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(71) Applicant (for all designated States except US): **BIOS-
TRATUM, INC.** [US/US]; 4620 Creekstone Drive, Suite
#200, Durham, NC 27703 (US).

(71) Applicant and

(72) Inventor: **SALO, Sirpa** [FI/FI]; University of Oulu, Box
3000, FIN-90014 Oulu (FI).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **TRYGGVASON,**
Karl [IS/SE]; Division of matrix Biology, Department

of Medical Biochemistry and Biophysics, Karolinska
Institutet, S-171 77 Stockholm (SE).

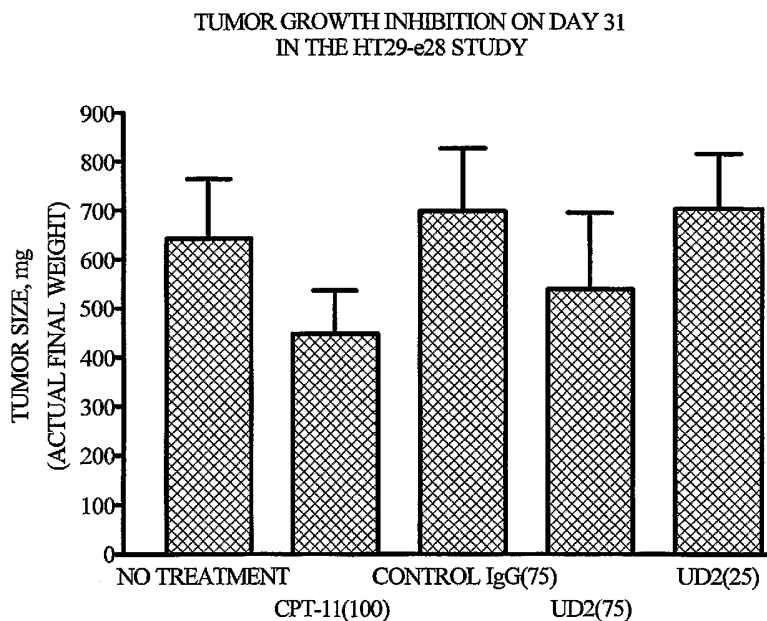
(74) Agent: **HARPER, David, S.**; McDonnell Boehnen
Hulbert & Berghoff LLP, 300 South Wacker Drive, Suite
#3100, Chicago, IL 60606 (US).

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(54) Title: USE OF ANTIBODIES TO THE GAMMA 2 CHAIN OF LAMININ 5 TO INHIBIT TUMOR GROWTH AND METAS-
TASIS



(57) Abstract: The present invention provides antibodies against the laminin 5 γ 2 chain, compositions thereof and methods for inhibiting tumor growth and/or metastasis using such antibodies and compositions.

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Use of antibodies to the gamma 2 chain of laminin 5 to inhibit tumor growth and metastasis

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CROSS REFERENCE

This application claims priority to U.S. Provisional Patent Application 60/523,895 filed November 20, 2003, which is incorporated herein by reference in its entirety.

10

BACKGROUND OF THE INVENTION

Laminins are basement membrane glycoproteins with diverse biological functions including cell adhesion, proliferation, migration and differentiation. Thus far, 11
15 genetically distinct chains forming at least 12 laminin isoforms have been characterized. Every member of this growing protein family has a heterotrimeric chain composition of α , β , and γ chains that are formed through an intracellular self-assembly mechanism.

Laminin-5 is a specific component of epithelial basement membranes with the chain composition $\alpha 3\beta 3\gamma 2$ (Kallunki, et al., J. Cell Biol. 119: 679-93, 1992). The $\gamma 2$
20 chain has a mass of ≈ 130 kD and is thus smaller than the "classical" ≈ 200 kD $\beta 1$ and $\gamma 1$ light chains of laminin 1. Expression of laminin 5 chains is often up-regulated in epithelial cancers, such as squamous cell carcinomas and gastric carcinomas, but not in mesenchymally derived cancers (Larjava, et al., J. Clin. Invest. 92: 1425-35, 1993) (Pyke, et al., Am. J. Pathol. 145: 782-91, 1994) (Pyke, et al., Cancer Res. 55: 4132-9,
25 1995) (Tani, et al., Am. J. Pathol. 149: 781-93, 1996) (Orian-Rousseau, et al., J. Cell. Sci. 111: 19932004, 1998) (Sordat, et al., J. Pathol. 185: 44-52, 1998). However, down-regulation has been reported in epithelial prostate and breast carcinomas (Hao, J., Yang, Am. J. Pathol. 149: 1341-9, 1996) (Martin, et al., Mol. Med. 4: 602-613, 1998). In colon adenocarcinomas, both gene and protein expression of the $\gamma 2$ chain seem to be
30 a characteristic of cancer cells with a budding phenotype (Larjava, et al., J. Clin. Invest. 92: 1425-35, 1993) (Pyke, et al., Am. J. Pathol. 145: 782-91, 1994) (Pyke, et al., Cancer Res. 55: 4132-9, 1995). Tumor cell budding in colorectal carcinoma has also been associated with the presence of intracellular laminin-5 (Sordat, et al., J. Pathol. 185: 44-52, 1998).

The $\gamma 2$ chain of laminin-5 has also been shown to be strongly expressed in malignant cells located at the invasion front of several human carcinomas, as determined by *in situ* hybridization and immunohistochemical staining (Pyke, C., Romer, J., Kallunki, P., Lund, L.R., Ralfkiaer, E., Dano, K. & Tryggvason, K. (1994) Am. J. Pathol. 145: 782-791; Pyke, C., Salo, S., Ralfkiaer, E., Romer, J., Dano, K. & Tryggvason, K. (1995) Cancer Res. 55: 4132-4139). However, no studies have shown that antibodies to the $\gamma 2$ chain of laminin 5 can be used to inhibit tumor cell growth.

SUMMARY OF THE INVENTION

10 The present invention provides antibodies, compositions and methods for inhibiting tumor growth and/or metastasis. In one aspect, the present invention provides antibodies that bind to one or more epitopes of the laminin 5 $\gamma 2$ chain contained within **SEQ ID NO: 6**.

15 In another aspect, the present invention provides a method for inhibiting tumor growth and/or metastasis comprising administering to a subject with a laminin 5-secreting tumor an amount effective to inhibit tumor growth and/or metastasis of an antibody that binds to one or more epitopes of the laminin 5 $\gamma 2$ chain contained within **SEQ ID NO: 6**.

20 In a further aspect, the present invention provides a pharmaceutical composition comprising an antibody that binds to more epitopes of the laminin 5 $\gamma 2$ chain contained within **SEQ ID NO: 6** and a pharmaceutically acceptable carrier. In a further embodiment, the pharmaceutical composition further comprises one or more other anti-tumor agents.

25

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the efficiency of human laminin-5 and recombinant human laminin $\gamma 2$ chain for attachment of HaCat keratinocytes and KLN205 squamous carcinoma cells *in vitro*. Attachment efficiency was compared to the efficiency with which the cells bound to laminin-1. Substrate concentrations (10 $\mu\text{g/ml}$) providing maximum attachment to laminin-1 and laminin-5 were used. The results are presented as means $\pm$ SD calculated from at least four duplicate series; the values for laminin-1 were given the arbitrary value of 100%.

Figures 2A-B show the effects of polyclonal $\gamma 2$ chain antibodies on the migration of KLN205 squamous carcinoma cells in Boyden and Transwell chamber assays of migration.

Figure 3 shows tumor growth inhibition using mAb 5D5 and CPT-11 on day 31 in the HT29-e28 cell line.

Figures 4A-E show tumor growth curves for individual mice in the HT29-e28 study.

Figures 5-6 are graphs showing the inhibition of HSC-3 cell migration by various monoclonal antibodies employed at either 25 ug/ml or 100 ug/ml.

DETAILED DESCRIPTION OF THE INVENTION

In one aspect, the present invention provides isolated antibodies that specifically bind to one or more epitopes of the laminin 5 $\gamma 2$ chain contained within **SEQ ID NO: 5** (gamma 2 amino acids 382-608; numbering based on amino acid sequence of the full gamma 2 chain shown in **SEQ ID NO:4**):

CICPVG YKGQFCQDCASGYKRDSARLGPF GTCIPCNCQGGGACDPDTGDCYS
GDENPDIECADCPIGFYNDPHDPRSCKPCPC HNGFSCSVIPETEEVVCNNCP PG
VTGARCELCADGYFGDPFGEHGPVRPCQPCQ CNSNVDPSASGNCDRLTGRCL
KCIHNTAGIYCDQCKAGYFGDPLAPNPADKCRACNCNPMGSEPVGCRSDGTC
VCKPGFGGPNCEHGAFS (**SEQ ID NO: 5**)

It is well established in the literature that the alpha 3 and gamma 2 chains of laminin 5 undergo extracellular proteolysis. The processing of the gamma 2 chain converts it from a 155-kDa form to a 105-kDa form (Marinkovich, et al, JBC 267, 17900-17906). Processing occurs within domain III at amino acid 434 and removes domains IV and V (Amano et al, JBC 275, 22728-22735).

Gianelli, et al (Science 277, 225-228) and Koshikawa, et al (J. Cell Bio 148, 615-624) demonstrated that proteolytic processing of the rat gamma 2 chain may be by MMP2 and MT1-MMP, both of which are present in the invading front of tumors. A more recent study by Veitch, et al (JBC 278, 15661-15668) indicates that processing of human laminin 5 may require BMP-1 isoenzymes.

The functional significance of the gamma 2 cleavage remains uncertain. However, the processed form of gamma 2 is the predominant extracellular form found in tissue. Thus, the processed gamma 2 form is likely to be the preferred form of gamma 2 for targeting with a monoclonal antibody to prevent tumor cell migration.

5 The data presented herein indicate that antibodies which target an epitope contained within residues 494-534 of Domain III, downstream of the processing site at residue 434 have a greater effect on inhibiting cell migration compared to an antibody which targets an epitope which spans the processing site (residues 391-461). This data supports the preferential targeting of sequences contained within a specific region of
10 Domain III which is downstream to the cleavage site at residue 434 and remains as part of the laminin 5 molecule after proteolytic processing has occurred.

Using various techniques, potential antigenic regions have been identified within domain III of the gamma 2 chain at the following positions: AA 380-400; AA 420-460; AA 520-550; and AA 560-590 (numbering relative to full length gamma 2
15 (SEQ ID NO:4)). Taken together with the processing site information and the experimental data disclosed herein, peptide fragments containing all or a portion of AA 520-550 and/or AA 560-590 are further embodiments of the present invention.

Furthermore, EGF-like domains, often speculated to be involved in protein-protein interactions, are present within domain III at amino acid positions (numbering
20 relative to full length gamma 2 (SEQ ID NO:4)) 382-415; 416-461; 462-516; 517-572; and 573-602. Thus, taken together with the processing site information and the experimental data provided herein, peptide fragments containing all or a portion of AA 462-516, 517-572; and/or 573-602 are further embodiments of the present invention.

In various preferred embodiment, the antibodies specifically bind to one or
25 more epitopes of the laminin 5 γ 2 chain contained within any of the following polypeptide sequences (all amino acid sequence numbering is relative to full length gamma 2 (SEQ ID NO:4)):

SEQ ID NOS:

6: 435-608

30 DENPDIECADCPIGFYNDPHDPRCKPCPCHNGFSCSVIPETEEVVCNNCP
PGVTGARCELCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRLTGR
CLKCIHNTAGIYCDQCKAGYFGDPLAPNPADKCRACNCNPMGSEPVGCRSDG
TCVCKPGFGGPNCEHGAFS

7: 435-602

35 DENPDIECADCPIGFYNDPHDPRCKPCPCHNGFSCSVIPETEEVVCNNCP
PGVTGARCELCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRLTGRC

LKCIHNTAGIYCDQCKAGYFGDPLAPNPADKCRACNCNPMGSEPVGCRSDGTC
VCKPGFGGPNC
8: 435-590
DENPDIECADCPIGFYNDPHDPRSCCKPCPCHNGFSCSVIPETEEVVCNNCP
5 PGVTGARCELCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRLTGRC
LKCIHNTAGIYCDQCKAGYFGDPLAPNPADKCRACNCNPMGSEPVGCRSDGT
9: 435-572
DENPDIECADCPIGFYNDPHDPRSCCKPCPCHNGFSCSVIPETEEVVCNNCP
PGVTGARCELCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRLTGRC
10 LKCIHNTAGIYCDQCKAGYFGDPLAPNPADKCRAC
10: 435-567
DENPDIECADCPIGFYNDPHDPRSCCKPCPCHNGFSCSVIPETEEVVCNNCP
PGVTGARCELCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRLTGRC
LKCIHNTAGIYCDQCKAGYFGDPLAPNPA
15 11: 435-550
DENPDIECADCPIGFYNDPHDPRSCCKPCPCHNGFSCSVIPETEEVVCNNCP
PGVTGARCELCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRLTGRC
LKCIHNTAGIYC
12: 435-534
20 DENPDIECADCPIGFYNDPHDPRSCCKPCPCHNGFSCSVIPETEEVVCNNCP
PGVTGARCELCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRL
13: 462-608
CPCHNGFSCSVIPETEEVVCNNCPPGVTGARCELCADGYFGDPFGEHGP
VRPCQPCQCNSNVDPSASGNCDRLTGRCCLKCIHNTAGIYCDQCKAGYFGDPLA
25 PNPADKCRACNCNPMGSEPVGCRSDGTCVCKPGFGGPNC
14: 462-602
CPCHNGFSCSVIPETEEVVCNNCPPGVTGARCELCADGYFGDPFGEHGP
VRPCQPCQCNSNVDPSASGNCDRLTGRCCLKCIHNTAGIYCDQCKAGYFGDPLA
PNPADKCRACNCNPMGSEPVGCRSDGTCVCKPGFGGPNC
30 15: 462-590
CPCHNGFSCSVIPETEEVVCNNCPPGVTGARCELCADGYFGDPFGEHGP
VRPCQPCQCNSNVDPSASGNCDRLTGRCCLKCIHNTAGIYCDQCKAGYFGDPLA
PNPADKCRACNCNPMGSEPVGCRSDGT
16: 462-572
35 CPCHNGFSCSVIPETEEVVCNNCPPGVTGARCELCADGYFGDPFGEHGP
VRPCQPCQCNSNVDPSASGNCDRLTGRCCLKCIHNTAGIYCDQCKAGYFGDPLA
PNPADKCRAC
17: 462-567
CPCHNGFSCSVIPETEEVVCNNCPPGVTGARCELCADGYFGDPFGEHGP
40 VRPCQPCQCNSNVDPSASGNCDRLTGRCCLKCIHNTAGIYCDQCKAGYFGDPLA
PNPA
18: 462-550
CPCHNGFSCSVIPETEEVVCNNCPPGVTGARCELCADGYFGDPFGEHGP
VRPCQPCQCNSNVDPSASGNCDRLTGRCCLKCIHNTAGIYC
45 19: 462-534
CPCHNGFSCSVIPETEEVVCNNCPPGVTGARCELCADGYFGDPFGEHGP
VRPCQPCQCNSNVDPSASGNCDRL
20: 494-608
LCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRLTGRCCLKCIH
50 NTAGIYCDQCKAGYFGDPLAPNPADKCRACNCNPMGSEPVGCRSDGTCVCKP

GFGGPNCEHGAFS
 21: 494-602
 LCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRLTGRCLKCIH
 NTAGIYCDQCKAGYFGDPLAPNPADKCRACNCNPMGSEPVGCRSDGTCVCKP
 5 GFGGPNC
 22: 494-590
 LCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRLTGRCLKCIH
 NTAGIYCDQCKAGYFGDPLAPNPADKCRACNCNPMGSEPVGCRSDGT
 23: 494-572
 10 LCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRLTGRCLKCIH
 NTAGIYCDQCKAGYFGDPLAPNPADKCRA
 24: 494-567
 LCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRLTGRCLKCIH
 NTAGIYCDQCKAGYFGDPLAPNPA
 15 25: 494-550
 LCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRLTGRCLKCIH
 NTAGIYC
 26: 494-534
 LCADGYFGDPFGEHGPVRPCQPCQCNSNVDPSASGNCDRL
 20

The nucleic acid sequences encoding each of these polypeptides can be easily determined by one of skill in the art by referring to the nucleic acid sequence of the full length gamma 2 chain presented as SEQ ID NO:3.

25 These various embodiments of the antibodies of the invention are useful for all of the aspects of the present invention disclosed below.

The term antibody as used herein is intended to include antibody fragments thereof which are specifically reactive with one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO: 5, such as the various epitopes disclosed above, or peptide fragments thereof. The antibody can be a polyclonal antibody or a monoclonal antibody, but preferably is a monoclonal antibody. The antibodies can be humanized, fully human, or murine forms of the antibodies.

As used herein the term "isolated" means that the antibodies are separated from their in vivo environment.

35 As used herein "specific binding" means that the antibodies recognize one or more epitope within one or more of the disclosed polypeptide sequences, but possess little or no detectable reactivity with other laminin 5 gamma 2 epitopes under standard conditions, such as those disclosed herein.

40 Antibodies can be made by well-known methods, such as described in Harlow and Lane, Antibodies; A Laboratory Manual, Cold Spring Harbor Laboratory, Cold

Spring Harbor, N.Y., (1988). In one example, pre-immune serum is collected prior to the first immunization. A peptide portion of the one or more of the polypeptides disclosed herein, together with an appropriate adjuvant, is injected into an animal in an amount and at intervals sufficient to elicit an immune response. Animals are bled at
5 regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the initial immunization. At about 7 days after each booster immunization, or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots are stored at about -20° C.

Polyclonal antibodies against the polypeptides can then be purified directly by passing
10 serum collected from the animal through a column to which non-antigen-related proteins prepared from the same expression system without the polypeptides bound.

Monoclonal antibodies can be produced by obtaining spleen cells from the animal. (See Kohler and Milstein, *Nature* 256, 495-497 (1975)). In one example, monoclonal antibodies (mAb) of interest are prepared by immunizing inbred mice with
15 a polypeptide as disclosed herein, or portion thereof. The mice are immunized by the IP or SC route in an amount and at intervals sufficient to elicit an immune response. The mice receive an initial immunization on day 0 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of by the intravenous (IV) route. Lymphocytes from antibody positive mice are obtained by
20 removing spleens from immunized mice by standard procedures known in the art. Hybridoma cells are produced by mixing the splenic lymphocytes with an appropriate fusion partner under conditions that allow formation of stable hybridomas. The antibody producing cells and fusion partner cells are fused in polyethylene glycol at concentrations from about 30% to about 50%. Fused hybridoma cells are selected by
25 growth in hypoxanthine, thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected from growth positive wells and are screened for antibody production by an immunoassay such as solid phase immunoradioassay. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson,
30 Soft Agar Techniques, in *Tissue Culture Methods and Applications*, Kruse and Paterson, Eds., Academic Press, 1973.

To generate such an antibody response, a polypeptide as disclosed herein, or a fragment thereof, is typically formulated with a pharmaceutically acceptable carrier for

parenteral administration. Such acceptable adjuvants include, but are not limited to, Freund's complete, Freund's incomplete, alum-precipitate, water in oil emulsion containing *Corynebacterium parvum* and tRNA. The formulation of such compositions, including the concentration of the polypeptide and the selection of the vehicle and other components, is within the knowledge of those of skill of the art.

Antibodies can be fragmented using conventional techniques, and the fragments screened for utility in the same manner as described herein for whole antibodies. For example, F(ab')₂ fragments can be generated by treating antibody with pepsin. The resulting F(ab')₂ fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

As used herein, the term "epitope" refers to a specific site within the protein that is bound by the antibody, which includes both linear and non-linear epitopes. An epitope can be of any length capable of being recognized by an antibody, but preferably is at least 6 amino acids in length for a linear epitope, more preferably at least 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, or 40 amino acids in length for a linear epitope. Alternatively, the antibodies may include those that recognize a non-linear epitope, such as a structural epitope.

In a further preferred embodiment of this aspect of the invention, isolated monoclonal antibodies are of the IgG isotype. In a further preferred embodiment, the isolated monoclonal antibodies are selected from the group consisting of those designated herein as 5D5 and 6C12, and the hybridomas expressing these monoclonals, which are deposited with DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH – Hanover, Germany) under deposit numbers DSM ACC2652, and DSM ACC2653, respectively. Monoclonal antibody 4G1 is commercially available, since January of 2004, from DAKO CYTOMATION – Stockholm, Sweden).

In a further aspect, the present invention provides cells that produce the monoclonal antibodies of the invention, such as hybridoma cells. Such hybridoma cells are produced as described above.

In a further aspect, the present invention provides isolated polypeptides consisting of one or more of the polypeptides selected from the group consisting of SEQ ID NOS: 6-26. These polypeptides are useful for the production of antibodies specific for epitopes within the polypeptide sequence, as disclosed herein.

In another aspect, the present invention provides pharmaceutical compositions comprising one or more of the antibodies disclosed above and a pharmaceutically acceptable carrier. For administration, the antibody is ordinarily combined with one or more adjuvants appropriate for the indicated route of administration. The compounds
5 may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanolic acids, stearic acid, talc, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, acacia, gelatin, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and tableted or encapsulated for conventional administration. Alternatively, the antibody may be dissolved in saline, water, polyethylene glycol,
10 propylene glycol, carboxymethyl cellulose colloidal solutions, ethanol, corn oil, peanut oil, cottonseed oil, sesame oil, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well known in the pharmaceutical art. The carrier or diluent may include time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax, or other materials well known in the art.

15 The antibody may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (*e.g.*, solutions, suspensions, or emulsions). Antibody may be applied in a variety of solutions. Suitable solutions for use in accordance with the invention are sterile, dissolve sufficient amounts of the antibody, and are not harmful for the proposed application. The antibody may be subjected to conventional
20 pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc.

In a further embodiment, the pharmaceutical compositions of the invention comprise one or more additional anti-tumor agents, for example, a chemotherapeutic agent, such as one or more of those described below. The components of such
25 pharmaceutical compositions may be pre-mixed together or may be combined at any time prior to administration to a patient in need thereof.

The antibodies and pharmaceutical compositions of the invention are useful both in tumor diagnostics and in anti-tumor therapy. Thus, in another aspect, the present invention provides methods for detecting the presence of invasive cells in a tissue,
30 comprising

- (a) contacting a tissue with an antibody according to the present invention to form an immunocomplex between the antibody and a laminin 5 $\lambda 2$ epitope in the tissue; and

(b) detecting formation of the immunocomplex, wherein the formation of the immunocomplex correlates with the presence of invasive cells in the tissue.

The contacting can be performed *in vivo*, using labeled isolated antibodies and standard imaging techniques, or can be performed *in vitro* on tissue samples. In a preferred embodiment of the *in vitro* diagnostic method, unbound antibodies are removed, such as by appropriate wash steps known to those of skill in the art.

In a preferred embodiment, the tissue is a tumor tissue. In a further preferred embodiment, the tumor tissue is a laminin 5 secreting tumor tissue. More preferably, the tumor tissue is a carcinoma, including but are not limited to squamous cell carcinomas (including but not limited to squamous cell carcinoma of skin, cervix, and vulva), gastric carcinomas, colon adenocarcinomas, colorectal carcinomas, and cervical carcinomas.

In another aspect, the present invention provides methods for inhibiting tumor growth and/or metastasis in an individual in need thereof, comprising contacting the tumor with an amount effective to inhibit tumor growth and/or metastasis of one or more antibodies or pharmaceutical compositions of the present invention. In a preferred embodiment, the tumor is a laminin 5-secreting tumor. In a further preferred embodiment, the subject is a mammal; in a more preferred embodiment, the subject is human.

As used herein, the term "inhibiting tumor growth" means to reduce the amount of tumor growth that would occur in the absence of treatment, and includes decrease in tumor size and/or decrease in the rate of tumor growth.

As used herein, the term "inhibiting tumor metastasis" means to reduce the amount of tumor metastasis that would occur in the absence of treatment, and includes decrease in the number and/or size of metastases.

As used herein, the term "laminin-5 secreting tumor" means a tumor that expresses detectable amounts of laminin 5. Such tumors include, but are not limited to, carcinomas. Such carcinomas include, but are not limited to squamous cell carcinomas (including but not limited to squamous cell carcinoma of skin, cervix, and vulva), gastric carcinomas, colon adenocarcinomas, colorectal carcinomas, and cervical carcinomas.

In another embodiment, the methods of the invention can be used in combination with surgery on the subject, wherein surgery includes primary surgery for

removing one or more tumors, secondary cytoreductive surgery, and palliative secondary surgery.

In a further embodiment, the methods of the invention further comprise treating the subject with chemotherapy and/or radiation therapy. One benefit of such a method is that use of the antibody permits a reduction in the chemotherapy and/or radiation dosage necessary to inhibit tumor growth and/or metastasis. As used herein, "radiotherapy" includes but is not limited to the use of radio-labeled compounds targeting tumor cells. Any reduction in chemotherapeutic or radiation dosage benefits the patient by resulting in fewer and decreased intensity of side effects relative to standard chemotherapy and/or radiation therapy treatment.

In this embodiment, the antibody may be administered prior to, at the time of, or shortly after a given round of treatment with chemotherapeutic and/or radiation therapy. In a preferred embodiment, the antibody is administered prior to or simultaneously with a given round of chemotherapy and/or radiation therapy. In a most preferred embodiment, the antibody is administered prior to or simultaneously with each round of chemotherapy and/or radiation therapy. The exact timing of antibody administration will be determined by an attending physician based on a number of factors, but the antibody is generally administered between 24 hours before a given round of chemotherapy and/or radiation therapy and simultaneously with a given round of chemotherapy and/or radiation therapy.

The methods of the invention are appropriate for use with chemotherapy using one or more cytotoxic agent (ie: chemotherapeutic), including, but not limited to, cyclophosphamide, taxol, 5-fluorouracil, adriamycin, cisplatin, methotrexate, cytosine arabinoside, mitomycin C, prednisone, vindesine, carbaplatin, and vincristine. The cytotoxic agent can also be an antiviral compound which is capable of destroying proliferating cells. For a general discussion of cytotoxic agents used in chemotherapy, see Sathe, M. et al., Cancer Chemotherapeutic Agents: Handbook of Clinical Data (1978), hereby incorporated by reference.

The methods of the invention are also particularly suitable for those patients in need of repeated or high doses of chemotherapy and/or radiation therapy.

In practicing the invention, the amount or dosage range of antibody employed is one that effectively inhibits tumor growth and/or metastasis. The actual dosage range is based on a variety of factors, including the age, weight, sex, medical condition of the individual, the severity of the condition, and the route of administration. An inhibiting

amount of antibody that can be employed ranges generally between 0.01 µg/kg body weight and 15 mg/kg body weight, preferably ranging between 0.05 µg/kg and 10 mg/kg body weight, more preferably between 1 µg /kg and 10 mg/kg body weight, and even more preferably between about 10 µg /kg and 5 mg/kg body weight.

5 The antibody may be administered by any suitable route, but is preferably administered parenterally in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term "parenteral" as used herein includes, subcutaneous, intravenous, intraarterial, intramuscular, intrasternal, intratendinous, intraspinal, intracranial, intrathoracic, infusion techniques or
10 intraperitoneally. In preferred embodiments, antibody is administered intravenously or subcutaneously.

The examples below are meant by way of illustration, and are not meant to be limiting as to the scope of the instant disclosure.

15

EXAMPLE 1

The following example demonstrates the effect of laminin-5, including the γ2 chain of laminin-5, on cell adhesion and cell migration.

20 Materials and Methods

Cells and Cell Culture

A mouse squamous cell carcinoma cell line, KLN205 (cat. no. ATCC CRL-1453) was obtained from American Type Culture Collection (Rockville, MD). The cells were maintained as monolayer cultures in Eagle's minimum essential medium
25 (MEM) containing non-essential amino acids and Earle's BSS supplemented with 10% fetal calf serum (FCS). The HaCat human keratinocyte cell line was a kind gift from Dr. Fuzenig (Heidelberg, Germany). The HaCat cells were cultured in Dulbecco's MEM supplemented with 10% FCS. However, when the cells were cultured for the production of laminin-5, the medium was replaced by serum-free medium.

30

Preparation of Proteins

Mouse EHS laminin (laminin-1) was obtained from GIBCO BRL. Fibronectin was purified from FCS using a gelatin-Sepharose 4B column (Sigma) as described in

Vuoto, M. & Vaheri, A. (1979) Biochem. J. 183: 331-337.34 and Gillies, R. J., Didier, N. & Denton, M. (1986) Anal. Biochem. 159: 109-113. Human laminin-5 was immunoaffinity purified from the media of HaCat cells cultured for three days in the absence of serum. Briefly, the medium was first passed through a 5 ml gelatin-
5 Sepharose column (Sigma, St. Louis, MO) to ensure the complete absence of fibronectin from the protein preparation, after which the medium was passed through a 10 ml anti-laminin γ 2-Sepharose affinity column in order to bind laminin-5 molecules. Both columns were equilibrated in phosphate-buffered saline. The anti-laminin γ 2-Sepharose affinity column was prepared by coupling a Protein A-purified anti- γ 2 IgG (8
10 mg/ml) to 10 ml of CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden). The anti- γ 2 IgG was purified from a rabbit polyclonal antiserum prepared against a GST-fusion protein containing domain III of the γ 2 chain (Pyke, C., Salo, S., Ralfkiaer, E., Romer, J., Dano, K. & Tryggvason, K. (1995) Cancer Res. 55: 4132-4139). The laminin-5 was eluted from the immunoaffinity column using 50 mM triethanolamine,
15 pH 11.25, 0.1% Triton X-100 and neutralized directly with 1 M Tris-HCl, pH 7.0. Collected fractions were analyzed by SDS-PAGE and Western blotting using the same polyclonal antibodies as used for the preparation of the affinity column. Fractions containing laminin-5 were pooled and dialyzed against 50 mM Tris-HCl, 0.1 M NaCl, pH 7.4. Some batches of laminin-5 were denatured with 5 M urea and renatured to
20 study the effects of the treatment on adhesion and migration properties.

Generation of Recombinant Baculovirus and Expression of Recombinant Laminin 2 Chain

The γ 2 chain of laminin-5 was expressed as recombinant protein using the
25 baculovirus system and purified for studies on its functional properties. A full-length human laminin γ 2 chain cDNA containing 6 bp of the 5' UTR and 822 bp of the 3' UTR was constructed from four overlapping cDNA clones L52, HT2-7, L15 and L61 (Kallunki, P., Sainio, K., Eddy, R., Byers, M., Kallunki, T., Sariola, H., Beck, K., Hirvonen, H., Shows, T.B. & Tryggvason, K. (1992) J. Cell Biol. 119: 679-693). The
30 resulting 4,402 bp cDNA was analyzed by restriction enzyme mapping and partial sequencing, and cloned into the pVL1393 recombinant transfer plasmid prior to transfer into the AcNPV- γ 2 baculovirus vector kindly provided by Max Summers (Texas A&M University). This baculovirus vector containing the human laminin γ 2 chain cDNA

under the transcriptional control of the polyhedrin promoter was produced and purified following standard procedures, except that it was first enriched according to the method of Pen, et al. (Pen, J., Welling, G.W. & Welling-Wester, S. (1989), Nucl. Acid. Res. 17: 451) from the virus containing medium obtained by co-transfecting Sf9 cells with the wild-type virus (AcNPV) DNA and the recombinant transfer vector pVL 1393- γ 2. For expression of the recombinant protein, High Five (H5) cells were infected with the recombinant virus at a multiplicity of infection (MOI) of 5-10 pfu per cell by using standard protocols.

The recombinant γ 2 chain was purified by first resuspending the cells in 10 volumes of 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 2.5 mM EDTA, 1% Triton X-100, 1 mM PMSF and 1 mM NEM followed by homogenization in a Dounce homogenizer. The protein was extracted for 60 minutes on ice and solubilized proteins were removed by centrifugation at 1500 x g for 10 minutes at 4° C. The pellet was extracted again with buffer containing 1-3 M urea. The recombinant γ 2 chain was extracted with a buffer containing 5 M urea, and renatured by dialysis against 50 mM Tris-HCl, pH 7.4, 100 mM NaCl.

Preparation of Antibodies

Polyclonal antiserum against domain III of the laminin γ 2 chain was prepared and characterized as described previously. Briefly, rabbits were immunized s.c. four times using a γ 2-GST fusion protein as antigen. The antigen contained 177 amino acid residues (res. # 391-567) from domain III of the γ 2 (SEQ ID NO:27) (Kallunki, P., Sainio, K., Eddy, R., Byers, M., Kallunki, T., Sariola, H., Beck, K., Hirvonen, H., Shows, T.B. & Tryggvason, K. (1992) J. Cell Biol. 119: 679-693). Antibodies against the GST-epitopes were removed from the antisera by negative immunoabsorption with GST-Sepharose made by coupling *E. coli* expressed GST protein to CNBr-activated Sepharose. The removal of anti-GST IgG was ensured by Western blotting analysis with GST-specific antibodies. The specificity of the antibody against the laminin γ 2 chain was also tested by Western blotting as well as by ELISA.

Polyclonal antibody against the C-terminus of the laminin γ 2 chain was produced in rabbits essentially as above for domain III using a γ 2-GST fusion protein as antigen. The antigen contained 161 amino acids (res. # 1017-1178) from domain I/II of

the $\gamma 2$ chain and antibodies against the GST-epitopes were removed from the antisera by negative immunoadsorption with GST-Sepharose. The specificity of the antibody was tested by Western blotting and ELISA.

Polyclonal antiserum against laminin-1 was a kind gift of Dr. Foidart (University of Liege, Belgium). Normal rabbit serum was obtained prior to immunization from the rabbits used for immunization. IgG from the laminin-1 and laminin $\gamma 2$ chain antisera, as well as from normal rabbit serum, was purified using Protein A Sepharose (Pharmacia, Uppsala, Sweden).

10 *Cell Adhesion Assay*

Microtiter plates (96 wells: Nunc, Copenhagen, Denmark) were coated with 100 μ l/well of laminin-1 (10 μ g/ml), laminin-5 (10 μ g/ml), or recombinant laminin $\gamma 2$ chain (10 μ g/ml) in PBS or 50 mM Tris-HCl, pH 7.4 by incubating the plates overnight at 4° C. Control wells were uncoated or coated with the same amounts of BSA. In some experiment the proteins were first denatured by dialysis overnight against 5 M urea, 50 mM Tris-HCl, pH 7.5 and then renatured by dialysis against 50 mM Tris-HCl, pH 7.5. Potential remaining active sites on the plates were blocked with 150 μ l of 10 mg/ml BSA in PBS for 2 hours at room temperature. The wells were washed with PBS, and 100 μ l of Eagle's MEM containing 5 mg/ml BSA was added. For the adhesion assays, KLN205 cells were detached from subconfluent cell culture dishes with trypsin-EDTA (0.25%-0.03%) and resuspended in Eagle's MEM/BSA (5 mg/ml) at a concentration of 2×10^5 cells/ml and allowed to recover for 20 minutes at 37° C. A total of 20,000 cells were then added to each well and allowed to attach for an additional 90 minutes at 37° C. The extent of cell adhesion was determined by measuring color yields at 600 nm, following fixation with 3% paraformaldehyde and staining with 0.1% crystal violet. For inhibition assays with the anti- $\gamma 2$ antibody, the substrate coated wells were incubated with 20 μ g/ml of anti- $\gamma 2$ chain IgG in PBS for 60 minutes prior to incubations with the cells.

30 *Migration Assay*

The effect of endogenous laminin-5 on migration of KLN205 cells was determined by using a modified Boyden chamber assay, as described by Hujanen and Terranova (Hujanen, E. & Terranova, V.P. (1985) Cancer Res. 45: 3517-3521), and the

effect of exogenous laminin-5 by using a modified Transwell assay, as described by Pelletier, et al. (Pelletier, A.J., Kunicki, T. and Quaranta, V. (1996), J. Biol. Chem. 271:364).

The Boyden chamber assay was carried out as follows. Polycarbonate filters (pore size 10 μm , diameter 12 mm; Costar, Cambridge, MA) were coated with 2.5 μg of EHS type IV collagen, and used to separate the upper and lower compartments of the 50 μl chamber. A total of 1×10^5 cells in Eagle's MEM containing 0.1% BSA were placed in the upper compartment, and the lower compartment was filled with medium with or without chemoattractants (50 $\mu\text{g/ml}$ laminin-1 or fibronectin). To study the effect of the laminin $\gamma 2$ chain antibodies on cell migration, anti- $\gamma 2$ (III) IgG or anti- $\gamma 2$ (C-term) IgG was added to the upper compartment together with the cells at a concentration of 20 $\mu\text{g/ml}$. Normal rabbit IgG was used as a negative control. After an 8-hour incubation at 37° C in a humidified atmosphere, the filters were removed, fixed and stained (Diff-Quick, Baxter Diagnostics, Tübingen, Germany). The cells that had not migrated were removed from the upper surface of the filter with cotton swabs. Migration of cells was quantified by counting the cells on the lower surface of each filter in 10 randomly selected high power fields (x400). All assays were performed in triplicate.

The "Transwell" plate assay (Transwell plates with pore size 12 μm , diameter 12 mm; Costar, Cambridge, MA) was used to determine the effect of exogenous laminin-5 on cell migration. The lower side of the membrane was coated with 2.5 μg of EHS type IV collagen for 3 hours at room temperature. Both sides were blocked with 1% bovine serum albumin for 1 hour. A total of 1×10^5 cells were added per well in the upper compartment in Eagle's MEM containing 10% FCS, and the lower compartment was filled with 2.5 $\mu\text{g/ml}$ laminin-5 as a chemoattractant. Antibodies against the C-terminus and domain III of the $\gamma 2$ chains or nonimmune IgG were added to the upper compartment, together with the cells at a concentration 20 $\mu\text{g/ml}$. Following a 16-hour incubation at 37° C the cells were fixed and stained. Cells on the top surface of the membrane were removed with cotton swabs, and cells that had migrated to the lower side of the membrane were counted (12 fields $\pm$ S.D.).

30

Immunohistochemical Staining

Five μm thick paraffin sections were stained with polyclonal antibodies against laminin-1 or the $\gamma 2$ chain of laminin-5. In brief, the paraffin sections were first incubated with 0.4% pepsin in 0.1 M HCl at 37° C for 20 minutes to expose the antigens, blocked for nonspecific binding with 5% newborn rabbit serum, 0.1% BSA, and then incubated for 1 hour at 37° C with the polyclonal IgG diluted in TBS to 5-10 $\mu\text{g/ml}$. Subsequently, a biotinylated swine-anti-rabbit antibody was applied, followed by incubation with a 1:400 dilution of Horseradish-Peroxidase-Avidin-Biotin-Complex (DAKO, Copenhagen, Denmark). The color was developed in diaminobentsamidine (DAB), followed by counterstaining of the slides with hematoxylin.

Results

Characterization of Proteins and Epithelium-Derived Cells

Immunopurified trimeric laminin-5, isolated from the culture medium of HaCat cells contained two major bands when analyzed by SDS-PAGE. These bands corresponded, respectively, to the 165 kDa $\gamma 2$ chain, and the 155 kDa and 140 kDa $\gamma 2$ and $\beta 3$ chains migrating as a single band, as reported previously. Additionally, a weak band of about 105 kDa corresponding to the processed $\gamma 2$ chain could be observed.

Full-length human recombinant laminin $\gamma 2$ chain was produced in High-5 *Spodoptera frugiperda* insect cells using the baculovirus system. Since the $\gamma 2$ chain was not secreted to the culture medium, possibly because it was not assembled intracellularly into a normal heterotrimer, it was isolated from the cell fraction as described in *Materials and Methods*. The protein was extracted under denaturing conditions using 5 M urea, renatured by extensive dialysis against 50 mM Tris-HCl, 100 mM NaCl, pH 7.4, and purified. The purified recombinant $\gamma 2$ chain was full length (approximately 155 kDa) and highly pure as determined by SDS-PAGE analysis.

The HaCat human keratinocytes and mouse KLN205 squamous carcinoma cells were shown to express laminin-5, based on Northern blot analyses and immunostaining, using a cDNA probe and/or polyclonal antibodies specific for the $\gamma 2$ chain, respectively. Furthermore, the KLN205 cells developed $\gamma 2$ chain positive primary tumors and metastases in mice *in vivo* (data not shown). Following intramuscular or subcutaneous inoculations, large primary tumors developed in 4 weeks with numerous lung

metastases after 4-6 weeks. KLN205 cells injected into the tail vein produced multiple lung tumors (experimental metastases) in four weeks. Consequently, both cell types were considered appropriate for the cell attachment and migration experiments carried out in this study.

5

Laminin-5 Molecule, but not Recombinant Laminin $\gamma 2$ Chain, Promotes Cell Adhesion

The laminin-5 and recombinant $\gamma 2$ chain prepared in this study, as well as commercial laminin-1, were used as substrata in attachment assays (**Figure 1**) with the two epithelium-derived HaCat and KLN205 cell lines that both express laminin-5. Both
10 cell lines attached about 2.5 times more readily to laminin-1 than to plastic. Adhesion of the cells to laminin-5 appeared to be slightly higher than that to laminin-1, but the differences were not statistically significant. The cells attached equally well to laminin-5 preparations denatured in 5 M urea and then renatured by dialysis against 50 mM Tris-HCl, 100 mM NaCl, pH 7.4, as described for the recombinant $\gamma 2$ chain above,
15 indicating that this treatment did not affect the binding properties of the trimeric molecule. The attachment to laminin-5 did not significantly decrease in the presence of two different polyclonal antibodies made against the short or long arms of the $\gamma 2$ chain or pre-IgG. Different amounts of the antibody against the short arm of the $\gamma 2$ chain were also tested (up to 50 $\mu\text{g/ml}$), but no effects on cell adhesion were observed. When
20 the cells were plated on the recombinant $\gamma 2$ chain alone, the attachment was not significantly higher than that to plastic, this attachment not being influenced by polyclonal antibodies against the $\gamma 2$ chain. The data confirm previous results showing that trimeric laminin-5 promotes adhesion of epithelial cells, but the present results further strongly suggest that this adhesion is not mediated by the $\gamma 2$ chain.

25

Antibodies Against Laminin $\gamma 2$ Domain III, But Not Domain I/II, Inhibit Cell Migration

The potential role of the $\gamma 2$ chain of laminin-5 in cell migration was examined for the KLN205 cells *in vitro* using Boyden and Transwell chamber assays as described in *Materials and Methods*.

30

Migration was first studied in the Boyden chamber assay using laminin-1 and fibronectin in the lower chamber as chemoattractants (See **Figure 2A**). The two compartments of the chemotactic Boyden chambers were separated by a type IV

collagen coated porous filter (pore size 8 μm). The cells (1×10^5) in MEM containing 0.1% BSA were placed in the upper compartment, and laminin-1 (+/-) or fibronectin (-/+) in MEM containing 0.1% BSA were added as chemoattractants to the lower compartment. IgG against $\gamma 2$ chain domains III, I/II or preimmune IgG was added to the upper compartment with the cells at a concentration of 20 $\mu\text{g/ml}$. After an 8-hour incubation at 37°C the filters were removed and migration of cells to the lower surface of the filter was quantitated. The data are expressed as percentage of migrated cells (+/- SD (bars)) per high power field, setting migration in the presence of pre-immune IgG as 100%. Cells were counted in ten randomly selected high power fields to triplicate assays. When polyclonal IgG against the short arm of the $\gamma 2$ chain was added to the upper compartment containing the cells, the migration of cells through the filter was decreased to about 35 to 45% of that observed with the preimmune serum. In contrast, the polyclonal IgG against C-terminal domain I/II did not affect migration of the cells.

The effects of the two antibodies were similarly used in the Transwell assay using native laminin-5 as chemoattractant in the lower compartment (See **Figure 2B**). The lower side of the membrane was coated with EHS type IV collagen, and the lower compartment was filled with 2.5 $\mu\text{g/ml}$ laminin-5 as a chemoattractant. Pre-immune IgG, IgG against the $\gamma 2$ chain domains III or I/II were added to the upper chamber containing the cells. Following a 16-hour incubation the cells were fixed and cells at the lower side of the membrane were counted (12 fields +/- SD). The results were essentially the same as in the Boyden chamber assay. Thus, addition of IgG raised against domain III of the $\gamma 2$ chain inhibited the migration to about 50% as compared with preimmune IgG, while the polyclonal IgG against domain I/II did not affect the cell migration.

These *in vitro* results demonstrate that laminin-5 have a role in the locomotion of epithelium-derived cells, and that this function can be inhibited by antibodies directed against domain III of the $\gamma 2$ chain.

Thus, antibodies against the short arm of the laminin $\lambda 2$ chain inhibited the migration of KLN2O5 squamous carcinoma cells by about 55-65% as determined in the Boyden chamber migration assay. Interestingly, the antibodies used here were directed against 177 amino acid residues of domain III (SEQ ID NO:27) that when deleted by mutation cause lethal junctional epidermolysis bullosa. Accordingly, the short arm of

the laminin $\lambda 2$ chain is important for the interaction of this laminin isoform to other extracellular matrix proteins and this interaction is also involved in the cell migration process.

5 EXAMPLE 2

The following example describes, in detail, the preparation of monoclonal antibodies according to the invention as well as demonstrating their use in inhibiting tumor cell growth in laminin-5 secreting tumors.

Monoclonal antibodies against the $\gamma 2$ chain of laminin-5 were produced by
10 immunizing Balb/c mice with 100 ug GST-laminin- $\gamma 2$ -III fusion protein as antigen. The GST-laminin- $\gamma 2$ -III fusion protein contains human laminin- $\gamma 2$ -chain amino acid residues 391-567 (SEQ ID NO:27). Subsequent to immunization, spleen cells from the immunized mice were fused with mouse myeloma cell obtained from cell line P3X63Ag.8.653 (ATCC #CRL-1580). The hybridoma clones were then screened in
15 immunohistology on frozen and paraffin sections (human cervix carcinoma, normal cervix and normal skin) for the production of the anti-laminin- $\gamma 2$ antibody. The staining result was compared to negative control, mouse normal serum and IgG, and to the positive result obtained with well-characterized anti-laminin-5, $\gamma 2$ chain polyclonal antibody (described in Pyke, et al., 1995). The hybridoma clones were also screened in
20 ELISA. The best hybridoma clones were picked and cloned again twice (single cell cloning) to ensure that the produced hybridoma cell line was monoclonal.

The following describes the details of the production of three specific hybridoma clones and corresponding monoclonal antibodies produced therefrom. Characterization studies were conducted with respect to the 4G1, 5D5 and 6C12
25 monoclonal antibodies. Western blot analysis and ELISAs were carried out to confirm the specificity of the antibodies to the $\gamma 2$ chain of laminin 5. Western blot analysis involved running recombinant laminin 5 $\gamma 2$ chain (as well as appropriate controls) in an SDS-PAGE gel, blotting the gel on a nylon membrane, and incubating the membrane with the antibodies

30 For ELISA, plates were coated with 100 μ l GST- $\gamma 2$ -III fusion protein (antigen) (Salo et al., Matrix Biology 18:197-210 (1999) at a concentration of 2.5 ug/ml in 0.1M carbonate/bi-carbonate buffer (pH 9) overnight at 4° C (0.25ug/well). The ELISA plate was then washed three times with a PBST solution (200 μ l) (10mM potassium

phosphate, 150 mM NaCl), pH 7.5, and 0.05% Tween-20. Non-specific binding was then blocked by addition of BSA-PBS (1% bovine serum in PBS buffer (10mM K₃P0₄, 150 mM NaCl, pH 7.5)) (200 m/well) for a period of 90 minutes. To this, a dilution of negative controls (normal mouse serum) and a sample diluted in BSA-PBS (Mab 4G1, 5D5 or 6C12) were added and then the ELISA plate was incubated for 1 hour at room temperature. After incubation, the ELISA plate was then washed with PBST three times. Next, HRP-conjugated anti-mouse IgG secondary antibody (Peroxidase (HRP) conjugated Rabbit Anti-Mouse IgG (H+L), Jackson Laboratories #315-035-045) was added and the plate was incubated at room temperature for 30 minutes. The ELISA plate was then washed again three times with PBST solution (200 ml). An ABTS-peroxide substrate was then added to the wells (ABTS diluted in 0.1 M Na-citrate, pH5; diluted immediately before assay use 1ml to 10ml with Na citrate buffer + 2 μ l 30% hydrogen peroxide) and then the plate was allowed to incubate in the dark for 30 minutes. The absorbance was then read with a micro plate reader at 405 nm at 30 and 60 minutes.

These analyses demonstrated the specificity of the monoclonal antibodies for domain III of the laminin 5 γ 2 chain. Epitope mapping of the epitopes recognized by Mab 4G1, 5D5 or 6C12 indicated that they each bound epitopes within the amino acid sequence of **SEQ ID NO:6**.

Monoclonal antibodies against the γ 2 chain of laminin-5 were then tested for efficacy in inhibiting tumor cell growth in laminin-5 secreting tumors.

Study 1: Tumor Growth in Immunosuppressed Mice

The following study demonstrates the ability of IgG immunoglobulin against human laminin-5, γ 2-III-domain (Mab 5D5) to affect the number and size of metastases in immune deficient mice.

10⁶ human squamous epithelial carcinoma cells were injected into the tail vein of immunosuppressed mice for tumor implantation. The cell lines used were human squamous epithelial carcinoma cells, cell line A431 and HSC-3. The cells were provided in suspensions in a medium containing DMEM-glutamax, 1% penicillin-streptomycin, 1% Na-pyruvate, 5% FCS. The cells were re-suspended in sterile Ca and Mg free PBS for inoculation. A control cell count was performed for the cell suspension at arrival and the cell density and the injected volume was recorded. The

origin of the cells is HSC-3: Japan Health Science Research Resources Bank, JCRB 0623 A431: ATCC catalog number CRL-1555. The immunosuppressed mice were selected as they are susceptible to grow cells of human origin as is well known in the field. The tumor cells in groups 3 and 6 were injected into mice with test item (test item was 50 μ g/ml) for tumor implantation. The tumor cells were allowed to grow for one week after which the animal received intravenous injections of the test item twice a week for four weeks.

Table 1. Study Layout

Group	Mouse Strain		Animal Number	Cell Line	Treatment
1	Balb/c~nudet	5	1-5	-	-control, no treatment
2	Balb/c-nude ¹	5	6-10	HSC-3	+control, no treatment
3	Balb/c-nude ¹	5	11-15	HSC-3	Test item treatment: 50 μ g 5D5/mouse injection
4	SCID ²	5	21-25	-	-control, no treatment
5	SCID~	5	26-30	A431	+control, no test item
6	SCIDZ	5	31-35	A431	Test item treatment: 50 μ g 5D5/mouse injection

¹ Balb/c-nude (BALBicABom-nu, M&B A/S, Denmark)

² Fox Chase Scid (C.B-17/Icr scid/scid, M&B A/S, Denmark) immunodeficient mice.

After the treatment period, the animals were killed and tissue samples were collected. Number and size of the tumors in different tissues were counted and compared.

Test Items and Dosing Solutions

The test item was IgG immunoglobulin against human laminin-5, γ 2-III-domain (Mab 5D5). The test item was produced with monoclonal hybridoma method *in vitro* as set forth above. The test item (Mab 5D5) was suspended in sterile phosphate buffered saline (PBS) with a concentration of 1 mg/ml. The vehicle was sterilized using a 0.2 μ m filter. The delivered test item was diluted with sterile PBS 50:50 to give a dosing concentration of 500 μ g/ml.

The test item was administered intravenously into the lateral tail vein of the

immunosuppressed mice in a volume of 0.1 ml/animal. The dosing was twice a week on Mondays and Thursdays. The first dose of test item was administered one week after the induction of experimental metastasis.

After four weeks of treatment (eight doses of test item), the animals were killed by exsanguination with cardiac puncture in CO₂ anesthesia. Blood was collected and serum separated and frozen in -20° C. A gross necropsy was performed and the macroscopic signs were recorded with special attention to macroscopic tumor masses, which were calculated and measured if possible. The following organs/tissues were collected and weighed: lungs, lymph nodes (cervical and mesenteric), liver, and spleen. The organs/tissues were rinsed in PBS and fixed in 4% phosphate buffered formalin.

Clinical Signs

Animal number 6 had a thickening of the tail from day 5 through the whole study. The tail of animal number 11 turned dark/black after tumor cell inoculation and eventually turned necrotic. Half of the tail was missing from day 7 onward. No other treatment related clinical signs were recorded. One animal (number 8, group 2) was found dead on the morning of the day following tumor cell inoculation. Gross necropsy did not reveal any macroscopic changes. All other animals survived in good condition during the whole study.

Necropsy

The injected tumor cells induced tumor growth almost only in the lungs. Other tissues with macroscopic metastases include spleen, liver, small intestine, and preputial gland. The SCID mice had changes in the liver which might be of microbial origin. In the lungs, the metastases were so numerous and so small that it was impractical to calculate or measure individual metastases.

The following Table 2 represents a summarization of the results of the mice treated from Table 1.

Table 2. Experimental Metastases in Lung

Group	Mouse Strain	N	Cell Line	Treatment	Number of Mice with Macroscopic Lung Metastases Observed
I	Balb/c-nude	5	-	-control, no treatment	-
2*	Balb/c-nude	5	HSC-3	+ control, no treatment	4/4 (full of metastases)
3	Balb/c-nude	5	HSC-3	Test item treatment	1/5
4	SCID ~	5	-	-control, no treatment	-
5	SCID	5	A431	+control, no test item	3/5
6	SCID	5*	A431	Test item treatment	4/5

* one mouse was dead at the end of the second study

- 5 As can be seen from Table 2 above, the treated Balb/c-nude mice had 1 of 5 mice with macroscopic lung metastases while 4 of 4 untreated control Balb/c-nude mice had macroscopic lung metastases.

EXAMPLE 3

- 10 Monoclonal antibody 5D5 was tested against HT29 carcinomas in a tumor growth inhibition assay. The assay compared immunotherapy with 75 and 25 $\mu\text{g}/\text{mouse}$ 5D5, qod x 15, to conventional chemotherapy with 100 mg/kg CPT-11 (irinotecan/Campostar), qwk x 3.

15 *Methods and Materials*

- Female nude athymic mice (Harlan) were 13 weeks of age on day 1 of the study. The animals were fed *ad libitum* water (reverse osmosis, 1 ppm Cl) and the NIH 31 Modified and Irradiated Lab Diet® consisting of 18.0% protein, 5.0% fat, and 5.0% fiber. Mice were housed in static microisolators on a 12-hour light cycle at 21-22 ° C
20 (70-72 ° F) and 40%-60% humidity.

Tumor Implantation

An HT29 carcinoma fragment (1 mm³) was implanted subcutaneously in the flank region of each mouse. When the tumors reached a size ranging from 62.5-126

mg, the mice were sorted into five treatment groups to provide a group mean tumor weights of 84.2-85.5 mg. Estimated tumor weight was calculated using the formula:

$$\text{Tumor Weight (mg)} = \frac{w^2 \times l}{2}$$

Where w = width and l = length in mm of the HT29 carcinoma.

Dosing solutions of 5D5 and control IgG were prepared fresh daily by dilution with phosphate-buffered saline. CPT-11 (Pharmacia; 20 mg/mL) was diluted with saline on each day of dosing.

On day 1, mice were sorted into five groups of animals ($n = 10/\text{group}$), and dosing was initiated according to the protocols listed in Table 3.

Table 3. Protocol Design for the HT29-e29 Study

Group	n	Treatment Regimen I			
		Agent	mg/kg	Route	Schedule
I	10	No treatment	n/a		
2	10	CPT-11	100	IP	Qwkx3
3	10	Control IgG	75 ug/ mouse	IV	Qod x I 5
4	10	5D5	75 ug/ mouse	IV	Qod x I 5
5	10	5D5	25 ug/ mouse	IV	Qod x I 5~

As a positive reference drug, CPT- 11 was administered once per week for three weeks (qwk x 3) in 100 mg/kg doses. CPT- 11 was delivered i.p. in volumes of 0.2 ml/20 g body weight, which were body-weight adjusted. Doses of 5D5 or control mouse IgG were delivered intravenously in volumes of 0.2 mL/mouse. The antibody doses were not body-weight adjusted. Untreated Group I mice served as controls for the CPT-11 therapy. Group 3 mice received 15 $\mu\text{g}/\text{mouse}$ doses of control IgG once daily on alternate days (qod x 15). Mice in groups 4 and 5 received 75 and 25 $\mu\text{g}/\text{mouse}$ doses of 5D5 x 15, respectively.

Endpoint

Efficacy was evaluated in a tumor growth inhibition assay. Tumors were measured twice weekly until the study was terminated on day 31. Each animal was then euthanized and its HT29 carcinoma was excised and weighed. Treatment may produce complete tumor regression (CR) or partial tumor regression (PR) in an animal. In a CR response, there is no measurable tumor mass at the completion of the study. In a PR response, the tumor weight is lower than the weight on day 1, but greater than 0 mg. All tumors that did not regress were included in the calculation of tumor growth inhibition.

The increase in tumor weight for each animal was calculated as the difference between the actual tumor weight at the end of the study and the calculated tumor weight on day 1. These values were used to calculate the group mean tumor weight increases. Tumor growth inhibition was calculated from the group mean tumor weight increases of treated and control mice by the following equation:

$$\%TGI = \left[1 - \left(\frac{\text{MeanNetTum or Weight}_{\text{Treated}}}{\text{MeanNet Turn or Weight}_{\text{control}}} \right) \right] \times 100 \%$$

Toxicity

The mice were weighed twice weekly until the end of the study. They were examined frequently for clinical signs of any adverse, drug-related side effects. Acceptable toxicity for cancer drugs in mice is defined by the NCI as a mean group weight loss of less than 20% during the test, and not more than one toxic death among ten treated animals.

Statistics and Graphical Analyses

The unpaired t-test and Mann-Whitney U-test (for analysis of means and medians, respectively) were used to determine the statistical significance of the difference in mean tumor weights for mice in a treatment group and mice in a control group. The two-tailed statistical analyses were conducted at $P = 0.05$.

Results

Efficacy: Growth of HT29 Colon Carcinomas in Control Mice

Treatment protocols are listed in Table 3. Group I mice received no treatment and served as controls for CPT-11 and 5D5 therapy. Group 3 mice received fifteen 75 μ g/mouse doses of irrelevant mouse IgG on alternate days (qod x 15). Table 4 summarizes the results for all groups in the study. The mean values for actual day 31

5 tumor weights in untreated and IgG-treated mice are 640.0 and 696.2 mg, respectively.

Table 4. Treatment Response Summary for the HT29-e28 Study

Gp.		Regimen 1				Final Tumor Weight Mean $\pm$ SEM (n)	Tumor Growth Inhibition	# CP	Mean % Tumor Decrease	# CR	Max. % BW Loss Day	# Death ^a	
		Agent	mg/kg	Route	Schedule							TR	NT R
1	10	No treatment	n/a			640.0 $\pm$ 124.9 mg (10)	0%	0	None	0	-0.4%; Day 4	0	0
2	10	CPT-11	100	IP	Qwk x 3	447.9 $\pm$ 91.8 mg (10)	34.7%	0	None	0	-5.8%; Day 4	0	0
3	10	Control IgG	75 μ g/mouse	IV	Qod x 15	696.2 $\pm$ 131.4 mg (10)	0%	0	None	0	-3.6%; Day 4	0	0
4	10	5D5	75 μ g/mouse	IV	Qod x 15	543.5 $\pm$ 149.3 mg (8)	17.8%		None	0	-2.0%; Day 4	0	0
5	10	5D5	25 μ g/mouse	IV	Qod x 15	700.7 $\pm$ 116.1 mg (10) ~	0%	0	None	0	-1.2%; Day 4	0	0

Response of HT29 Xenografts to Intraperitoneal CPT-11 Therapy

Group 2 mice were treated once weekly for three weeks (qwk x 3) with i.p. injections of 100 mg/kg CPT-11 (Table 3). No tumors regressed in response to CPT-11. The final mean tumor weight in Group 3 mice was 447.9 mg (Table 4). Group 2 mice experienced 34.7% tumor growth inhibition, relative to the untreated mice. This result, which is illustrated in a bar graph in **Figure 3**, was not statistically significant ($P = 0.23.11$, unpaired two-tailed t-test). **Figures 4A-E** shows the growth of individual tumors in all treatment groups, as calculated from caliper measurements. CPT-11 treatment caused a decrease in the slope of tumor growth.

Response of HT29 Xenografts to Intravenous 5D5 Immunotherapy

5D5 was administered intravenously to mice in Groups 4 and 5 on the qod x 15 schedule at 75 and 25 ug/mouse, respectively (Table 3). No tumor regressions were observed. The 75 and 25 $\mu\text{g}/\text{mg}$ mouse 5D5 treatments yielded final actual mean tumor weights of 543.5 and 700.7, respectively (Table 4). The high dose of 5D5 inhibited HT29 carcinoma growth by 17.8%, relative to tumor growth in untreated mice. Tumor growth inhibition in Group 4 mice, relative to untreated and IgG-treated mice, was not statistically significant ($P = 0.6241$ and 0.453 , respectively; t-test). Group 5 mice experienced no inhibition of tumor growth. **Figure 3** illustrates the lack of significant tumor growth inhibition, given the large error (SEM) bars. **Figures 4 A-E** shows that there was a modest decrease in the slopes of the tumor growth curves in animals treated with 75 ug/mouse 5D5.

Side Effects

All therapies were well tolerated. The highest group mean body-weight loss, an acceptable 5.8%, was recorded in mice treated with CPT-11. Body weight losses in antibody-treated mice were 3.6% or lower.

Discussion

The HT29 colon carcinoma xenograft model was appropriate for 5D5 evaluation because HT29 cells produce laminin. Growth of primary tumors can be impeded by anti-proliferative agents, such as CPT-11, as well as by agents that prevent invasion of the substratum. Combinational treatments using monoclonal antibodies

against the $\gamma 2$ chain of laminin-5, such as 5D5, with anti-proliferative agents such as CPT-11 are also contemplated as part of the invention. Treatment efficacy was based on tumor growth inhibition, i.e., the difference between the mean increase in tumor size in control and treated groups of animals during the 31-day study. Although there was no response to 5D5 at a dose of 25 $\mu\text{g}/\text{mouse}$, tumor growth was inhibited by 17.8% at 75 $\mu\text{g}/\text{mouse}$ (Table 4 and **Figure 3**). Thus, a 75 $\mu\text{g}/\text{mouse}$ dose of 5D5 produced some therapeutic effect against HT29 colon carcinomas. In general, there was a reduction in the slopes of the tumor growth curves in mice treated with CPT-11 and 5D5 (**Figures 4A-E**). Accordingly, these results indicate that anti-laminin immunotherapy has application in cancer treatment of laminin-5 secreting tumors.

In summary, established HT29 colon carcinomas responded to therapy with 75 $\mu\text{g}/\text{mouse}$ doses of 5D5. High dose 5D5 immunotherapy achieved 50% of the tumor growth inhibition that was produced by CPT-11 chemotherapy. The tumor growth shown in **Figures 4A-E** curves suggest that 5D5 immunotherapy can impair colon tumor growth at doses of 75 $\mu\text{g}/\text{mouse}$ or higher.

Example 4

Further in vitro migration experiments demonstrating the inhibitory effect of the monoclonal antibodies on HSC-3 cell migration are shown in Figures 5-6. The Transwell studies were carried out as described above, using either 25 $\mu\text{g}/\text{ml}$ or 100 $\mu\text{g}/\text{ml}$ of antibody and using approximately 10,000 cells per well (**Figure 5**) or approximately 30,000 cells per well (**Figure 6**). The incubation time in each case was 6 hours at 37° C. As can be seen from the data, each of the antibodies was effective at inhibiting cell migration relative to controls. The data further demonstrate that the 5D5 monoclonal antibody exhibited consistently improved efficacy relative to the 4G1 antibody, while the 6C12 monoclonal antibody exhibited improved efficacy relative to the 4G1 monoclonal in at least some cases.

Example 5

In an effort to identify the specific epitopes within domain III recognized by the monoclonal antibodies, ELISAs were performed by testing antibody binding to the following polypeptide fragments (all numbering is relative to the full length gamma 2

protein (SEQ ID NO:4): AA391-567; AA391-555; AA391-494; AA461-635; AA464-534 (expressed as GST fusion proteins). The assays were carried out as follows:

1. Coat ELISA plate with 100 ul of the protein at a concentration of 1 ug/ml in 0.1M carbonate/bicarbonate buffer, pH 9 overnight at 4 degrees C (100
5 ng/well). All subsequent incubations were carried out at room temperature.
2. Wash 3 times with PBST.
3. Block non-specific binding with BSA-PBS for 90 minutes (200
ul/well).
4. Add dilutions of reference standards, negative control, and sample
10 diluted in BSA-PBS; incubate for 1 hour at room temperature.
5. Wash 3 times with PBST.
6. Add HRP-conjugated anti-mouse IgG secondary antibody (diluted
1:5000 in PBS), incubate 30 minutes.
7. Wash 3 times with PBST.
- 15 8. Add ABTS-peroxidase substrate. Prepare substrate immediately before
use: (a) dilute ABTS stock (10X) with Citrate buffer; (b) Add 2 ul of 30% hydrogen
peroxidase and mix; (c) add substrate to wells and incubate in the dark for 30 minutes.
9. Read absorbance with a microplate reader at 405 nm after 30 and 60
minutes.

20

The results of this experiment demonstrated that the 4G1 monoclonal antibody recognizes one or more epitopes between AA391-461, while both the 6C12 and the 5D5 monoclonal antibodies recognized one or more epitopes between AA494-534.

We claim:

1. An antibody that specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO: 6.
2. The antibody of claim 1, wherein the antibody specifically binds to one or
5 more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:7.
3. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:8.
4. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO: 9.
- 10 5. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:10.
6. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:11.
7. The antibody of claim 1, wherein the antibody specifically binds to one or
15 more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:12.
8. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:13.
9. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:14.
- 20 10. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:15.
11. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:16.
12. The antibody of claim 1, wherein the antibody specifically binds to one or
25 more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:17.
13. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:18.
14. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:19.
- 30 15. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:20.

16. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:21.
17. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:22.
- 5 18. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:23.
19. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:24.
20. The antibody of claim 1, wherein the antibody specifically binds to one or
10 more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:25.
21. The antibody of claim 1, wherein the antibody specifically binds to one or more epitopes of the laminin 5 γ 2 chain contained within SEQ ID NO:26.
22. The antibody of any one of claims 1-21, wherein the antibody is a monoclonal antibody.
- 15 23. The antibody of any one of claims 1-21 wherein the antibody is a humanized or fully human antibody.
24. The antibody of any one of claims 1-23 wherein the one or more epitopes comprise one or more linear epitopes.
25. The antibody of any one of claims 1-23 wherein the one or more epitopes
20 comprise one or more non-linear epitopes.
26. An isolated cell that produces the antibody of claim 22.
27. A pharmaceutical composition comprising the antibody of any one of claims 1-21 and a pharmaceutically acceptable carrier.
28. The pharmaceutical composition of claim 27 wherein the antibody is a
25 monoclonal antibody.
29. The pharmaceutical composition of claim 27 wherein the antibody is a humanized or fully human antibody.
30. The pharmaceutical composition of any one of claims 27-29, further comprising an additional anti-tumor agent.
- 30 31. A method for detecting the presence of invasive cells, comprising
 - (a) contacting a tissue with an antibody according to any one of claims 1-21 to form an immunocomplex between the antibody and a laminin 5 λ 2 epitope in the tissue; and

- (b) detecting formation of the immunocomplex, wherein the formation of the immunocomplex correlates with the presence of invasive cells in the tissue.
32. The method of claim 31 wherein the tumor tissue comprises a carcinoma.
33. A method for inhibiting tumor growth, comprising administering to a patient
5 with a tumor an amount effective to inhibit tumor growth of an antibody according to any one of claims 1-21.
34. The method of claim 33 wherein the tumor comprises a carcinoma.
35. The method of claim 33 wherein the method is used in combination with one or more of the following treatments:
- 10 (a) primary surgery for removing one or more tumors;
(b) secondary cytoreductive surgery;
(c) palliative secondary surgery;
(d) radiation therapy; and
(e) chemotherapy.
- 15 36. A method for inhibiting tumor metastasis, comprising administering to a patient with a tumor an amount effective to inhibit tumor metastasis of an antibody according to any one of claims 1-21.
37. The method of claim 36 wherein the tumor comprises a carcinoma.
38. The method of claim 36 wherein the method is used in combination with one
20 or more of the following treatments:
- (a) primary surgery for removing one or more tumors;
(b) secondary cytoreductive surgery;
(c) palliative secondary surgery;
(d) radiation therapy; and
25 (e) chemotherapy.
39. The method of any one of claims 31-38 wherein the antibody is a monoclonal antibody.
40. The method of any one of claims 31-38 wherein the antibody is a humanized or fully human antibody.
- 30 41. Use of an antibody according to any one of claims 1-21 for the preparation of a medicament for inhibiting tumor metastasis.
42. Use of an antibody according to any one of claims 1-21 for the preparation of a medicament for inhibiting tumor growth.

43. The use of claim 41 or 42 wherein the antibody is a monoclonal antibody.
44. The use of claim 41 or 42 wherein the antibody is a humanized or fully human antibody.
45. The use of any one of claims 41-44 wherein the tumor comprises a carcinoma.
- 5 46. The use of any one of claims 41-44 wherein the medicament is prepared for use in combination with one or more of the following treatments:
- (a) primary surgery for removing one or more tumors;
 - (b) secondary cytoreductive surgery;
 - (c) palliative secondary surgery;
 - 10 (d) radiation therapy; and
 - (e) chemotherapy.
47. An isolated polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 6-26.

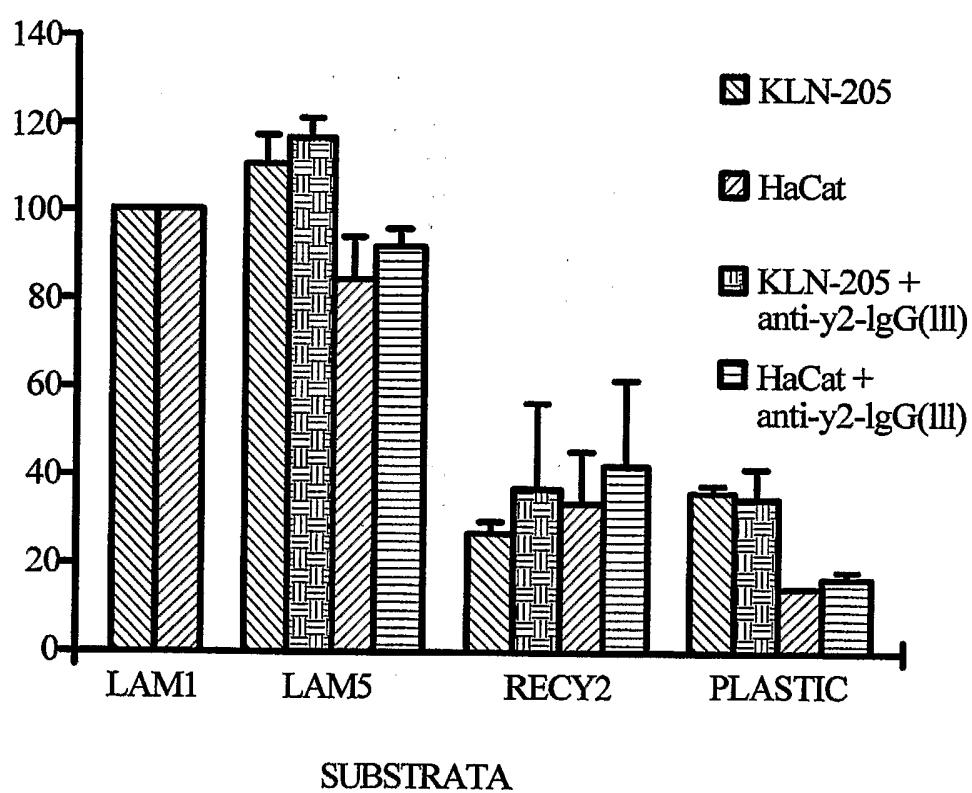
FIG. 1

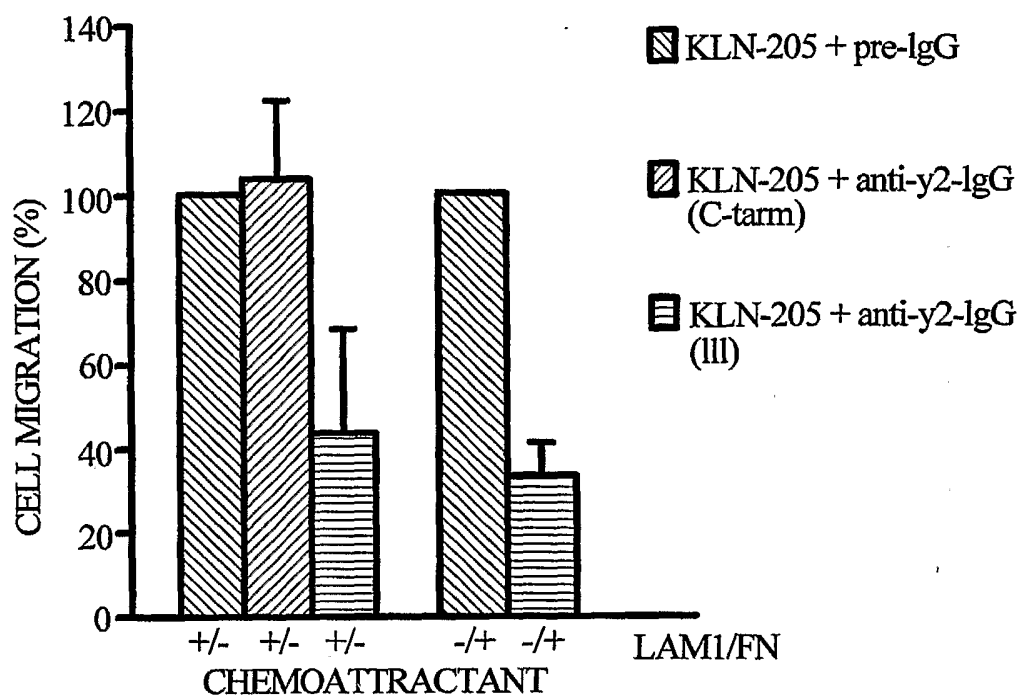
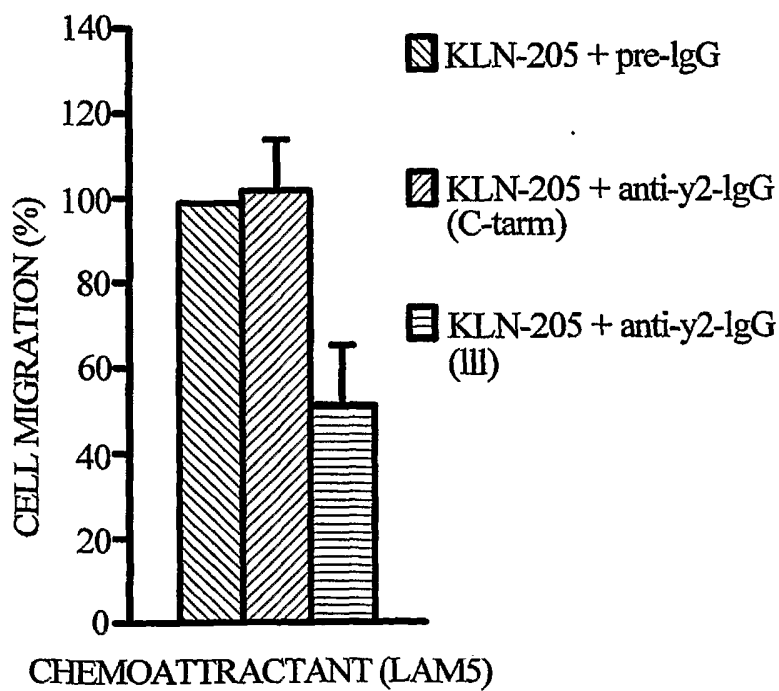
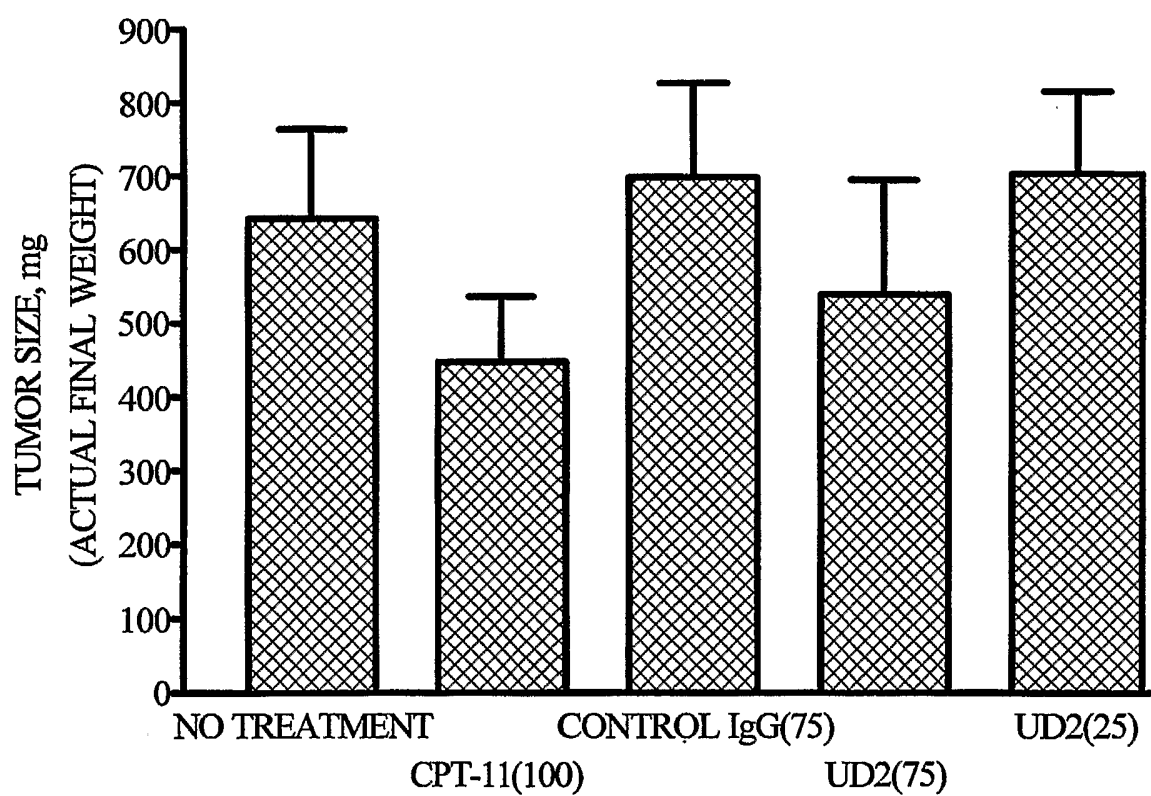
FIG. 2A*FIG. 2B*

FIG. 3

TUMOR GROWTH INHIBITION ON DAY 31
IN THE HT29-e28 STUDY



Tumor Growth Curves for Individual Mice in the HT29-e28 Study
Group 1: No Treatment

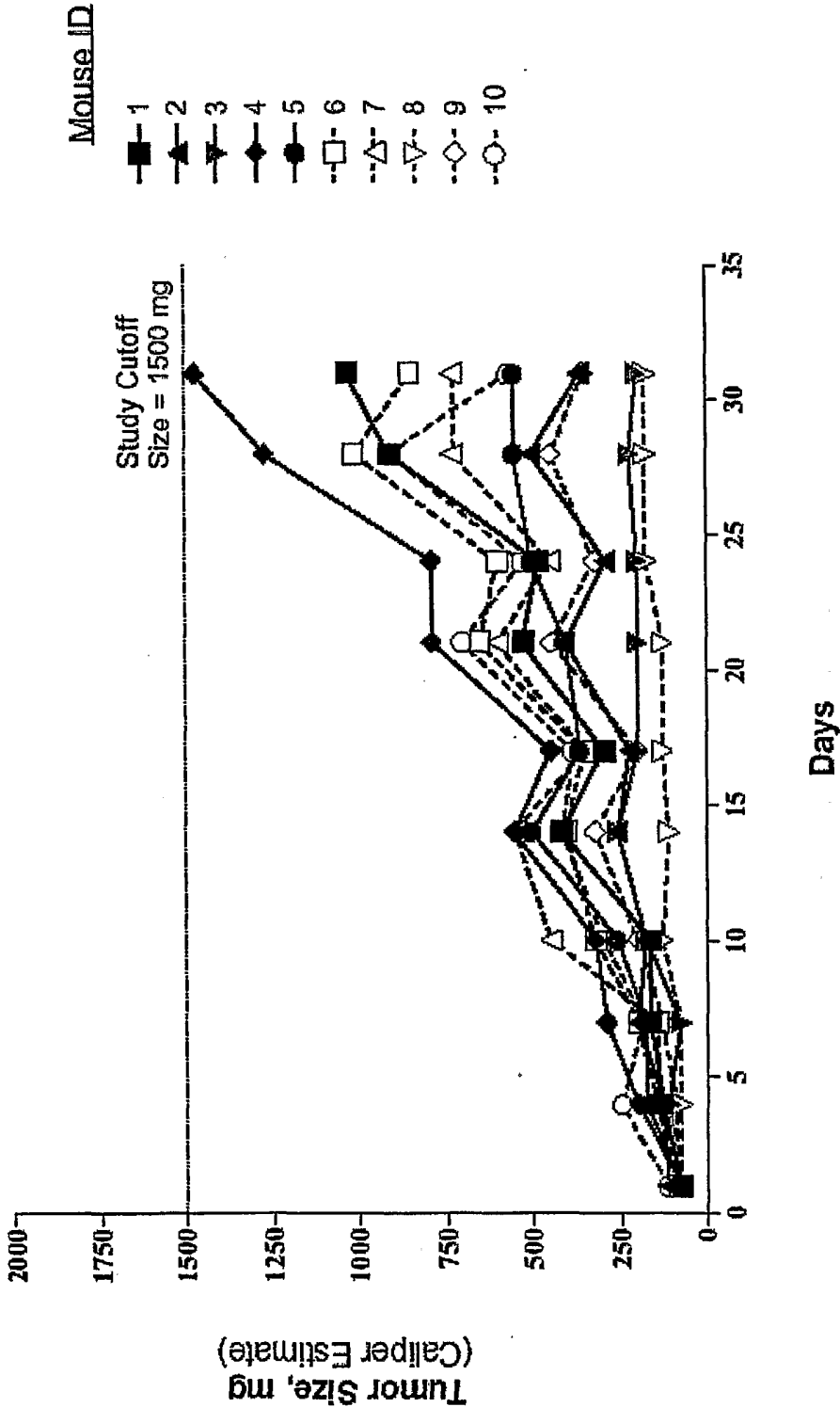


FIGURE 4A

Tumor Growth Curves for Individual Mice in the HT29-e28 Study
Group 2: CPT-11; 100 mg/kg; ip; qwk x 3

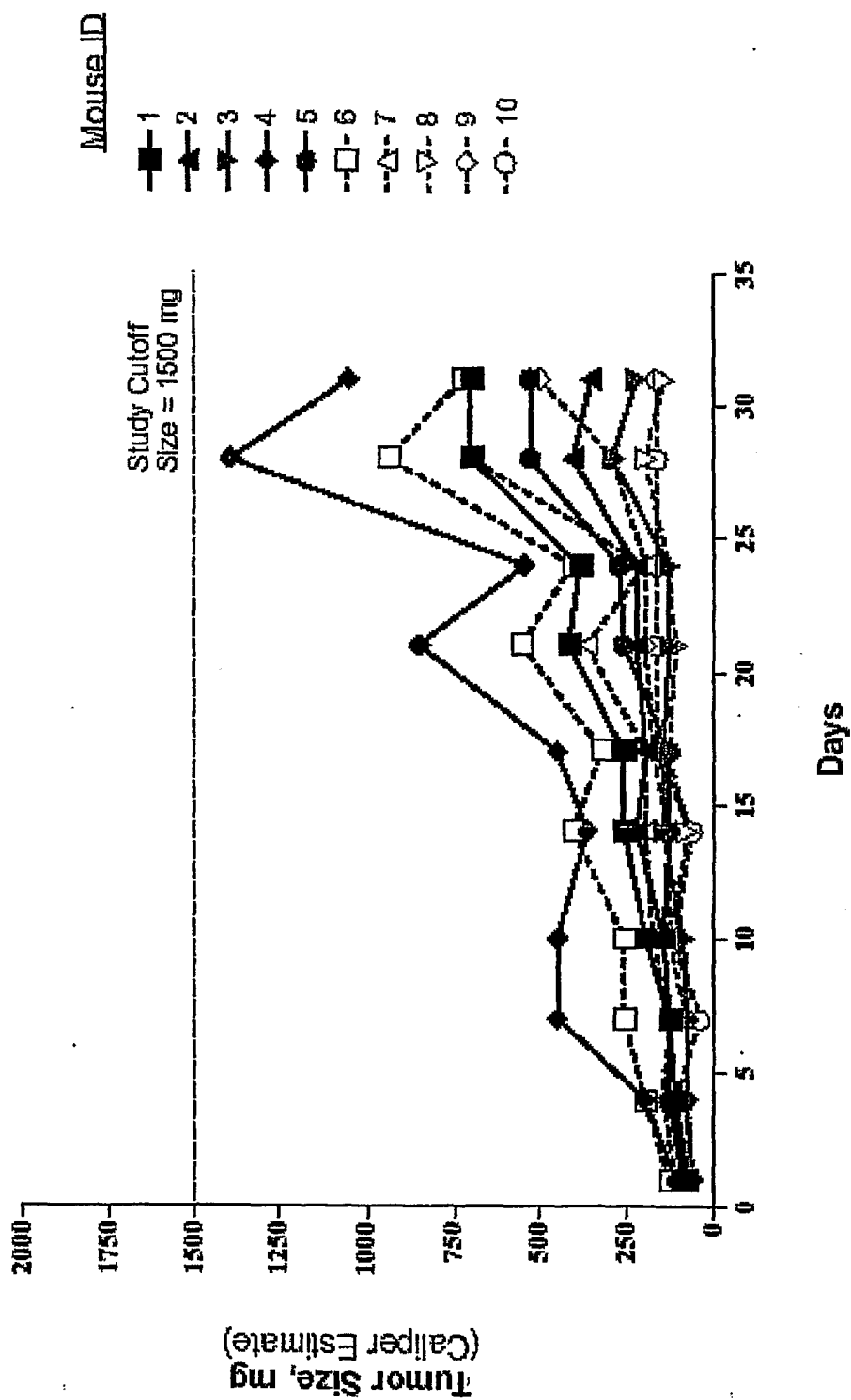


FIGURE 4B

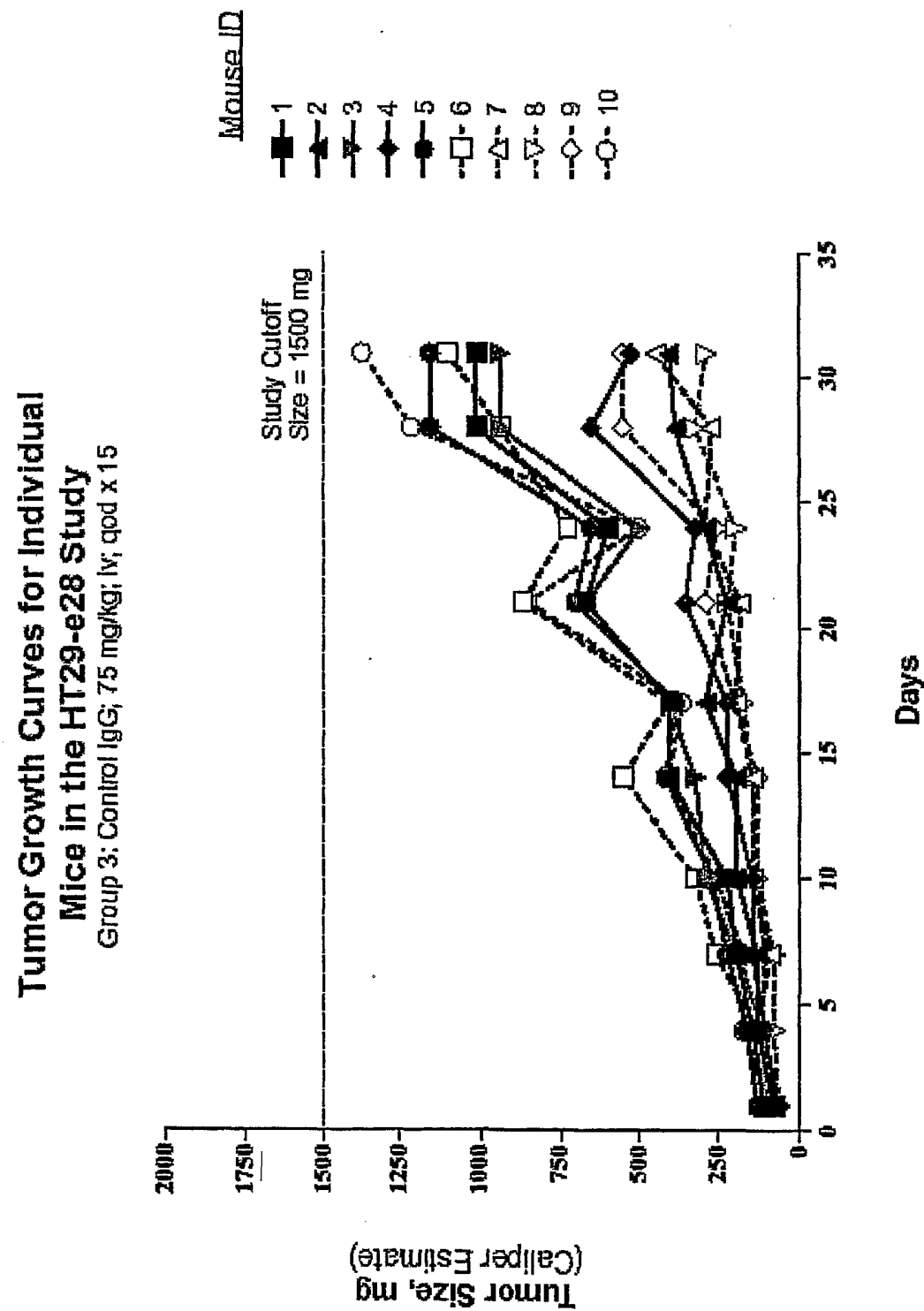


FIGURE 4C

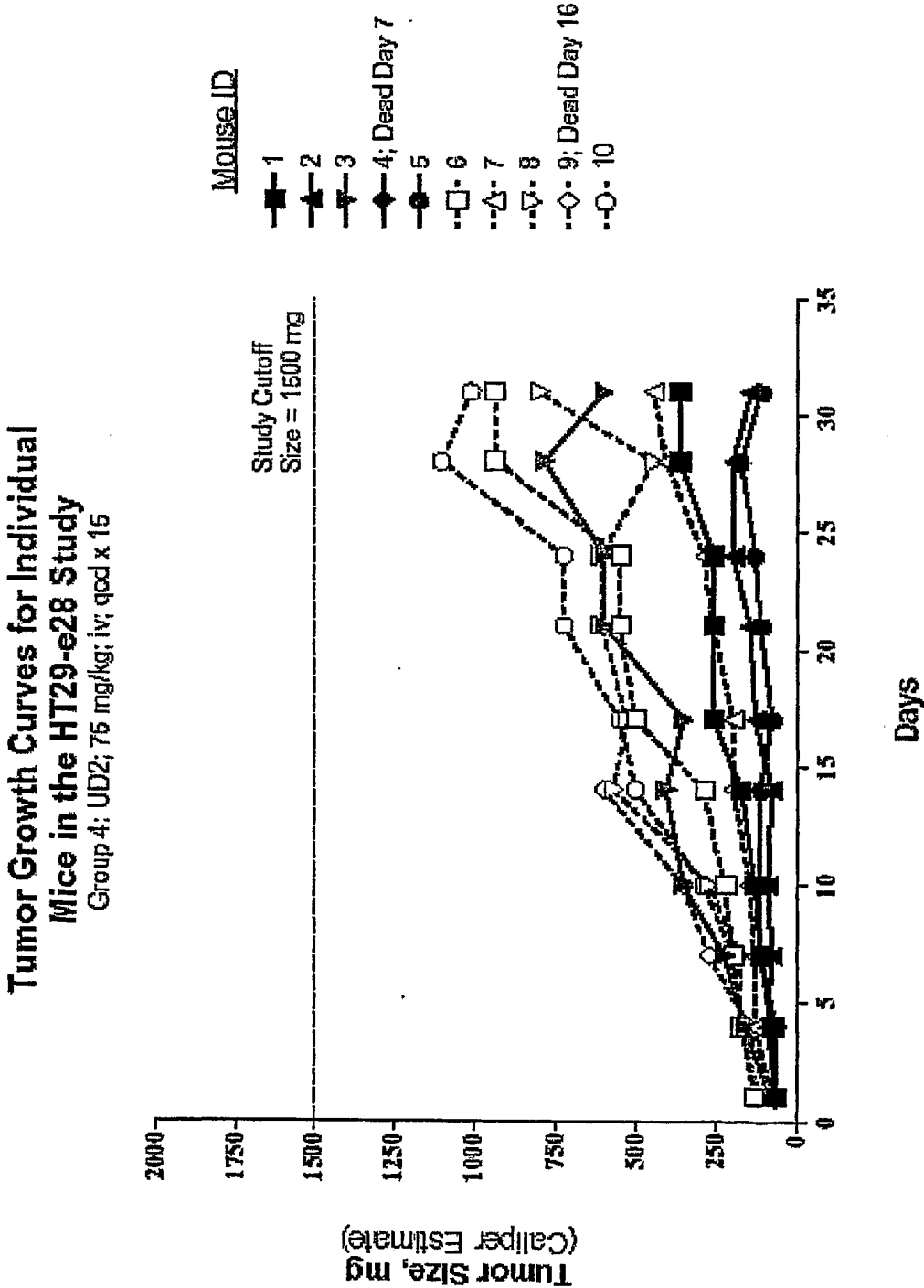


FIGURE 4D

Tumor Growth Curves for Individual Mice in the HT29-e28 Study
Group 5: UD2; 25 mg/kg; IV; qod x 15

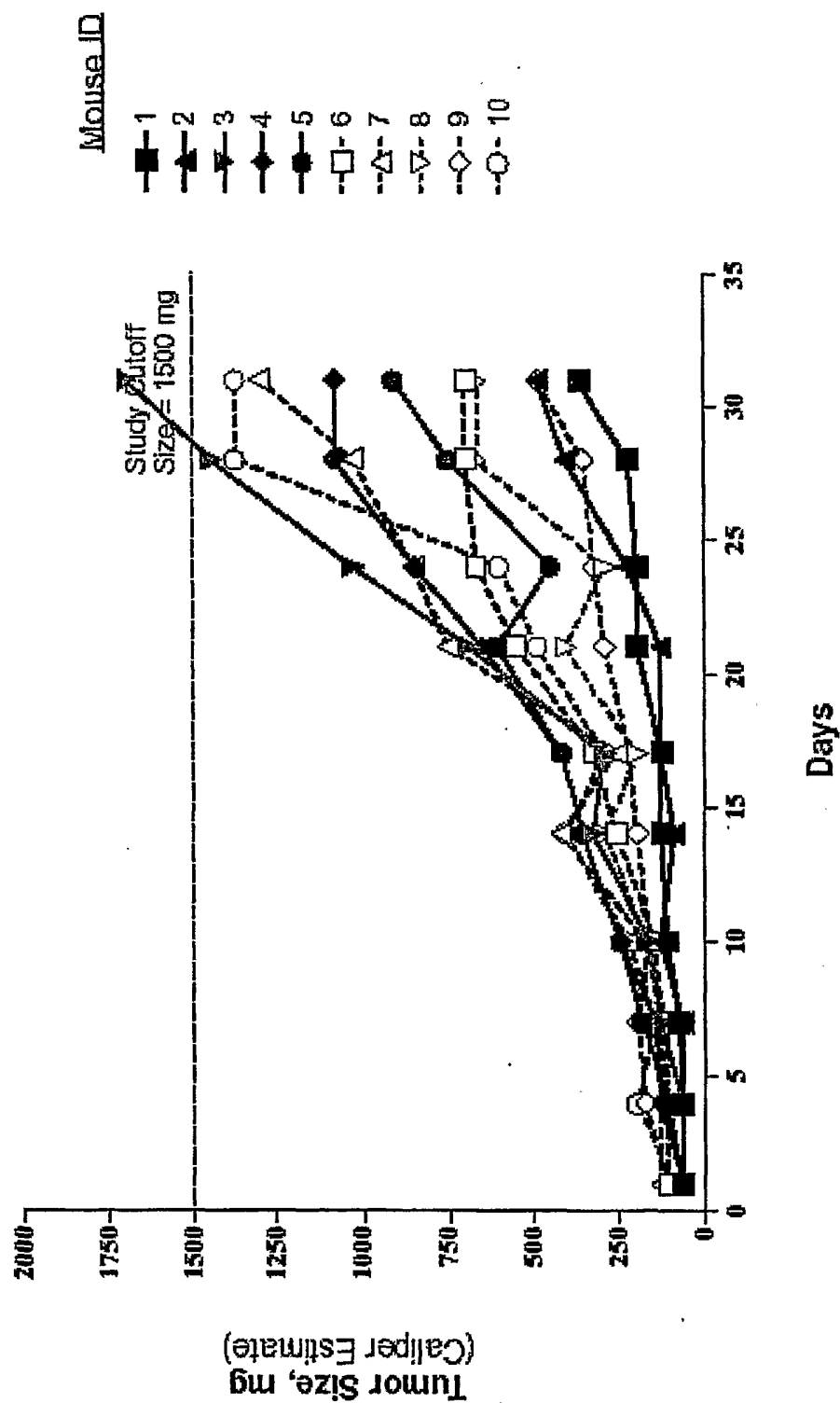


FIGURE 4E

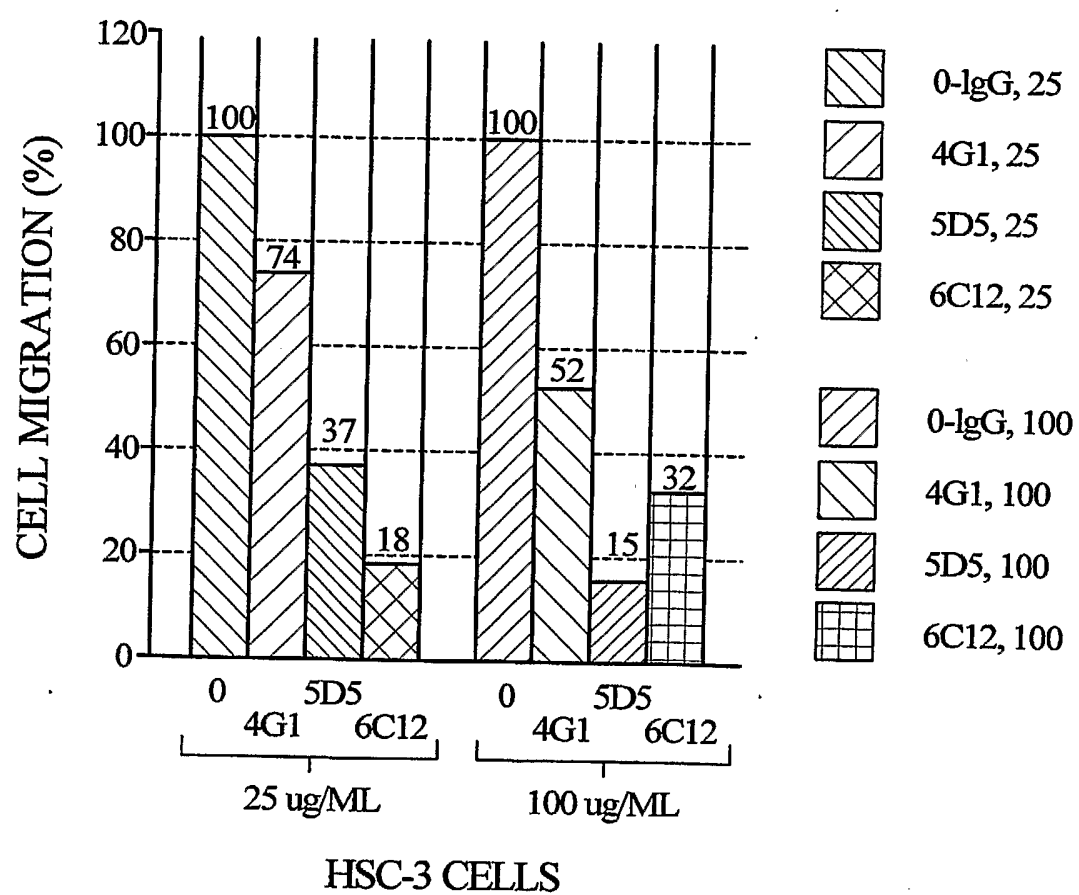
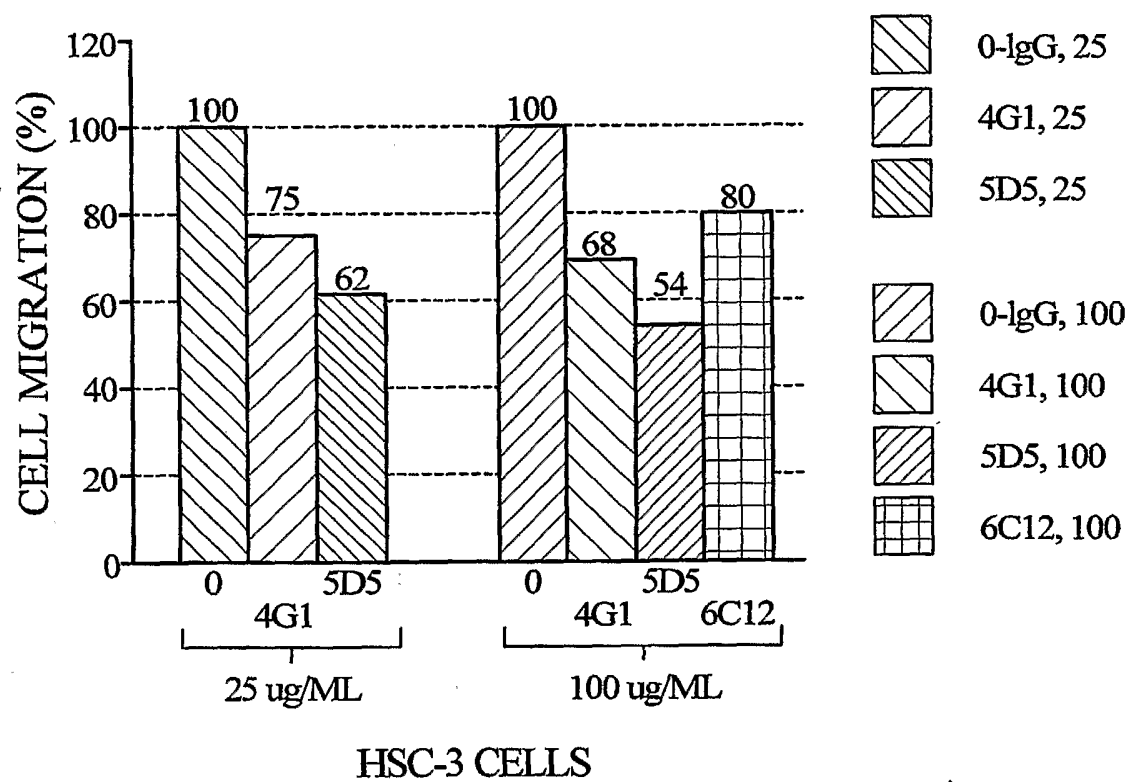
FIG. 5

FIG. 6

SEQUENCE LISTING

<110> Tryggvason, Karl
Salo, Sirpa

<120> Use of antibodies to the gamma 2 chain of laminin 5 to inhibit tumor growth and metastasis

<130> 02-1147-PCT2

<150> 60/523,895

<151> 2003-11-20

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Asn	Ser	Tyr	Gln	Ser	Arg	Leu	Asp	Asp	Leu	Lys	Met	Thr	Val	Glu	Arg	
		690				695					700					
gtt	cgg	gct	ctg	gga	agt	cag	tac	cag	aac	cga	gtt	cgg	gat	act	cac	2277
Val	Arg	Ala	Leu	Gly	Ser	Gln	Tyr	Gln	Asn	Arg	Val	Arg	Asp	Thr	His	
705					710					715					720	
agg	ctc	atc	act	cag	atg	cag	ctg	agc	ctg	gca	gaa	agt	gaa	gct	tcc	2325
Arg	Leu	Ile	Thr	Gln	Met	Gln	Leu	Ser	Leu	Ala	Glu	Ser	Glu	Ala	Ser	
				725					730					735		
ttg	gga	aac	act	aac	att	cct	gcc	tca	gac	cac	tac	gtg	ggg	cca	aat	2373
Leu	Gly	Asn	Thr	Asn	Ile	Pro	Ala	Ser	Asp	His	Tyr	Val	Gly	Pro	Asn	
			740					745					750			
ggc	ttt	aaa	agt	ctg	gct	cag	gag	gcc	aca	aga	tta	gca	gaa	agc	cac	2421
Gly	Phe	Lys	Ser	Leu	Ala	Gln	Glu	Ala	Thr	Arg	Leu	Ala	Glu	Ser	His	
		755					760					765				
gtt	gag	tca	gcc	agt	aac	atg	gag	caa	ctg	aca	agg	gaa	act	gag	gac	2469
Val	Glu	Ser	Ala	Ser	Asn	Met	Glu	Gln	Leu	Thr	Arg	Glu	Thr	Glu	Asp	
		770				775						780				

tat tcc aaa caa gcc ctc tca ctg gtg cgc aag gcc ctg cat gaa gga Tyr Ser Lys Gln Ala Leu Ser Leu Val Arg Lys Ala Leu His Glu Gly 785 790 795 800	2517
gtc gga agc gga agc ggt agc ccg gac ggt gct gtg gtg caa ggg ctt Val Gly Ser Gly Ser Gly Ser Pro Asp Gly Ala Val Val Gln Gly Leu 805 810 815	2565
gtg gaa aaa ttg gag aaa acc aag tcc ctg gcc cag cag ttg aca agg Val Glu Lys Leu Glu Lys Thr Lys Ser Leu Ala Gln Gln Leu Thr Arg 820 825 830	2613
gag gcc act caa gcg gaa att gaa gca gat agg tct tat cag cac agt Glu Ala Thr Gln Ala Glu Ile Glu Ala Asp Arg Ser Tyr Gln His Ser 835 840 845	2661
ctc cgc ctc ctg gat tca gtg tct ccg ctt cag gga gtc agt gat cag Leu Arg Leu Leu Asp Ser Val Ser Pro Leu Gln Gly Val Ser Asp Gln 850 855 860	2709
tcc ttt cag gtg gaa gaa gca aag agg atc aaa caa aaa gcg gat tca Ser Phe Gln Val Glu Glu Ala Lys Arg Ile Lys Gln Lys Ala Asp Ser 865 870 875 880	2757
ctc tca agc ctg gta acc agg cat atg gat gag ttc aag cgt aca caa Leu Ser Ser Leu Val Thr Arg His Met Asp Glu Phe Lys Arg Thr Gln 885 890 895	2805
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gga aaa agt ggg aga gag aaa tca gat cag ctg ctt tcc cgt gcc aat Gly Lys Ser Gly Arg Glu Lys Ser Asp Gln Leu Leu Ser Arg Ala Asn 915 920 925	2901
ctt gct aaa agc aga gca caa gaa gca ctg agt atg ggc aat gcc act Leu Ala Lys Ser Arg Ala Gln Glu Ala Leu Ser Met Gly Asn Ala Thr 930 935 940	2949
ttt tat gaa gtt gag agc atc ctt aaa aac ctc aga gag ttt gac ctg Phe Tyr Glu Val Glu Ser Ile Leu Lys Asn Leu Arg Glu Phe Asp Leu 945 950 955 960	2997
cag gtg gac aac aga aaa gca gaa gct gaa gaa gcc atg aag aga ctc Gln Val Asp Asn Arg Lys Ala Glu Ala Glu Glu Ala Met Lys Arg Leu 965 970 975	3045
tcc tac atc agc cag aag gtt tca gat gcc agt gac aag acc cag caa Ser Tyr Ile Ser Gln Lys Val Ser Asp Ala Ser Asp Lys Thr Gln Gln 980 985 990	3093
gca gaa aga gcc ctg ggg agc gct gct gct gat gca cag agg gca aag Ala Glu Arg Ala Leu Gly Ser Ala Ala Ala Asp Ala Gln Arg Ala Lys 995 1000 1005	3141
aat ggg gcc ggg gag gcc ctg gaa atc tcc agt gag att gaa cag Asn Gly Ala Gly Glu Ala Leu Glu Ile Ser Ser Glu Ile Glu Gln 1010 1015 1020	3186
gag att ggg agt ctg aac ttg gaa gcc aat gtg aca gca gat gga Glu Ile Gly Ser Leu Asn Leu Glu Ala Asn Val Thr Ala Asp Gly 1025 1030 1035	3231
gcc ttg gcc atg gaa aag gga ctg gcc tct ctg aag agt gag atg	3276

Ala Leu Ala Met Glu Lys Gly Leu Ala Ser Leu Lys Ser Glu Met
 1040 1045 1050

agg gaa gtg gaa gga gag ctg gaa agg aag gag ctg gag ttt gac 3321
 Arg Glu Val Glu Gly Glu Leu Glu Arg Lys Glu Leu Glu Phe Asp
 1055 1060 1065

acg aat atg gat gca gta cag atg gtg att aca gaa gcc cag aag 3366
 Thr Asn Met Asp Ala Val Gln Met Val Ile Thr Glu Ala Gln Lys
 1070 1075 1080

gtt gat acc aga gcc aag aac gct ggg gtt aca atc caa gac aca 3411
 Val Asp Thr Arg Ala Lys Asn Ala Gly Val Thr Ile Gln Asp Thr
 1085 1090 1095

ctc aac aca tta gac ggc ctc ctg cat ctg atg ggt atg tga 3453
 Leu Asn Thr Leu Asp Gly Leu Leu His Leu Met Gly Met
 1100 1105 1110

accacaacc cacaacottc cagctccatg ctccagggct ttgctccaga acactcacta 3513

tacctagccc cagcaaaggg gagtctcagc tttccttaag gatatcagta aatgtgcttt 3573

gtttccaggc ccagataact ttcggcagggt tcccttacat ttactggacc ctgttttacc 3633

gttgctaaga tgggtcactg aacacctatt gcacttgggg gtaaagggtct gtggggccaaa 3693

gaacagggtgt atataagcaa cttcacagaa cacgagacag cttgggaatc ctgctaaaga 3753

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gagagaaggc aggttgagac tacttaagat attgttgaaa taattgaaga gagaaatgac 3933

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gtctgtgaag tcaagaatca cttgccggcc ggggtgtggtg gctcacgcct gtaattctag 4053

cactttggga gactgaagcg ggtggatcac ccgaggtcag gagttgaaga ccagcctggc 4113

caacatggtg aaacctgtc totactaaaa gtacaaaaat tagctggatg atgggtggtgg 4173

gcgcctgtaa ttccagctac tcaggagtct gaggcaggag aatcgcttga acccaggagg 4233

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cctaaaaaaaa aaaaaaaaaa aaa 4316

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<211> 1111

<212> PRT

<213> Homo sapiens

<400> 4

Met Pro Ala Leu Trp Leu Gly Cys Cys Leu Cys Phe Ser Leu Leu Leu
 1 5 10 15

Pro Ala Ala Arg Ala Thr Ser Arg Arg Glu Val Cys Asp Cys Asn Gly
 20 25 30

Lys Ser Arg Gln Cys Ile Phe Asp Arg Glu Leu His Arg Gln Thr Gly

35

40

45

Asn Gly Phe Arg Cys Leu Asn Cys Asn Asp Asn Thr Asp Gly Ile His
 50 55 60

Cys Glu Lys Cys Lys Asn Gly Phe Tyr Arg His Arg Glu Arg Asp Arg
 65 70 75 80

Cys Leu Pro Cys Asn Cys Asn Ser Lys Gly Ser Leu Ser Ala Arg Cys
 85 90 95

Asp Asn Ser Gly Arg Cys Ser Cys Lys Pro Gly Val Thr Gly Ala Arg
 100 105 110

Cys Asp Arg Cys Leu Pro Gly Phe His Met Leu Thr Asp Ala Gly Cys
 115 120 125

Thr Gln Asp Gln Arg Leu Leu Asp Ser Lys Cys Asp Cys Asp Pro Ala
 130 135 140

Gly Ile Ala Gly Pro Cys Asp Ala Gly Arg Cys Val Cys Lys Pro Ala
 145 150 155 160

Val Thr Gly Glu Arg Cys Asp Arg Cys Arg Ser Gly Tyr Tyr Asn Leu
 165 170 175

Asp Gly Gly Asn Pro Glu Gly Cys Thr Gln Cys Phe Cys Tyr Gly His
 180 185 190

Ser Ala Ser Cys Arg Ser Ser Ala Glu Tyr Ser Val His Lys Ile Thr
 195 200 205

Ser Thr Phe His Gln Asp Val Asp Gly Trp Lys Ala Val Gln Arg Asn
 210 215 220

Gly Ser Pro Ala Lys Leu Gln Trp Ser Gln Arg His Gln Asp Val Phe
 225 230 235 240

Ser Ser Ala Gln Arg Leu Asp Pro Val Tyr Phe Val Ala Pro Ala Lys
 245 250 255

Phe Leu Gly Asn Gln Gln Val Ser Tyr Gly Gln Ser Leu Ser Phe Asp
 260 265 270

Tyr Arg Val Asp Arg Gly Gly Arg His Pro Ser Ala His Asp Val Ile
 275 280 285

Leu Glu Gly Ala Gly Leu Arg Ile Thr Ala Pro Leu Met Pro Leu Gly
 290 295 300

Lys Thr Leu Pro Cys Gly Leu Thr Lys Thr Tyr Thr Phe Arg Leu Asn
 305 310 315 320
 Glu His Pro Ser Asn Asn Trp Ser Pro Gln Leu Ser Tyr Phe Glu Tyr
 325 330 335
 Arg Arg Leu Leu Arg Asn Leu Thr Ala Leu Arg Ile Arg Ala Thr Tyr
 340 345 350
 Gly Glu Tyr Ser Thr Gly Tyr Ile Asp Asn Val Thr Leu Ile Ser Ala
 355 360 365
 Arg Pro Val Ser Gly Ala Pro Ala Pro Trp Val Glu Gln Cys Ile Cys
 370 375 380
 Pro Val Gly Tyr Lys Gly Gln Phe Cys Gln Asp Cys Ala Ser Gly Tyr
 385 390 395 400
 Lys Arg Asp Ser Ala Arg Leu Gly Pro Phe Gly Thr Cys Ile Pro Cys
 405 410 415
 Asn Cys Gln Gly Gly Gly Ala Cys Asp Pro Asp Thr Gly Asp Cys Tyr
 420 425 430
 Ser Gly Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly
 435 440 445
 Phe Tyr Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys
 450 455 460
 His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu Glu Val Val
 465 470 475 480
 Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys Glu Leu Cys
 485 490 495
 Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro Val Arg
 500 505 510
 Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser Ala Ser
 515 520 525
 Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile His Asn
 530 535 540
 Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe Gly Asp
 545 550 555 560
 Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys Asn Cys Asn

565

570

575

Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly Thr Cys Val
580 585 590

Cys Lys Pro Gly Phe Gly Gly Pro Asn Cys Glu His Gly Ala Phe Ser
595 600 605

Cys Pro Ala Cys Tyr Asn Gln Val Lys Ile Gln Met Asp Gln Phe Met
610 615 620

Gln Gln Leu Gln Arg Met Glu Ala Leu Ile Ser Lys Ala Gln Gly Gly
625 630 635 640

Asp Gly Val Val Pro Asp Thr Glu Leu Glu Gly Arg Met Gln Gln Ala
645 650 655

Glu Gln Ala Leu Gln Asp Ile Leu Arg Asp Ala Gln Ile Ser Glu Gly
660 665 670

Ala Ser Arg Ser Leu Gly Leu Gln Leu Ala Lys Val Arg Ser Gln Glu
675 680 685

Asn Ser Tyr Gln Ser Arg Leu Asp Asp Leu Lys Met Thr Val Glu Arg
690 695 700

Val Arg Ala Leu Gly Ser Gln Tyr Gln Asn Arg Val Arg Asp Thr His
705 710 715 720

Arg Leu Ile Thr Gln Met Gln Leu Ser Leu Ala Glu Ser Glu Ala Ser
725 730 735

Leu Gly Asn Thr Asn Ile Pro Ala Ser Asp His Tyr Val Gly Pro Asn
740 745 750

Gly Phe Lys Ser Leu Ala Gln Glu Ala Thr Arg Leu Ala Glu Ser His
755 760 765

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

Tyr Ser Lys Gln Ala Leu Ser Leu Val Arg Lys Ala Leu His Glu Gly
785 790 795 800

Val Gly Ser Gly Ser Gly Ser Pro Asp Gly Ala Val Val Gln Gly Leu
805 810 815

Val Glu Lys Leu Glu Lys Thr Lys Ser Leu Ala Gln Gln Leu Thr Arg
820 825 830

Glu Ala Thr Gln Ala Glu Ile Glu Ala Asp Arg Ser Tyr Gln His Ser
 835 840 845

Leu Arg Leu Leu Asp Ser Val Ser Pro Leu Gln Gly Val Ser Asp Gln
 850 855 860

Ser Phe Gln Val Glu Glu Ala Lys Arg Ile Lys Gln Lys Ala Asp Ser
 865 870 875 880

Leu Ser Ser Leu Val Thr Arg His Met Asp Glu Phe Lys Arg Thr Gln
 885 890 895

Lys Asn Leu Gly Asn Trp Lys Glu Glu Ala Gln Gln Leu Leu Gln Asn
 900 905 910

Gly Lys Ser Gly Arg Glu Lys Ser Asp Gln Leu Leu Ser Arg Ala Asn
 915 920 925

Leu Ala Lys Ser Arg Ala Gln Glu Ala Leu Ser Met Gly Asn Ala Thr
 930 935 940

Phe Tyr Glu Val Glu Ser Ile Leu Lys Asn Leu Arg Glu Phe Asp Leu
 945 950 955 960

Gln Val Asp Asn Arg Lys Ala Glu Ala Glu Glu Ala Met Lys Arg Leu
 965 970 975

Ser Tyr Ile Ser Gln Lys Val Ser Asp Ala Ser Asp Lys Thr Gln Gln
 980 985 990

Ala Glu Arg Ala Leu Gly Ser Ala Ala Ala Asp Ala Gln Arg Ala Lys
 995 1000 1005

Asn Gly Ala Gly Glu Ala Leu Glu Ile Ser Ser Glu Ile Glu Gln
 1010 1015 1020

Glu Ile Gly Ser Leu Asn Leu Glu Ala Asn Val Thr Ala Asp Gly
 1025 1030 1035

Ala Leu Ala Met Glu Lys Gly Leu Ala Ser Leu Lys Ser Glu Met
 1040 1045 1050

Arg Glu Val Glu Gly Glu Leu Glu Arg Lys Glu Leu Glu Phe Asp
 1055 1060 1065

Thr Asn Met Asp Ala Val Gln Met Val Ile Thr Glu Ala Gln Lys
 1070 1075 1080

Val Asp Thr Arg Ala Lys Asn Ala Gly Val Thr Ile Gln Asp Thr

1085

1090

1095

Leu Asn Thr Leu Asp Gly Leu Leu His Leu Met Gly Met
 1100 1105 1110

<210> 5
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 <212> PRT
 <213> Homo sapiens

<400> 5

Cys Ile Cys Pro Val Gly Tyr Lys Gly Gln Phe Cys Gln Asp Cys Ala
 1 5 10 15

Ser Gly Tyr Lys Arg Asp Ser Ala Arg Leu Gly Pro Phe Gly Thr Cys
 20 25 30

Ile Pro Cys Asn Cys Gln Gly Gly Gly Ala Cys Asp Pro Asp Thr Gly
 35 40 45

Asp Cys Tyr Ser Gly Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys
 50 55 60

Pro Ile Gly Phe Tyr Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro
 65 70 75 80

Cys Pro Cys His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu
 85 90 95

Glu Val Val Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys
 100 105 110

Glu Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly
 115 120 125

Pro Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro
 130 135 140

Ser Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys
 145 150 155 160

Ile His Asn Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr
 165 170 175

Phe Gly Asp Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys
 180 185 190

Asn Cys Asn Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly
 195 200 205

Thr Cys Val Cys Lys Pro Gly Phe Gly Gly Pro Asn Cys Glu His Gly
 210 215 220

Ala Phe Ser
 225

<210> 6
 <211> 174
 <212> PRT
 <213> Homo sapiens

<400> 6

Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly Phe Tyr
 1 5 10 15

Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys His Asn
 20 25 30

Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu Glu Val Val Cys Asn
 35 40 45

Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys Glu Leu Cys Ala Asp
 50 55 60

Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro Val Arg Pro Cys
 65 70 75 80

Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser Ala Ser Gly Asn
 85 90 95

Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile His Asn Thr Ala
 100 105 110

Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe Gly Asp Pro Leu
 115 120 125

Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys Asn Cys Asn Pro Met
 130 135 140

Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly Thr Cys Val Cys Lys
 145 150 155 160

Pro Gly Phe Gly Gly Pro Asn Cys Glu His Gly Ala Phe Ser
 165 170

<210> 7
 <211> 168
 <212> PRT
 <213> Homo sapiens

<400> 7

Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly Phe Tyr
 1 5 10 15
 Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys His Asn
 20 25 30
 Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu Glu Val Val Cys Asn
 35 40 45
 Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys Glu Leu Cys Ala Asp
 50 55 60
 Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro Val Arg Pro Cys
 65 70 75 80
 Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser Ala Ser Gly Asn
 85 90 95
 Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile His Asn Thr Ala
 100 105 110
 Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe Gly Asp Pro Leu
 115 120 125
 Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys Asn Cys Asn Pro Met
 130 135 140
 Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly Thr Cys Val Cys Lys
 145 150 155 160
 Pro Gly Phe Gly Gly Pro Asn Cys
 165

<210> 8
 <211> 156
 <212> PRT
 <213> Homo sapiens

<400> 8

Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly Phe Tyr
 1 5 10 15
 Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys His Asn
 20 25 30
 Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu Glu Val Val Cys Asn
 35 40 45
 Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys Glu Leu Cys Ala Asp
 50 55 60

Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro Val Arg Pro Cys
65 70 75 80

Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser Ala Ser Gly Asn
85 90 95

Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile His Asn Thr Ala
100 105 110

Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe Gly Asp Pro Leu
115 120 125

Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys Asn Cys Asn Pro Met
130 135 140

Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly Thr
145 150 155

<210> 9
<211> 138
<212> PRT
<213> Homo sapiens

<400> 9

Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly Phe Tyr
1 5 10 15

Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys His Asn
20 25 30

Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu Glu Val Val Cys Asn
35 40 45

Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys Glu Leu Cys Ala Asp
50 55 60

Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro Val Arg Pro Cys
65 70 75 80

Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser Ala Ser Gly Asn
85 90 95

Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile His Asn Thr Ala
100 105 110

Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe Gly Asp Pro Leu
115 120 125

Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala
130 135

<210> 10
 <211> 133
 <212> PRT
 <213> Homo sapiens

<400> 10

Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly Phe Tyr
 1 5 10 15

Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys His Asn
 20 25 30

Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu Glu Val Val Cys Asn
 35 40 45

Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys Glu Leu Cys Ala Asp
 50 55 60

Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro Val Arg Pro Cys
 65 70 75 80

Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser Ala Ser Gly Asn
 85 90 95

Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile His Asn Thr Ala
 100 105 110

Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe Gly Asp Pro Leu
 115 120 125

Ala Pro Asn Pro Ala
 130

<210> 11
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 <212> PRT
 <213> Homo sapiens

<400> 11

Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly Phe Tyr
 1 5 10 15

Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys His Asn
 20 25 30

Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu Glu Val Val Cys Asn
 35 40 45

Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys Glu Leu Cys Ala Asp
 50 55 60

Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro Val Arg Pro Cys
65 70 75 80

Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser Ala Ser Gly Asn
85 90 95

Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile His Asn Thr Ala
100 105 110

Gly Ile Tyr Cys
115

<210> 12
<211> 100
<212> PRT
<213> Homo sapiens

<400> 12

Asp Glu Asn Pro Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly Phe Tyr
1 5 10 15

Asn Asp Pro His Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys His Asn
20 25 30

Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu Glu Val Val Cys Asn
35 40 45

Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys Glu Leu Cys Ala Asp
50 55 60

Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro Val Arg Pro Cys
65 70 75 80

Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser Ala Ser Gly Asn
85 90 95

Cys Asp Arg Leu
100

<210> 13
<211> 141
<212> PRT
<213> Homo sapiens

<400> 13

Cys Pro Cys His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu
1 5 10 15

Glu Val Val Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys
20 25 30

Glu Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly
 35 40 45

Pro Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro
 50 55 60

Ser Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys
 65 70 75 80

Ile His Asn Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr
 85 90 95

Phe Gly Asp Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys
 100 105 110

Asn Cys Asn Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly
 115 120 125

Thr Cys Val Cys Lys Pro Gly Phe Gly Gly Pro Asn Cys
 130 135 140

<210> 14
 <211> 141
 <212> PRT
 <213> Homo sapiens

<400> 14

Cys Pro Cys His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu
 1 5 10 15

Glu Val Val Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys
 20 25 30

Glu Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly
 35 40 45

Pro Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro
 50 55 60

Ser Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys
 65 70 75 80

Ile His Asn Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr
 85 90 95

Phe Gly Asp Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys
 100 105 110

Asn Cys Asn Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly

115

120

125

Thr Cys Val Cys Lys Pro Gly Phe Gly Gly Pro Asn Cys
 130 135 140

<210> 15
 <211> 129
 <212> PRT
 <213> Homo sapiens

<400> 15

Cys Pro Cys His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu
 1 5 10 15

Glu Val Val Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys
 20 25 30

Glu Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly
 35 40 45

Pro Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro
 50 55 60

Ser Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys
 65 70 75 80

Ile His Asn Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr
 85 90 95

Phe Gly Asp Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys
 100 105 110

Asn Cys Asn Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly
 115 120 125

Thr

<210> 16
 <211> 111
 <212> PRT
 <213> Homo sapiens

<400> 16

Cys Pro Cys His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu
 1 5 10 15

Glu Val Val Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys
 20 25 30

Glu Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly

35

40

45

Pro Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro
 50 55 60

Ser Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys
 65 70 75 80

Ile His Asn Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr
 85 90 95

Phe Gly Asp Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala
 100 105 110

<210> 17
 <211> 106
 <212> PRT
 <213> Homo sapiens

<400> 17

Cys Pro Cys His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu
 1 5 10 15

Glu Val Val Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys
 20 25 30

Glu Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly
 35 40 45

Pro Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro
 50 55 60

Ser Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys
 65 70 75 80

Ile His Asn Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr
 85 90 95

Phe Gly Asp Pro Leu Ala Pro Asn Pro Ala
 100 105

<210> 18
 <211> 89
 <212> PRT
 <213> Homo sapiens

<400> 18

Cys Pro Cys His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu
 1 5 10 15

Glu Val Val Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys

20

25

30

Glu Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly
 35 40 45

Pro Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro
 50 55 60

Ser Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys
 65 70 75 80

Ile His Asn Thr Ala Gly Ile Tyr Cys
 85

<210> 19
 <211> 73
 <212> PRT
 <213> Homo sapiens

<400> 19

Cys Pro Cys His Asn Gly Phe Ser Cys Ser Val Ile Pro Glu Thr Glu
 1 5 10 15

Glu Val Val Cys Asn Asn Cys Pro Pro Gly Val Thr Gly Ala Arg Cys
 20 25 30

Glu Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly
 35 40 45

Pro Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro
 50 55 60

Ser Ala Ser Gly Asn Cys Asp Arg Leu
 65 70

<210> 20
 <211> 114
 <212> PRT
 <213> Homo sapiens

<400> 20

Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro
 1 5 10 15

Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser
 20 25 30

Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile
 35 40 45

His Asn Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe

50

55

60

Gly Asp Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys Asn
 65 70 75 80

Cys Asn Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly Thr
 85 90 95

Cys Val Cys Lys Pro Gly Phe Gly Gly Pro Asn Cys Glu His Gly Ala
 100 105 110

Phe Ser

<210> 21
 <211> 108
 <212> PRT
 <213> Homo sapiens

<400> 21

Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro
 1 5 10 15

Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser
 20 25 30

Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile
 35 40 45

His Asn Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe
 50 55 60

Gly Asp Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys Asn
 65 70 75 80

Cys Asn Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly Thr
 85 90 95

Cys Val Cys Lys Pro Gly Phe Gly Gly Pro Asn Cys
 100 105

<210> 22
 <211> 96
 <212> PRT
 <213> Homo sapiens

<400> 22

Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro
 1 5 10 15

Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser

20

25

30

Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile
 35 40 45

His Asn Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe
 50 55 60

Gly Asp Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala Cys Asn
 65 70 75 80

Cys Asn Pro Met Gly Ser Glu Pro Val Gly Cys Arg Ser Asp Gly Thr
 85 90 95

<210> 23

<211> 78

<212> PRT

<213> Homo sapiens

<400> 23

Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro
 1 5 10 15

Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser
 20 25 30

Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile
 35 40 45

His Asn Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe
 50 55 60

Gly Asp Pro Leu Ala Pro Asn Pro Ala Asp Lys Cys Arg Ala
 65 70 75

<210> 24

<211> 73

<212> PRT

<213> Homo sapiens

<400> 24

Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro
 1 5 10 15

Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser
 20 25 30

Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile
 35 40 45

His Asn Thr Ala Gly Ile Tyr Cys Asp Gln Cys Lys Ala Gly Tyr Phe

50

55

60

Gly Asp Pro Leu Ala Pro Asn Pro Ala
65 70

<210> 25
<211> 56
<212> PRT
<213> Homo sapiens

<400> 25

Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro
1 5 10 15

Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser
20 25 30

Ala Ser Gly Asn Cys Asp Arg Leu Thr Gly Arg Cys Leu Lys Cys Ile
35 40 45

His Asn Thr Ala Gly Ile Tyr Cys
50 55

<210> 26
<211> 40
<212> PRT
<213> Homo sapiens

<400> 26

Leu Cys Ala Asp Gly Tyr Phe Gly Asp Pro Phe Gly Glu His Gly Pro
1 5 10 15

Val Arg Pro Cys Gln Pro Cys Gln Cys Asn Ser Asn Val Asp Pro Ser
20 25 30

Ala Ser Gly Asn Cys Asp Arg Leu
35 40

<210> 27
<211> 177
<212> PRT
<213> Homo sapiens

<400> 27

Gln Phe Cys Gln Asp Cys Ala Ser Gly Tyr Lys Arg Asp Ser Ala Arg
1 5 10 15

Leu Gly Pro Phe Gly Thr Cys Ile Pro Cys Asn Cys Gln Gly Gly Gly
20 25 30

Ala Cys Asp Pro Asp Thr Gly Asp Cys Tyr Ser Gly Asp Glu Asn Pro
35 40 45

Asp Ile Glu Cys Ala Asp Cys Pro Ile Gly Phe Tyr Asn Asp Pro His
50 55 60

Asp Pro Arg Ser Cys Lys Pro Cys Pro Cys His Asn Gly Phe Ser Cys
65 70 75 80

Ser Val Ile Pro Glu Thr Glu Glu Val Val Cys Asn Asn Cys Pro Pro
85 90 95

Gly Val Thr Gly Ala Arg Cys Glu Leu Cys Ala Asp Gly Tyr Phe Gly
100 105 110

Asp Pro Phe Gly Glu His Gly Pro Val Arg Pro Cys Gln Pro Cys Gln
115 120 125

Cys Asn Ser Asn Val Asp Pro Ser Ala Ser Gly Asn Cys Asp Arg Leu
130 135 140

Thr Gly Arg Cys Leu Lys Cys Ile His Asn Thr Ala Gly Ile Tyr Cys
145 150 155 160

Asp Gln Cys Lys Ala Gly Tyr Phe Gly Asp Pro Leu Ala Pro Asn Pro
165 170 175

Ala