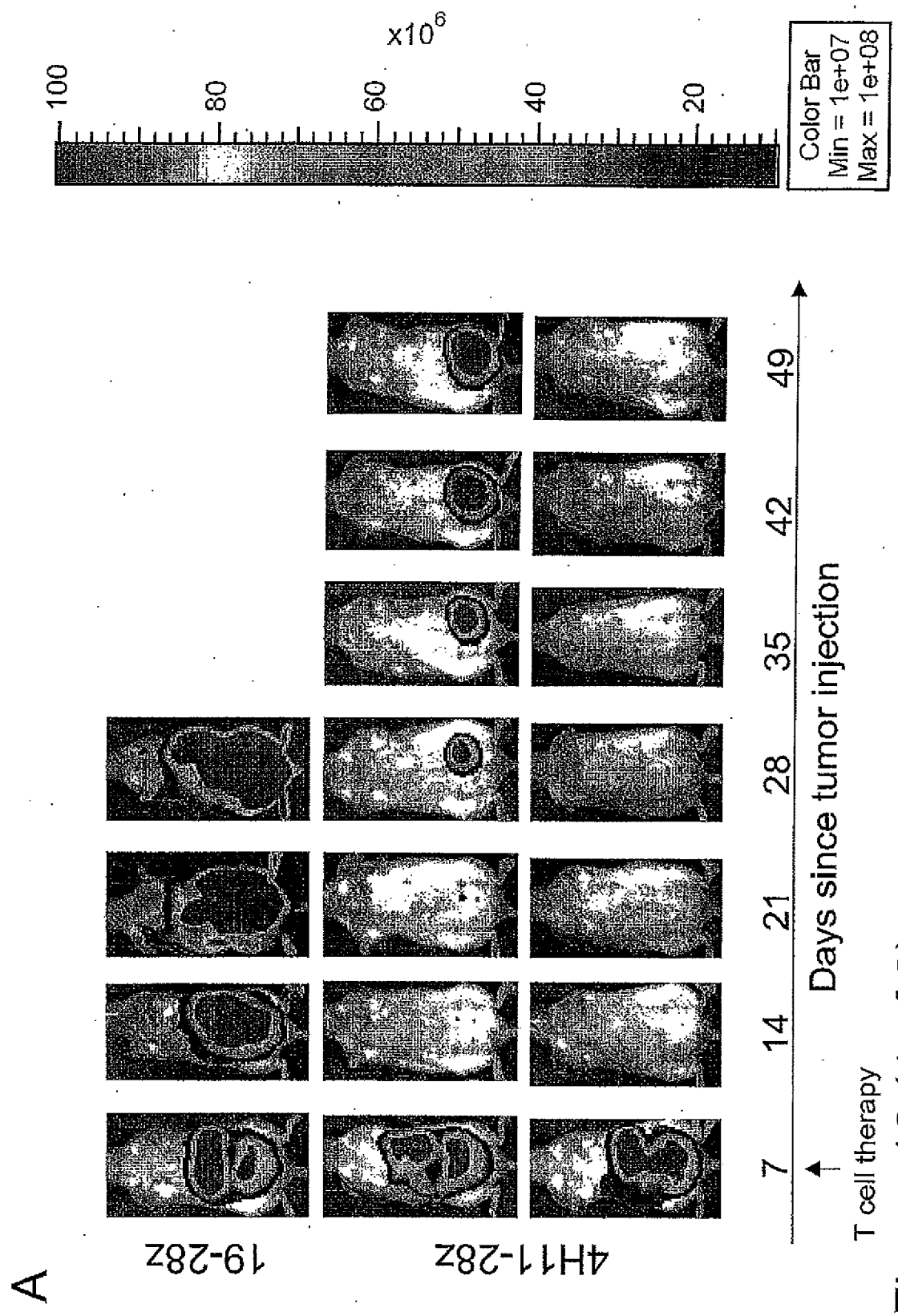

Figure 16 (1 of 2)

B

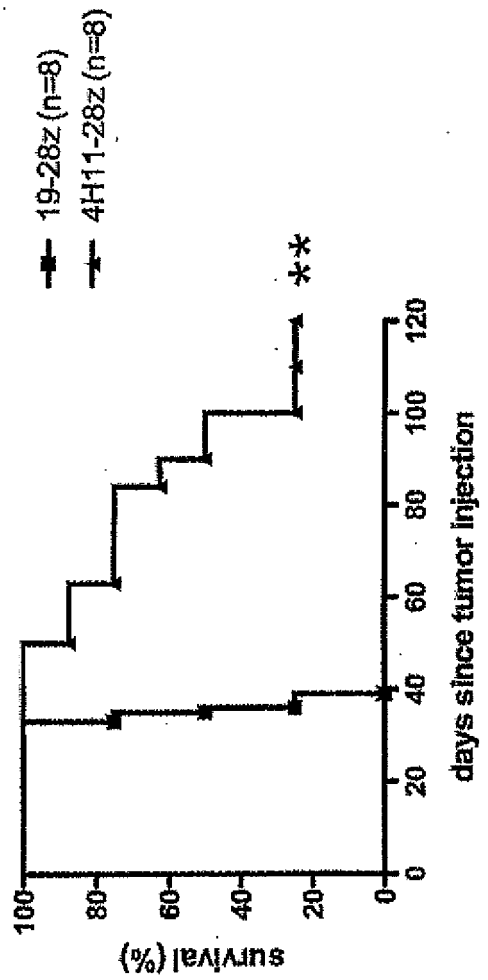

Figure 16 (2 of 2)

CD8 leader sequence
ATGGCTC TCCCAGTGAC TGCCCTACTG CTTCCCCTAG CGCTTCTCCT GCATGCAGAG

CD3 zeta chain intracellular domain
AGAGT GAAGTTCAGC AGGAGCGCAG AGCCCCCCGC GTACCAGCAG GGCCAGAACC AGCTCTATAA
CGAGCTCAAT CTAGGACGAA GAGAGGAGTA CGATGTTTTG GACAAGAGAC GTGGCCGGGA CCCTGAGATG
GGGGGAAAGC CGAGAAGGAA GAACCCTCAG GAAGGCCTGT ACAATGAACT GCAGAAAGAT AAGATGGCGG
AGGCCTACAG TGAGATTGGG ATGAAAGGCG AGCGCCGGAG GGGCAAGGGG CACGATGGCC TTTACCAGGG
TCTCAGTACA GCCACCAAGG ACACCTACGA CGCCCTTCAC ATGCAGGCCC
TGCCCCCTCG

(G4S)3 serine-glycine linker
GGTG GACGTCGATC AGGTGGAGGT GGATCTGGTGGAGGTGGATC T

CD8 transmembrane domain
GCGGCCGCAC CCACCACGAC GCCAGCGCCG CGACCACCAA CCCCGGCGCC CACGATCGCG TCGCAGCCCC
TGTCCCTGCG CCCAGAGGCG TGCCGGCCAG CGGCGGGGGG CGCAGTGCAC ACGAGGGGGC TGGACTTCGC
CTGTGATATCTACATCTGGG CGCCCTTGGC CGGGACTTGT GGGGTCCTTC TCCTGTCACT GGTTATCACC
CTTTACTGCA ACCAC

CD28 transmembrane + intracellular domains (-STOP)
CAA TTGAAGTTAT GTATCCTCCT CCTTACCTAG ACAATGAGAA GAGCAATGGA ACCATTATCC
ATGTGAAAGG GAAACACCTT TGTCCAAGTC CCCTATTTCC CGGACCTTCT AAGCCCTTTT GGGTGCTGGT
GGTGGTTGGT GGAGTCCTGG CTTGCTATAG CTTGCTAGTA ACAGTGGCCT TTATTATTTT CTGGGTGAGG
AGTAAGAGGA GCAGGCTCCT

Figure 17

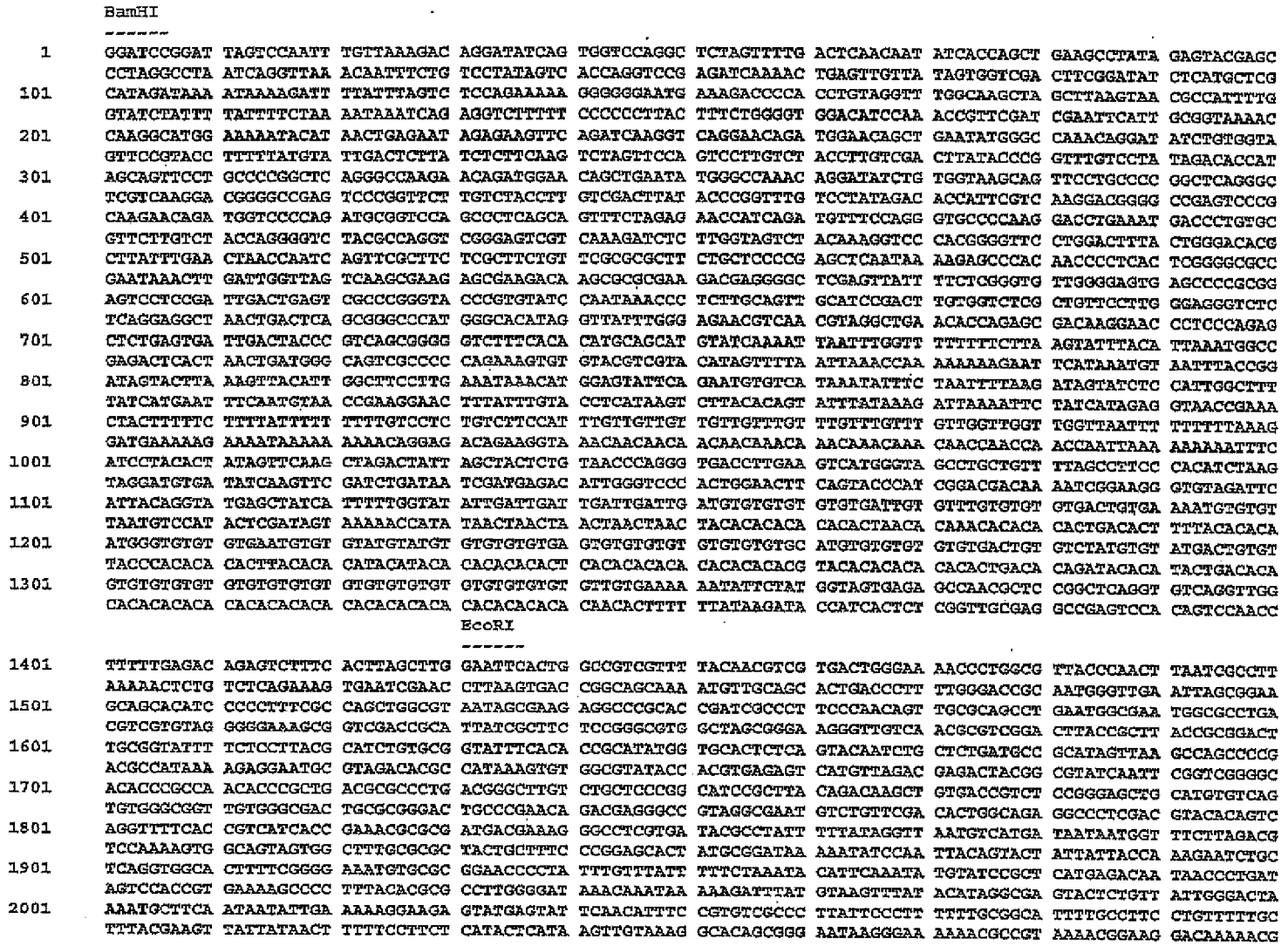

Figure 18 (1 of 5)

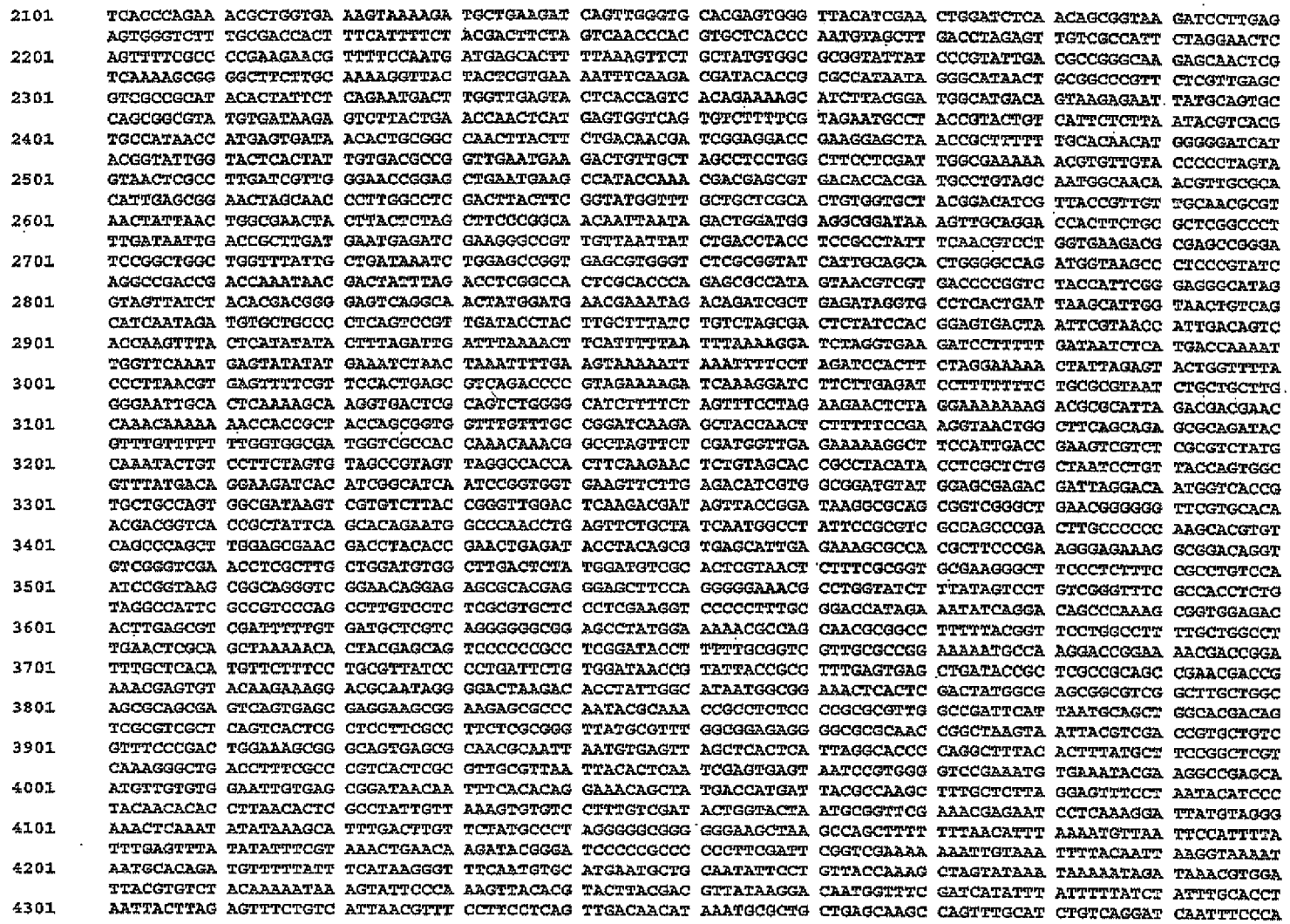

Figure 18 (2 of 5)

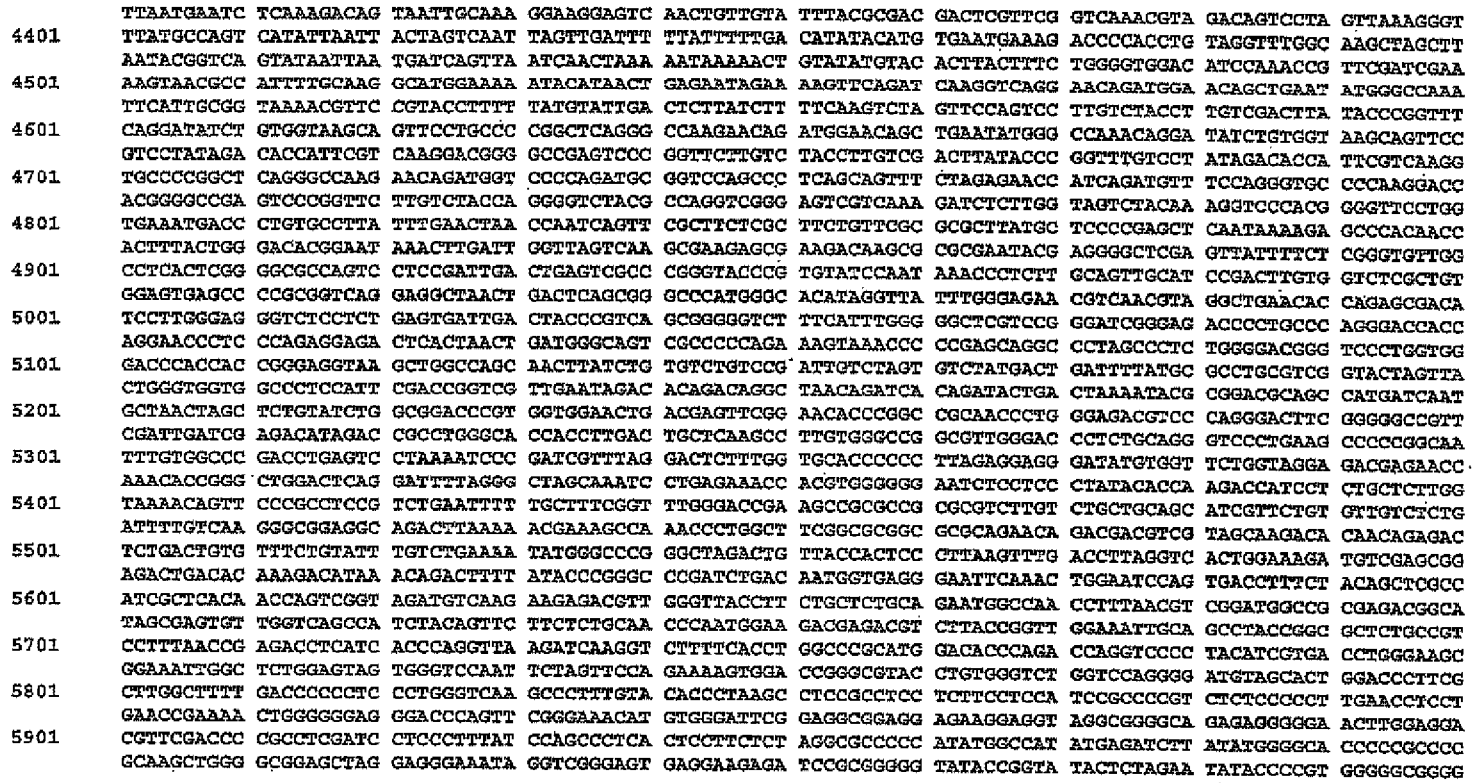

Figure 18 (3 of 5)

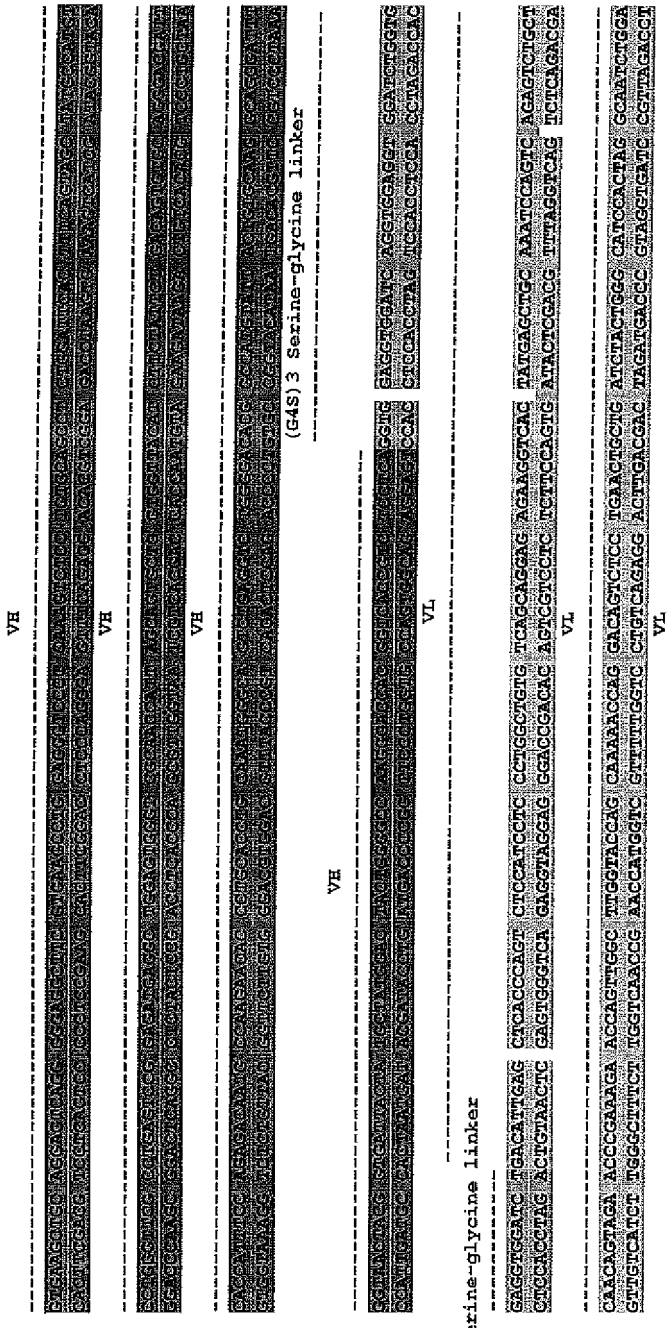

Figure 18 (4 of 5)

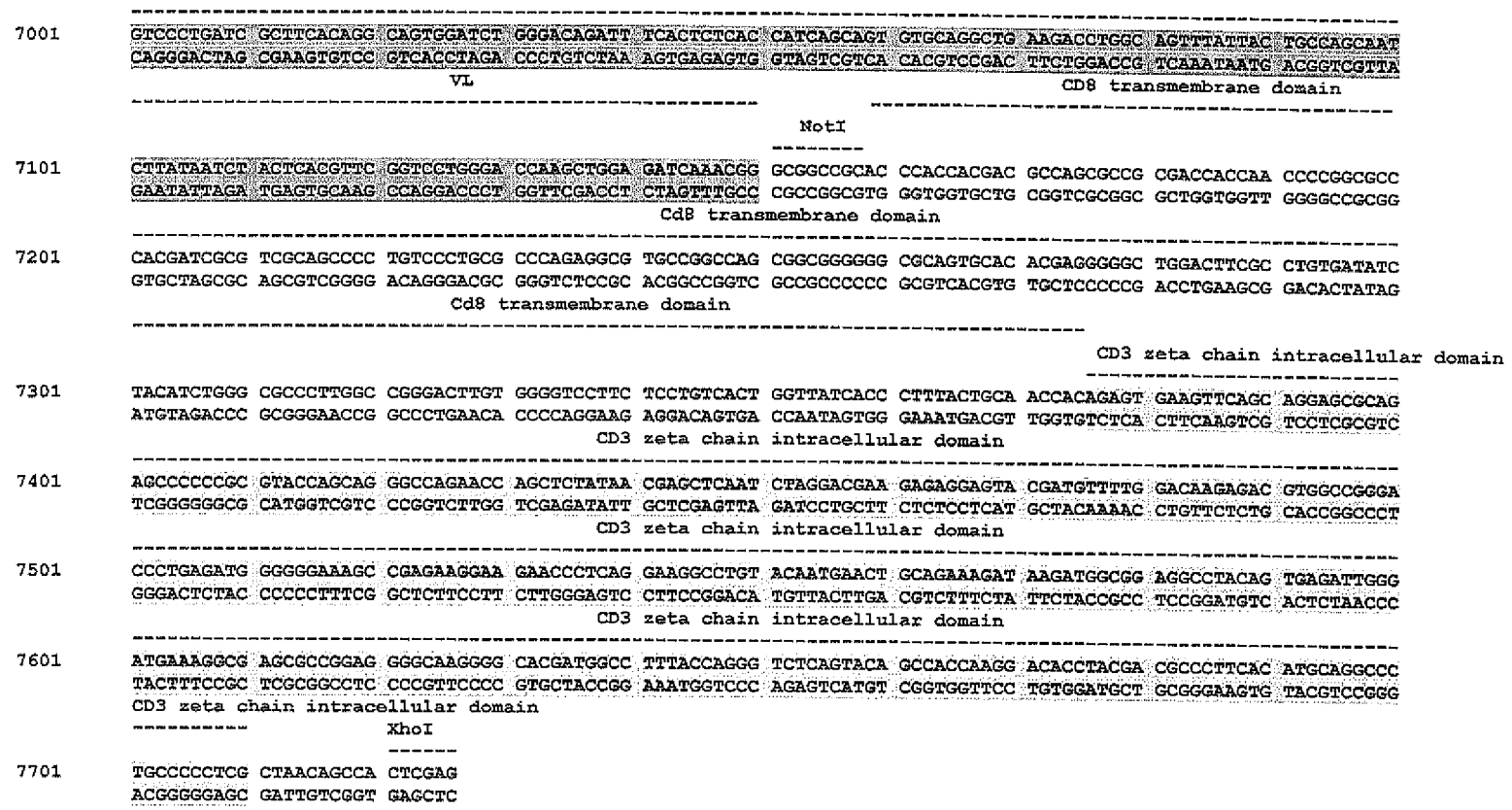

Figure 18 (5 of 5)

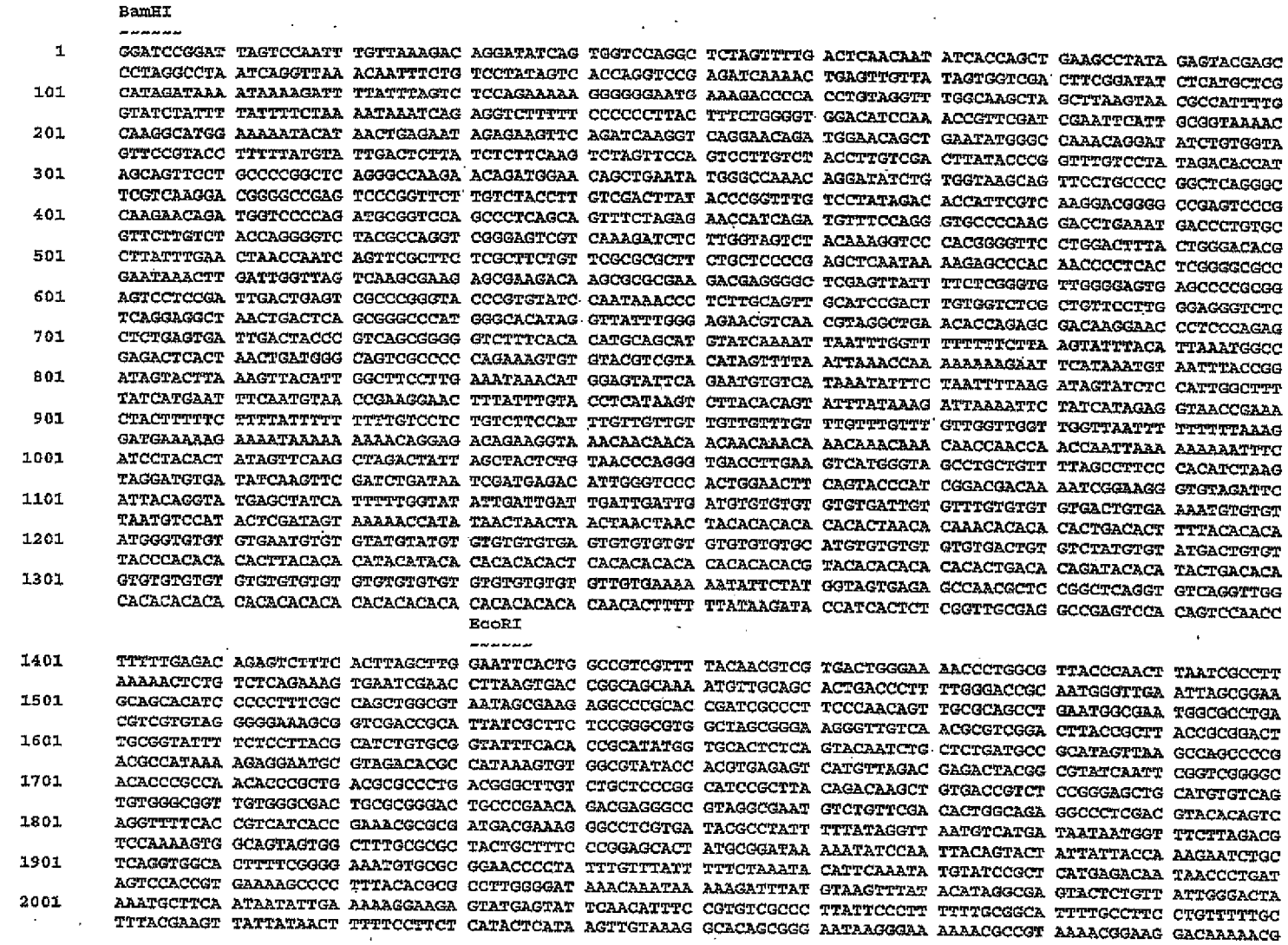

Figure 19 (1 of 6)

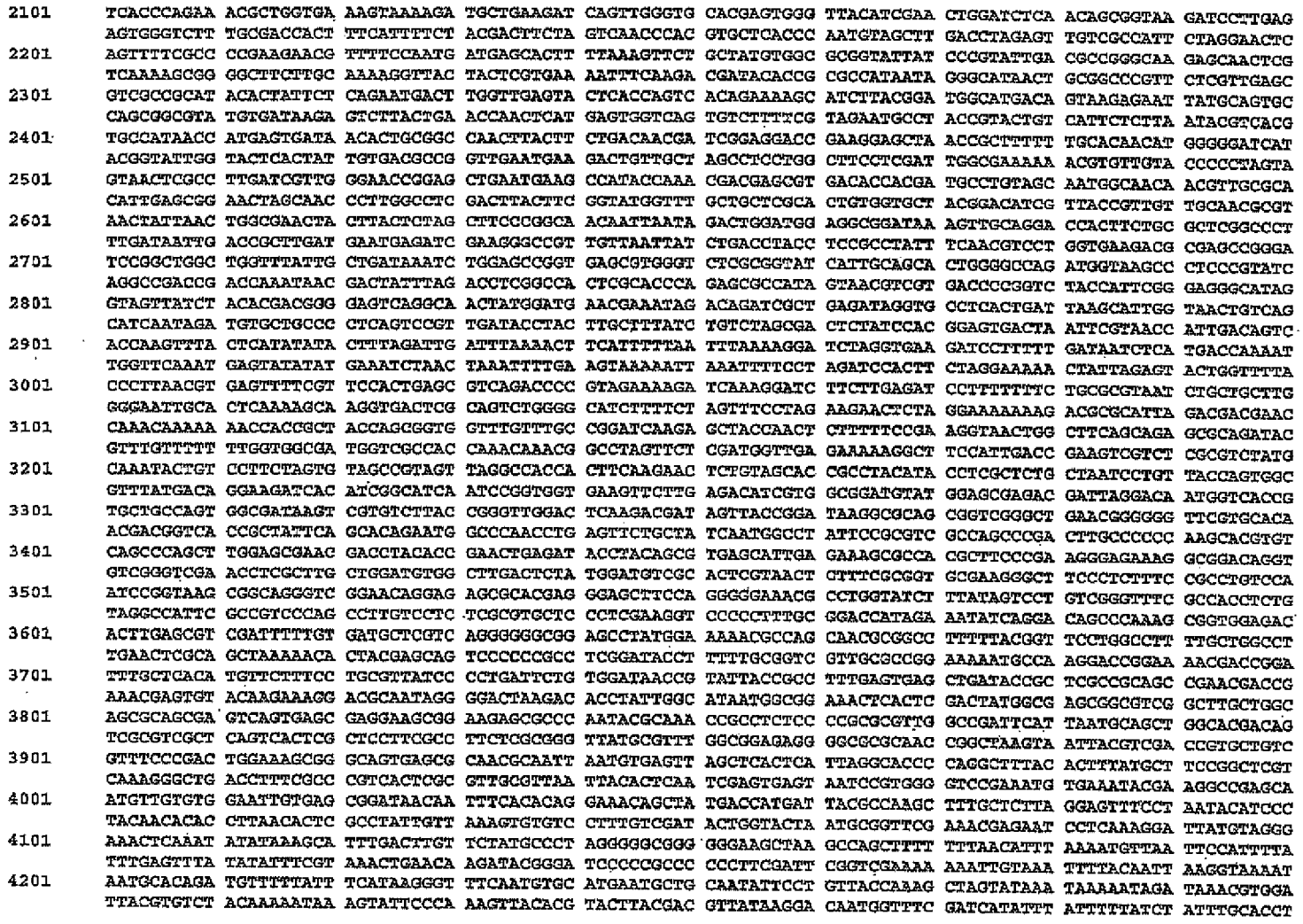

Figure 19 (2 of 6)

Figure 19 (3 of 6)

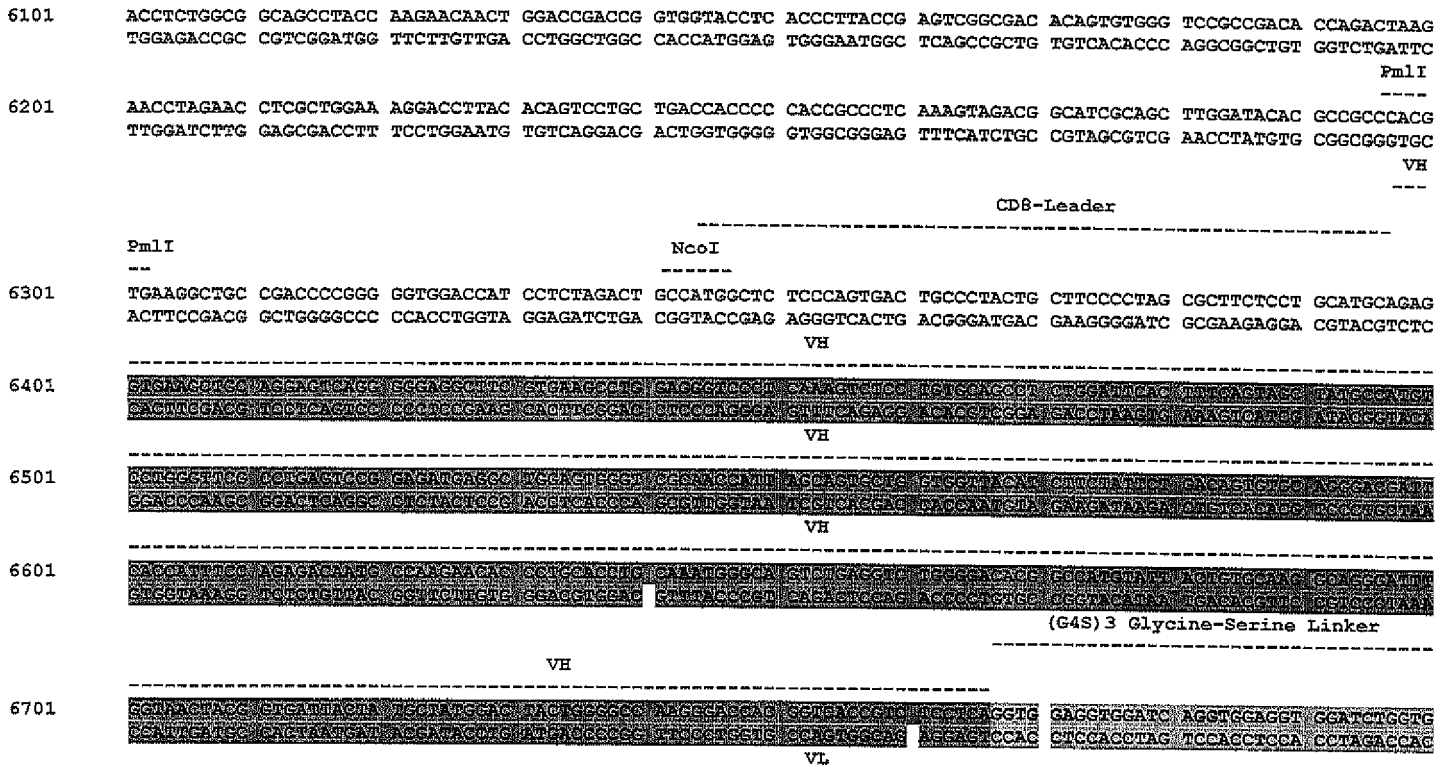

Figure 19 (4 of 6)

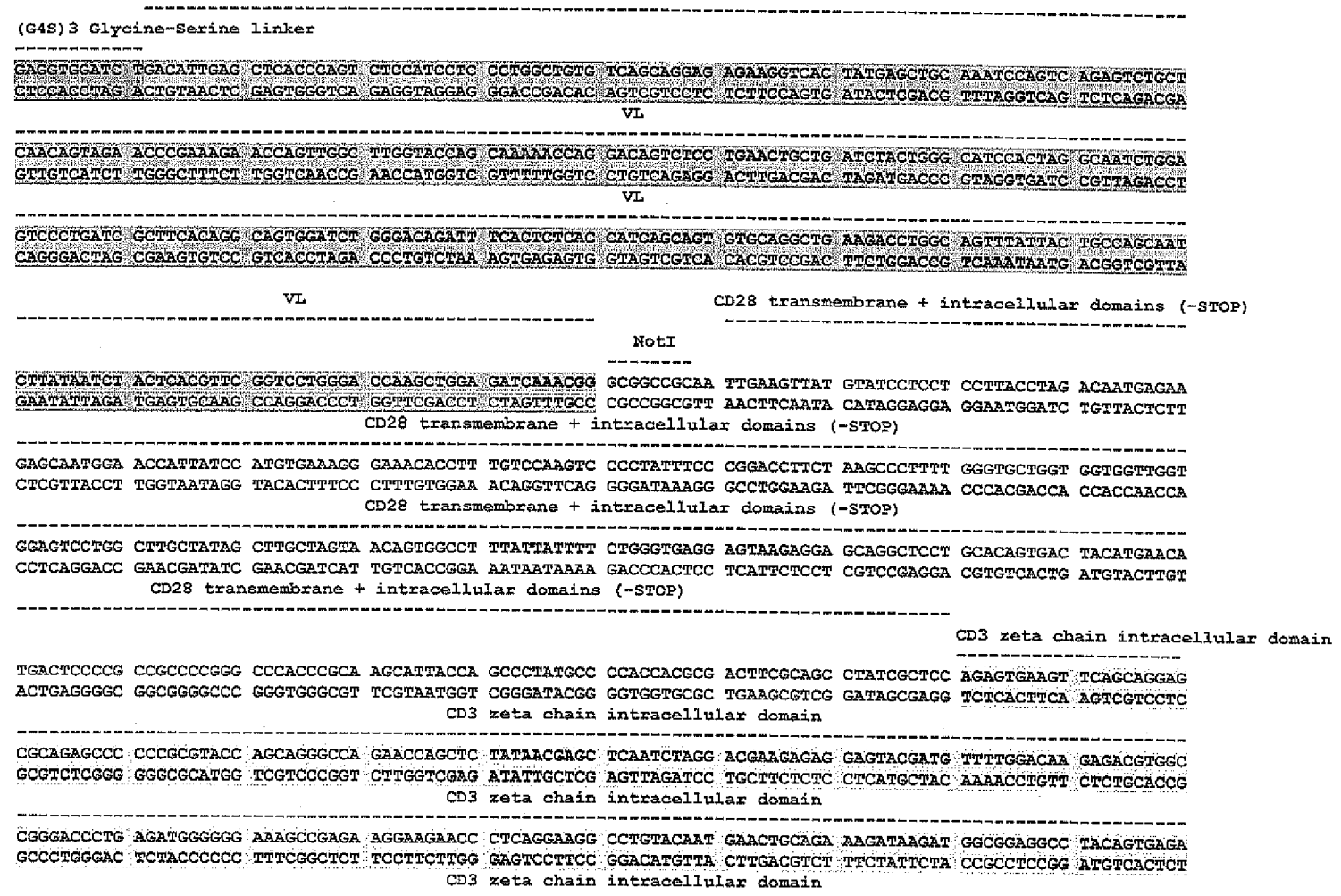

Figure 19 (5 of 6)

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        ----------------------------------------------------------------------------------------------------
7701    TTGGGATGAA AGGCGAGCGC CGGAGGGGCA AGGGGCACGA TGGCCTTTAC CAGGGTCTCA GTACAGCCAC CAAGGACACC TACGACGCCC TTCACATGCA
        AACCCTACTT TCCGCTCGCG GCCTCCCCGT TCCCCGTGCT ACCGGAAATG GTCCCAGAGT CATGTCGGTG GTTCCTGTGG ATGCTGCGGG AAGTGTACGT
        CD3 zeta chain intracellular domain
        ----------------
                                  XhoI
                                 -------
7801    GGCCCTGCCC CCTCGCTAAC AGCCACTCGA G
        CCGGGACGGG GGAGCGATTG TCGGTGAGCT C
```

Figure 19 (6 of 6)

Figure 20A

1. Mouse MUC16-CD Peptide 1 (SEQ ID NO:21):

TLDRKSVFVDGYSQNRDD **19 AA**

2. Mouse 1st Cysteine Loop peptide 2 (SEQ ID NO:22):

KSYFSDCQVLAFRSVSNNNNHTGVDSLCNFSPL **33 AA**

3. Mouse 2nd Cysteine Loop peptide 3 (SEQ ID NO:23):

SLYSNCRLASLRPKKNGTATGVNAICSYHQN **32 AA**

Figure 20B

Alignment of mouse MUC16 (SEQ ID NO:24) and human MUC16 (SEQ ID NO:25) amino acid sequences

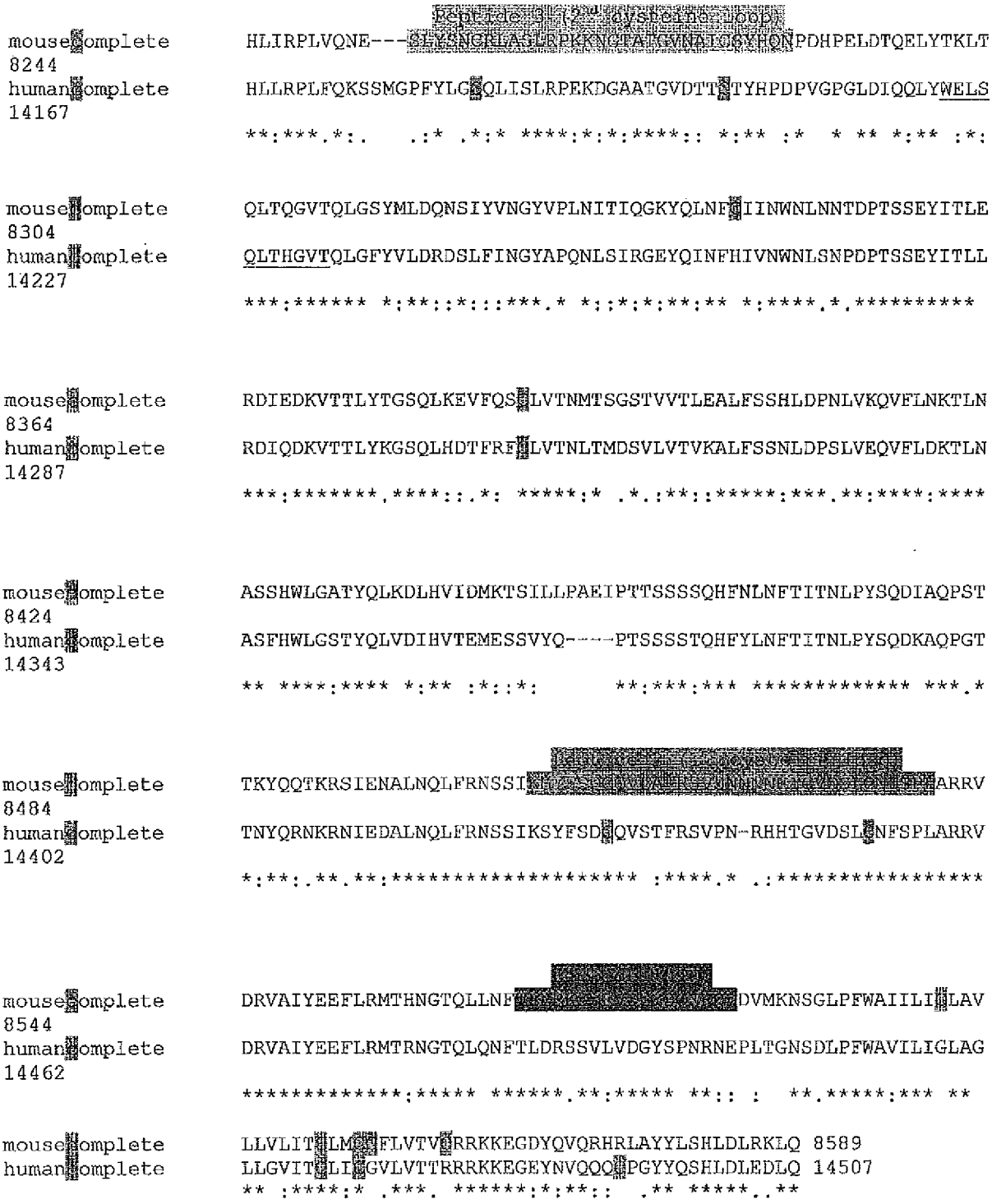


Figure 21

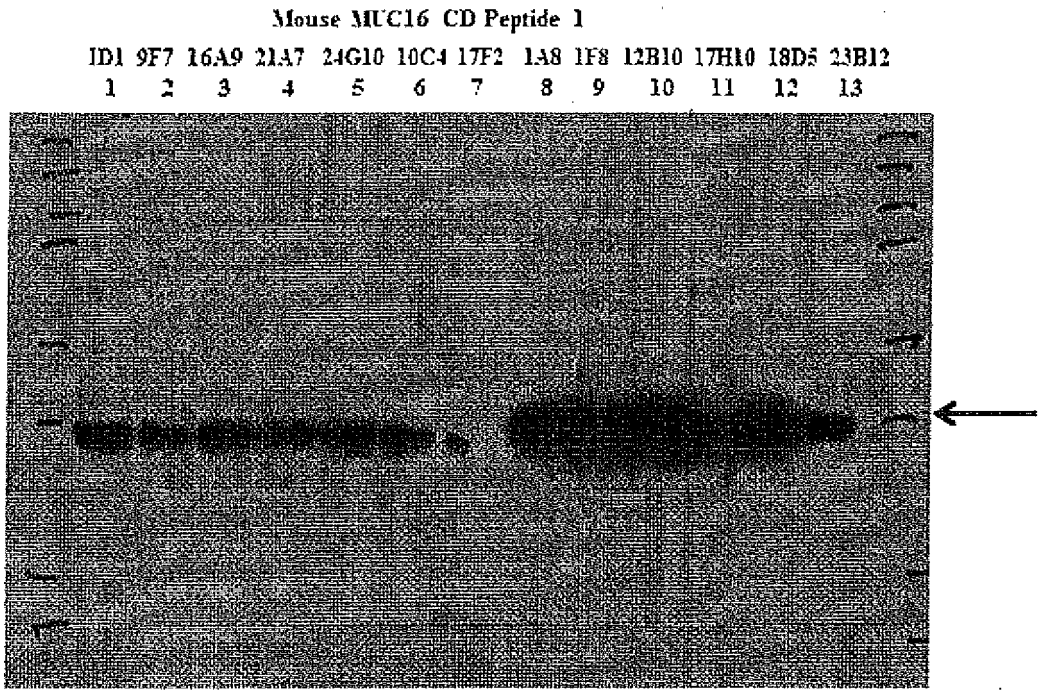

Mouse MUC16 CD Peptide 1
ID1 9F7 16A9 21A7 24G10 10C4 17F2 1A8 1F8 12B10 17H10 18D5 23B12
1 2 3 4 5 6 7 8 9 10 11 12 13
Mouse MUC16 CL Peptide 3
25E9 16F12 4A6 5D1 21B8 21E1 8A2 13E5 23G4 21D3 FB XX 4H11hu
14 15 16 17 18 19 20 21 22 23 24 25 26

Figure 22

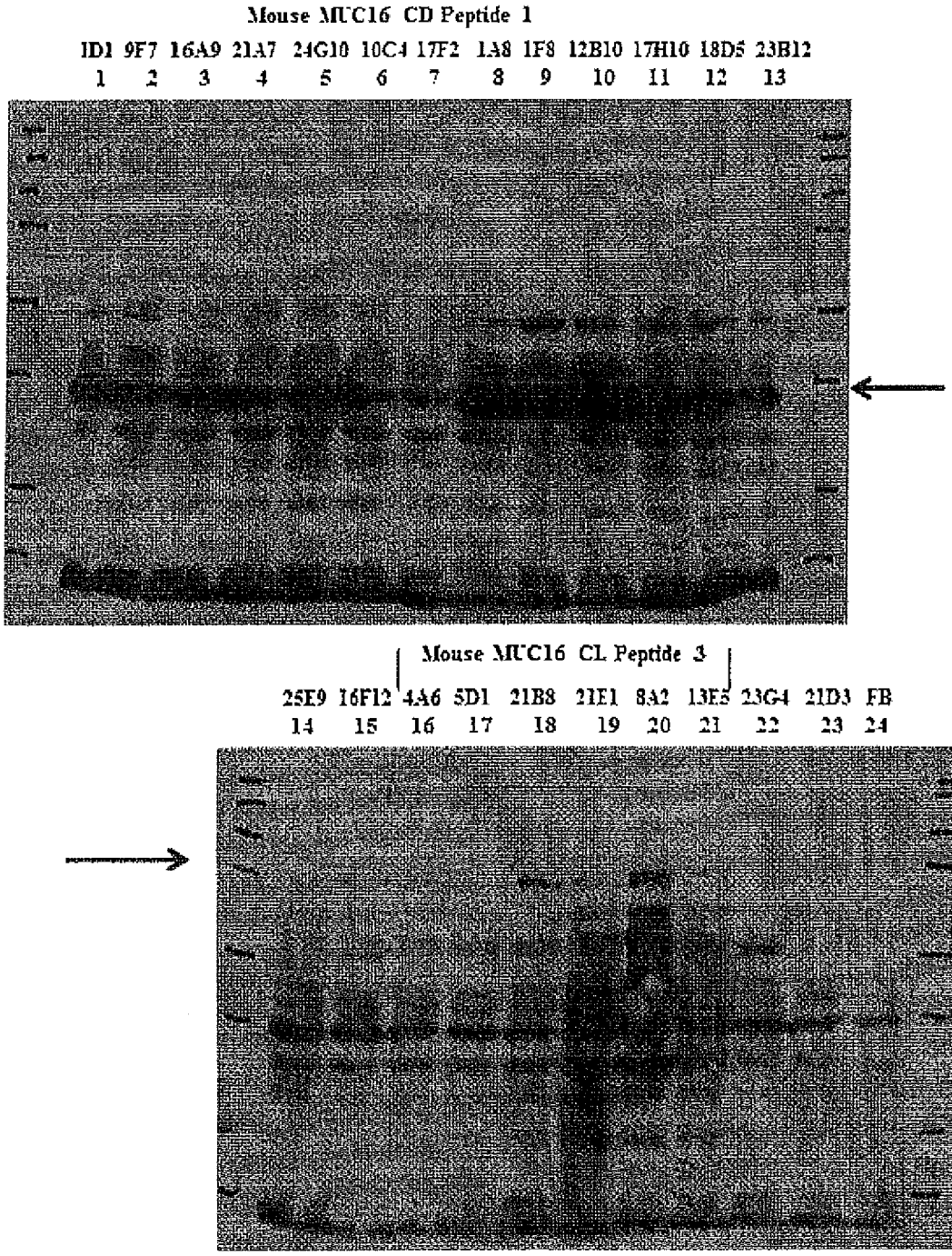
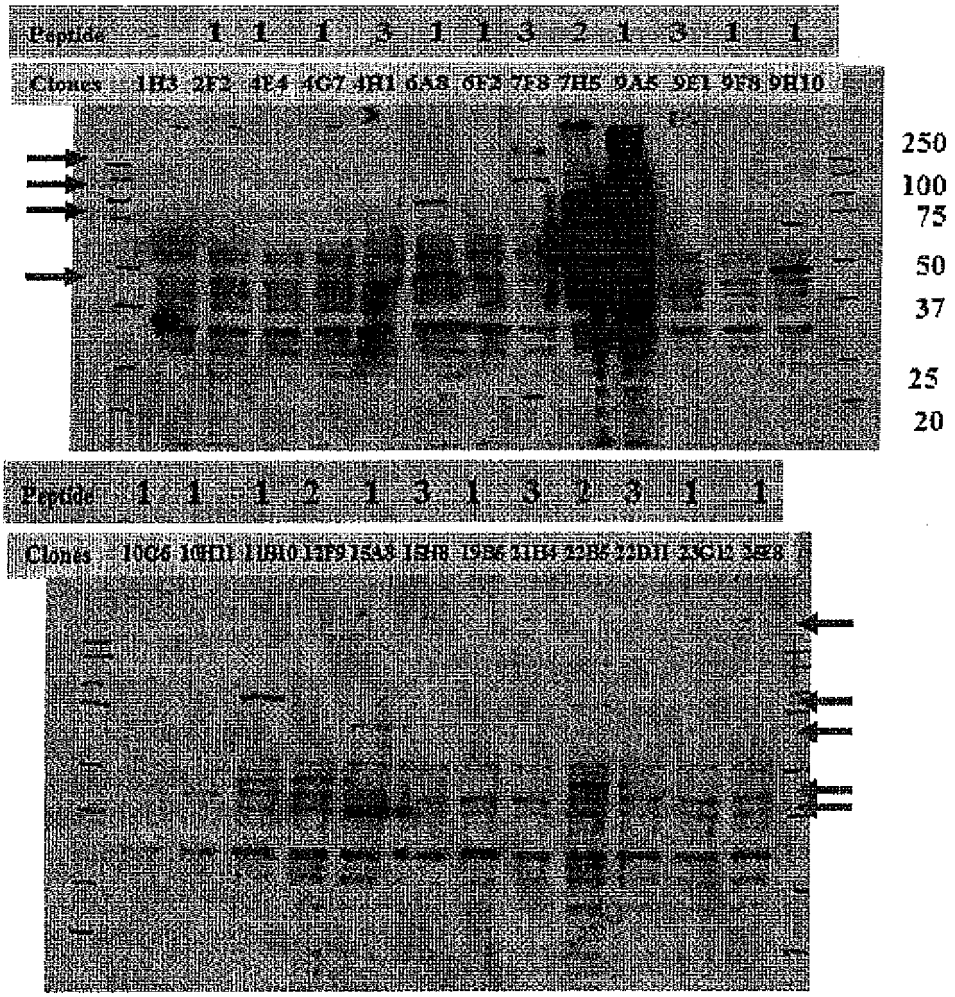
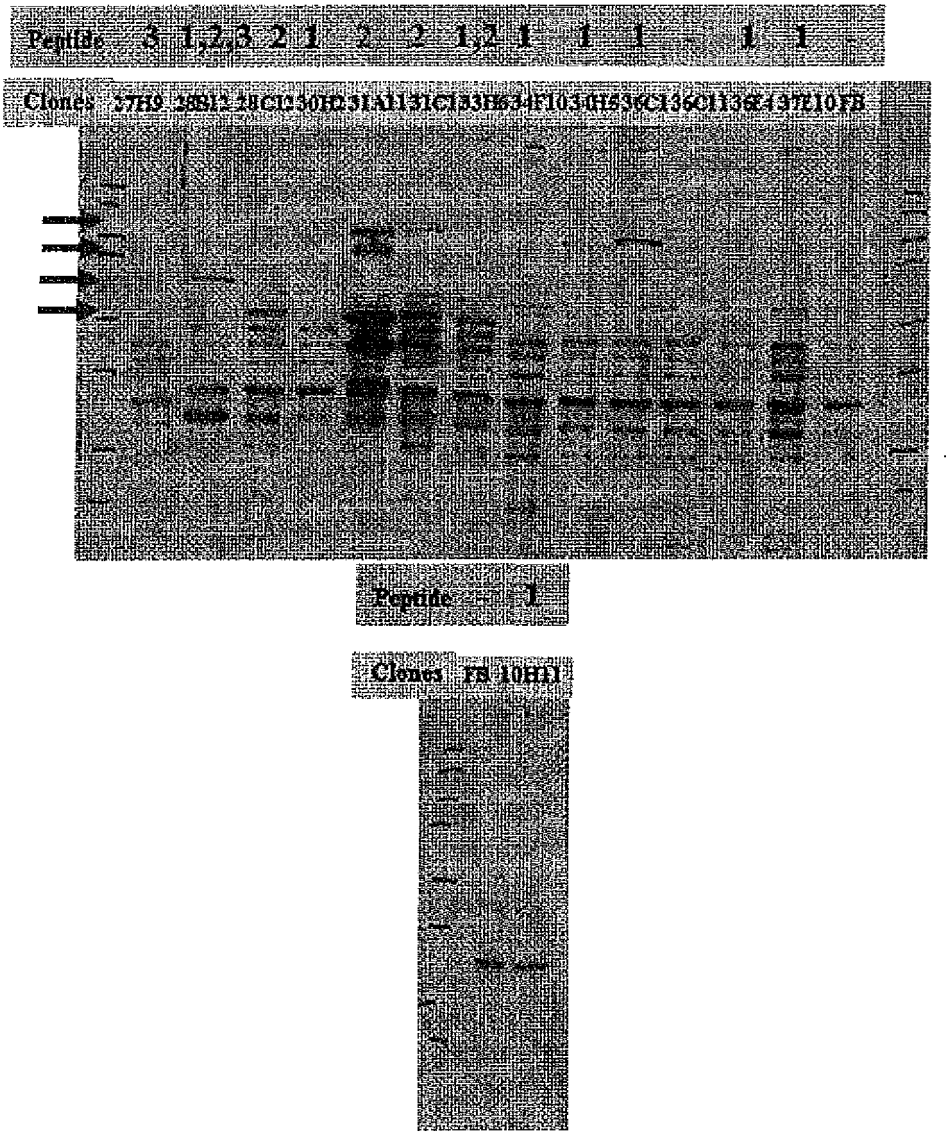

Mouse MUC16 CD Peptide 1
1D1 9F7 16A9 21A7 24G10 10C4 17F2 1A8 1F8 12B10 17H10 18D5 23B12
1 2 3 4 5 6 7 8 9 10 11 12 13
Mouse MUC16 CL Peptide 3
25E9 16F12 4A6 5D1 21B8 21E1 8A2 13E5 23G4 21D3 FB
14 15 16 17 18 19 20 21 22 23 24

Figure 23


Peptide 1 1 1 3 1 1 3 2 1 3 1 1
Clones 1H3 2F2 4F4 4G7 4H1 6A8 6F2 7E8 7H5 9A5 9E1 9F8 9H10
250
100
75
50
37
25
20
Peptide 1 1 1 2 1 3 1 3 2 3 1 1
Clones 10C6 10H1 11B10 13F9 15A3 15H8 19B6 21H4 22B6 22D11 23C12 2


Peptide 3 1,2,3 2 1 2 2 1,2 1 1 1 1 1
Clones 27H9 28B12 28C12 30H2 31A11 31C1 33H6 34F10 35H5 35C1 36C11 36E4 37E10 FB
Peptide 1
Clones FB-10H11

A. Nucleotide sequence encoding 12B10.3G10-V_H (SEQ ID NO:26)

GAGGTGAAGCTGGAGGAGTCAGGTGGAGGATTGGTGCAGCCTAAAGGATCATTGAAACTCTCATGTGCCGCCTCT
GGTTTCACCTTCAATACCTATGCCGTGCACTGGGTCCGCCAGGCTCCAGGAAAGGGTATGGAATGGGTTGCTCGC
ATAAGAAGTAAAAGTGGAAATTATGCAACATATTATGCCGATTCAGTGAAAGACAGATTCACCATCTCCAGAAAT
GATTCACAGAGCATGCTCTATCTGCAAATGAACAACCTGAAAACTGAGGACACAGCCATATATTACTGTGTGAGA
GCGGGTAACAACGGGGCCTTTCCTTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA

B. 12B10.3G10-V_H Amino Acid sequence (SEQ ID NO:27)

EVKLEESGGGLVQPKGSLKLSCAASGFTFNTYAVHWVRQAPGKGMEWVARIRSKSGNYAT
YYADSVKDRFTISRNDSQSMLYLQMNNLKTEDTAIYYCVRAGNNGAFPYWGQGTTVTVSS

C. Nucleotide sequence encoding 12B10.3G10-V_L (SEQ ID NO:28)

Note the VL has an optional *NotI* site added by the primer for cloning.

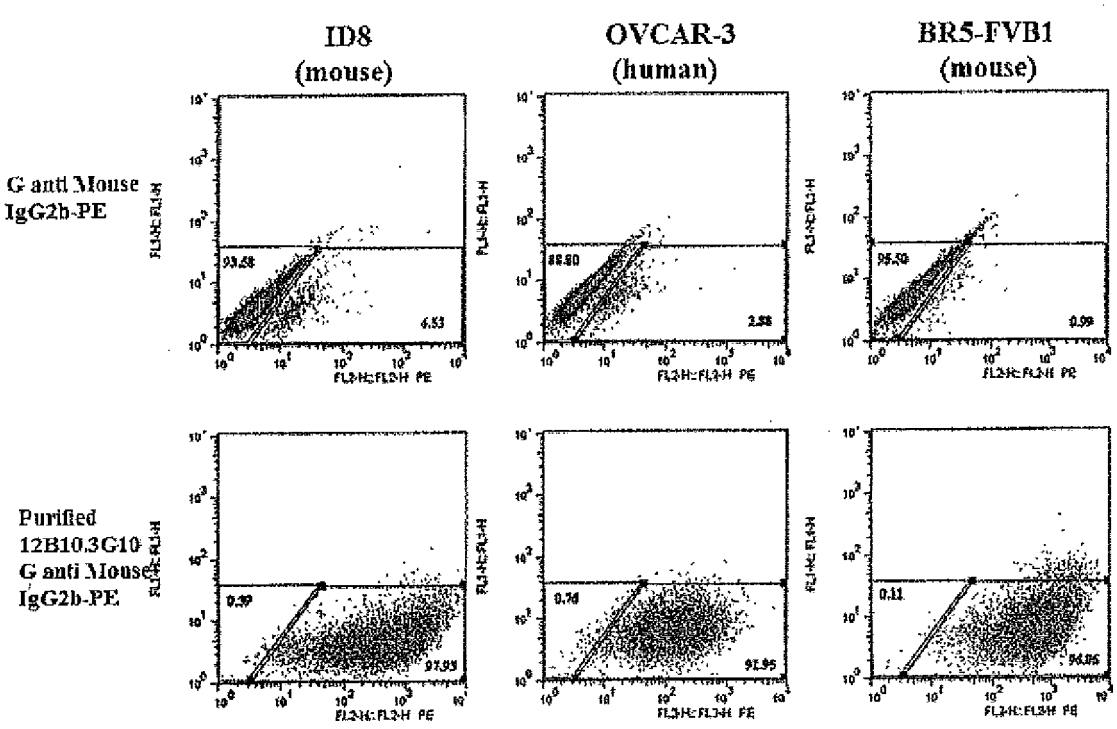
GACATTGAGCTCACCCAGTCTCCATCCTCACTGTCTGCATCTCTGGGAGGCAGAGTCACCATCACTTGCAAGGCT
AGCCAAGATATTAAGAAGTATATAGCTTGGTACCAACACAAGCCTGGAAAAACTCCTCGACTACTCATACATTTC
ACATCTACATTACAGACAGGCATCCCATCAAGGTTCAGTGGACGTGGGTCTGGGAGAGACTATTCCTTCAGCATC
AGCAACCTGGAGTCTGAAGATATTGCAACTTATTATTGTCTACAGTATGATAGTCTGTACACGTTCGGAGGGGGG
ACCAAGCTGGAGATCAAACGGGCGGCCGCA

D. 12B10.3G10-V_L Amino Acid sequence (SEQ ID NO:29)

DIELTQSPSSLSASLGGRVTITCKASQDIKKYIAWYQHKPGKTPRLLIHFTSTLQTGIPS
RFSGRGSGRDYSFSISNLESEDIATYYCLQYDSLYTFGGGTKLEIKRAAA

Figure 24

Figure 25


ID8
(mouse)
OVCAR-3
(human)
BR5-FVB1
(mouse)
G anti Mouse
IgG2b-PE
Purified
12B10.3G10
G anti Mouse
IgG2b-PE

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EUROPEAN SEARCH REPORT

Application Number
EP 17 15 0631

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X A	US 2004/057952 A1 (PAYNE GILLIAN [US] ET AL) 25 March 2004 (2004-03-25) * whole document, especially paragraphs [0011, 0014-0015, 0067, 0076, 0091-0095]; Examples 1, 8, 10, 13-14; Figure 2B; SEQ ID NO: 14 * -----	2,8-22 1,3-7	INV. C07K16/18 C12N15/13 C12N5/18 A61K39/395 A61P35/00 C07K16/30
X,D,P	RAO THAPI DHARMA ET AL: "Novel Monoclonal Antibodies Against the Proximal (Carboxy-Terminal) Portions of MUC16", APPLIED IMMUNOHISTOCHEMI, LIPPINCOTT WILLIAMS AND WILKINS, PHILADELPHIA, PA, US, vol. 18, no. 5, October 2010 (2010-10), pages 462-472, XP008163430, ISSN: 1062-3345, DOI: 10.1097/PAI.0B013E3181DBFCD2 * the whole document * -----	1-22	

TECHNICAL FIELDS SEARCHED (IPC)
C07K
A61K
A61P

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	11 July 2017	Luyten, Kattie

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EPO FORM 1503 03.82 (P04C01)

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摘要

本公開提供了特異性結合多肽或其抗原性部分的抗體及其抗原結合片段，其中所述多肽選自：a)MUC16 胞外域多肽，b)MUC16 細胞質結構域多肽，及 c)含有半胱氨酸環多肽的 MUC16 細胞外結構域多肽。本公開的抗體及含有它們的組合物可用於 MUC16 過表達的疾病例如癌症的診斷及治療應用。