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(54) Title: DETECTION OF URINARY MESOTHELIN-/MEGAKARYOCYTE POTENTIATING FACTOR-RELATED PEPTIDES FOR ASSESSMENT OF OVARIAN CANCER

(57) Abstract: The invention relates to methods and kits for assessing occurrence in woman urine of peptides having amino acid sequences related to those of mesothelin, megakaryocyte potentiating factor, and other peptides that have been associated with occurrence in the serum of ovarian cancer patients. The methods and kits can be used to diagnose ovarian cancer in a woman, to predict development of ovarian cancer in an otherwise asymptomatic woman, or to assess the efficacy of an ovarian cancer therapeutic method.

[0001]　　　Detection of Urinary Mesothelin-/Megakaryocyte Potentiating Factor-Related Peptides for Assessment of Ovarian Cancer

BACKGROUND OF THE INVENTION

[0002]　　　The invention relates generally to diagnosing ovarian cancer in women.

[0003]　　　Ovarian cancer is one of the foremost causes of cancer deaths among women in the U.S. One reason for the relatively high mortality rate among ovarian cancer patients is that the disease is often not diagnosed until it reaches an advanced phase.

[0004]　　　An ovarian tumor can be classified by the cell type from which the tumor arose, by the morphology of cells of the tumor, and by the stage to which the tumor has progressed.

[0005]　　　Ovarian tumors can arise from any of three ovarian cell types - epithelial, germ cells, and stromal cells. Epithelial ovarian tumors (EOTs) are the most common type of ovarian tumor, accounting for 85 percent or more of all ovarian tumors. EOTs arise from the epithelial cells that cover the outer surface of the ovary. Some EOTs are benign and cause relatively little medical harm. However, malignant EOTs can grow uncontrollably, invade nearby tissues, or metastasize to distant body locations. Germ cell ovarian tumors arise from ova-producing cells and can also be either benign or malignant. Stromal ovarian tumors arise from ovary connective tissue cells and are relatively rare.

[0006]　　　EOTs are further characterized by the type of cells that make up the EOT. Recognized cell types include serous, mucinous, endometrioid, and clear cell types. Some EOTs are not clearly differentiated among these cell types, and these non-differentiated EOTs tend to grow and spread more quickly than more clearly differentiated types of EOTs.

[0007]　　　EOTs are also classified by grade and stage. The grade of a tumor describes its macroscopic appearance. Grade 1 tumors tend to closely resemble normal ovarian tissue, and also tend to have a better prognosis. Grade 3 tumors tend to have an abnormal appearance, and usually have a worse prognosis. Grade 2 tumors are intermediate between Grades 1 and 3. The stage of a tumor describes the degree or extent to which the tumor appears to have spread from its origin. Stage I ovarian tumors are limited to one or both ovaries. Gradations within Stage I describe tumors limited to the interior of one (Stage IA)

or both ovaries (Stage IB) and tumors (Stage IC) which appear on the surface of one or both ovaries, in one or more ovaries having a ruptured capsule, or in association with tumor cells present in ascites or detected in a peritoneal wash. Stage II ovarian tumors have extended within the pelvis beyond the ovaries. Gradations within Stage II describe tumors that have extended to one or both of the uterus and the fallopian tubes (Stage IIA), to other pelvic organs (Stage IIB), or which are associated with tumor cells present in ascites or detected in a peritoneal wash. Stage III ovarian tumors have spread beyond the pelvis, to the lymph nodes, or both. Stage IV ovarian tumors have spread to organs beyond the peritoneal cavity.

[0008]   Early diagnosis of an ovarian tumor is important for effective treatment of the tumor and for extending patient survival. Early diagnosis of ovarian cancer is complicated by the fact that there are few macroscopic symptoms until the disease has progressed to an advanced phase. Existing diagnostic tests for ovarian tumors are not universally successful for detecting ovarian cancer at a sufficiently early phase that efficacious treatment can be administered. The reasons that existing tests do not detect ovarian cancer sufficiently early are not completely known. For example, some tests may rely on markers that are released from tumor cells at levels that are too low to be reliably detected until the tumor mass has grown relatively large.

[0009]   Markers that have been used by others to diagnose ovarian cancer include CA 125 (Halila et al., 1987, Br. J. Cancer 56(2):153-156), carcinoembryonic antigen (CEA; Juweid et al., 1997, Gynecol. Oncol. 67(3):259-271), vascular endothelial growth factor (VEGF; Candido Dos Reis et al., 2002, Gynecol. Obstet. Invest. 54(3):132-136), urinary gonadotropin peptide (UGP; Schutter et al., 1999, Anticancer Res. 19(6C):5551-5557), neopterin (Szarka et al., 1988, Acta Chir. Hung. 29(4):359-364), CD44 splice variants (Uhl-Steidl et al., 1995, Oncology 52(5):400-406), urine cysteine proteinase (UCP; Gao et al., 1996, Hua Xi Yi Ke Da Xue Xue Bao 27(3):291-294), anti-malignin antibody (Bogoch et al., 1991, Lancet 337:927), and tumor-associated trypsin inhibitor (TATI; Medl et al., 1995, Br. J. Cancer 71(5):1051-1054; Halila et al., 1988, Br. J. Cancer 57(3):304-307). Kudoh et al., (1999, Gynecol. Obstet. Invest. 47(1);52-57) compared the usefulness of several other serum markers for EOTs, including lactate dehydrogenase (LDH), alpha-hydroxybutyrate dehydrogenase (aHBDH), CA19-9, tissue polypeptide antigen (TPA), and sialyl TN (STN). Every markers listed in this paragraph is not present in abnormal amounts in every ovarian cancer patient.

[0010]   Mesothelin is a protein which is expressed on the surface of at least mesothelial cells, mesotheliomas, some squamous cell carcinomas, and ovarian cancers (Chang et al., 1996, Proc. Natl. Acad. Sci. USA 93:136-140). Mesothelin has been described in the literature (e.g., U.S. Patent No. 6,083,502). Antibodies that bind with mesothelin have also been described. For example, a monoclonal antibody designated K1 is described in U.S. Patent No. 5,320,956, and is secreted by the hybridoma that was deposited by others with the American Type Culture Collection (ATCC; 10801 University Boulevard; Manassas, VA 20110-2209; USA) as accession number HB 10570. Antibody K1 was found to bind with an approximately 40 kilodalton protein found on the surface of ovarian cancer and other cells (Chang et al., 1996, Proc. Natl. Acad. Sci. USA 93(1):136-140). That protein, designated mesothelin, is derived from a larger (approximately 69 kilodalton) precursor protein for which the sequence is shown in Fig. 1. Scholler et al. (1999, Proc. Natl. Acad. Sci. USA 96(20):11531-11536) recognized that an antigen having an amino acid sequence highly similar to the amino terminal sequences of mesothelin and of megakaryocyte potentiating factor (MPF) occurs in the serum of many patients afflicted with ovarian carcinoma. Scholler proposed that this antigen could be a useful serum marker for diagnosing ovarian carcinoma.

[0011]   A useful diagnostic test should not only be able to detect occurrence of ovarian cancer in a patient, it is also preferably inexpensive, non-invasive, and easy to use. Diagnostic tests that use blood or serum as a testing substrate require that blood be drawn from a patient to be screened, and sometimes require further separation, purification, or other treatment of the withdrawn blood sample. Drawing blood must be performed by a trained health care worker, is painful and invasive for the patient, requires specialized equipment and reagents for storage and transportation of blood samples, and carries the risk of transmission of blood-borne pathogens. By contrast, urine samples can be collected with little or no specialized equipment, do not require substantial oversight by a healthcare worker, are produced as a part of a patient's normal physiological processes, and generally are not significant sources of transmissible diseases. For these reasons, diagnostic testing using urine samples as a testing substrate can be preferable to comparable testing done with blood samples.

[0012]   The present invention provides an ovarian cancer diagnostic test that can be performed using urine samples and that can be used to detect ovarian cancer at an early phase of disease progression.

5   BRIEF SUMMARY OF THE INVENTION

[0013]   The invention relates to a method of diagnosing an ovarian cancer (i.e., one or more of an epithelial, stromal, and germ cell ovarian cancer) in a woman. The method comprises assessing occurrence of a mesothelin/megakaryocyte potentiating factor family (MMPFF) peptide in urine obtained from the woman. Occurrence of the MMPFF peptide in

10   the urine is an indication that the woman is afflicted with an ovarian cancer.

[0014]   In one embodiment, the MMPFF peptide is assessed in the woman's urine by contacting the urine (or centrifuged urine) with a first antibody that binds specifically with the MMPFF peptide. By assessing whether the MMPFF peptide has bound with the first antibody, occurrence of the MMPFF peptide in the woman's urine can be assessed. For

15   example, the first antibody can be bound to a substrate, such as a plastic multi-well plate of the type adapted for automated analysis in a robotic apparatus. Binding of the MMPFF peptide and the first antibody can, for example, be assessed by contacting the first antibody with a second antibody that binds specifically with the MMPFF peptide and assessing co-localization of the first and second antibodies. The second antibody can be detectably

20   labeled, such as with a compound selected from the group consisting of an enzyme, a radionuclide, a fluorophore, and a chromophore.

[0015]   By way of example, the method can comprise contacting a plate having an anti-MMPFF peptide 'capture' antibody bound thereto with urine obtained from a woman. The urine can, optionally, be incubated with the plate for a period such as 10 minutes or an hour.

25   The plate can then be contacted with a biotinylated second antibody that binds with an epitope of the MMPFF peptide than does the capture antibody. A streptavidin-linked enzyme can be bound to the biotinylated antibody to enable its detection in the presence of a chromogenic substrate (e.g., 3,3',5,5'-tetramethylbenzidine, which is a chromogenic substrate of horseradish peroxidase). Of course, the second antibody could instead be

30   fluorescently labeled, radiolabeled, or otherwise detectably labeled.

[0016]   In another embodiment of the method of diagnosing ovarian cancer, the first antibody can be contacted with a labeled substrate thereof contacting the first antibody with

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the urine. By comparing the amount of labeled ligand that binds with the first antibody that has been contacted with the urine with the amount of labeled ligand that binds with an equivalent amount of the first antibody that has not been contacted with the urine, one can assess how much of MMPFF from the urine has bound with the first antibody.

5   [0017]   The kits and methods described herein can be used to detect a variety of MMPFF peptides, including naturally occurring MMPFF peptides (i.e., both full length proteins such as mesothelin and fragments of such proteins that are naturally produced in the bodies of ovarian cancer patients) and synthetically produced MMPFF peptides. The amino acid sequence of the MMPFF peptide should comprise at least 10 (20, 50, or 200) consecutive

10   residues of a sequence selected from the group consisting of SEQ ID NOs: 1-5. Alternatively, the MMPFF peptide can comprise a portion of at least 20 consecutive amino acid residues, wherein the amino acid sequence of the portion is at least 90% (or 95%) identical to 20 consecutive residues of a sequence selected from the group consisting of SEQ ID NOs: 1-5.

15   [0018]   The kits and methods disclosed herein can be used in conjunction with known or hereafter developed kits and methods for assessing occurrence (e.g., in urine or serum) other ovarian cancer markers. By way of example, ovarian cancer markers known to occur in urine include urinary gonadotropin peptide, cysteine proteinase, neopterin, and tumor-associated trypsin inhibitor. Known serum ovarian cancer markers include CA 125,

20   carcinoembryonic antigen, vascular endothelial growth factor, tumor-associated trypsin inhibitor, a CD44 splice variant, anti-malignin antibody, lactate dehydrogenase, alpha-hydroxybutyrate dehydrogenase, CA19-9, tissue polypeptide antigen, and sialyl TN. CA 125 is known to be a surrogate marker for ovarian cancer occurrence and progression. Assessment of occurrence of MMPFF peptide in a woman's urine can likewise be performed

25   in conjunction with assessment of the MMPFF peptide in the woman's serum.

[0019]   Substantially the same kits and methods can be used to assess the likelihood that a woman will develop an ovarian cancer. That is, occurrence of an MMPFF peptide in urine obtained from a woman is an indication that the woman is more likely to develop an ovarian cancer than an otherwise identical woman in whose urine the MMPFF does not occur.

30   [0020]   Substantially the same kits and methods can also be used to assess the efficacy of a therapy for an ovarian cancer in a woman. The amount of an MMPFF peptide in urine obtained from the woman following the therapy can be compared with the amount of the

MMPFF in her urine before the therapy. A lower amount of the MMPFF peptide in the urine following the therapy is an indication that the therapy is efficacious. Similarly, a greater amount of the MMPFF peptide in the urine following the therapy is an indication that the therapy is not efficacious. Such kits and methods can be used to compare the

5   efficacy of various therapeutic agents or regimens.

[0021]   In another aspect, the invention relates to a kit for assessing occurrence of an MMPFF peptide in urine obtained from a woman. The kit comprises a first agent (e.g., an antibody) for binding the MMPFF peptide and an instructional material that describes contacting urine with the first agent. The format of the kit is not critical - many standard

10   formats can be used, such as ELISA, latex bead aggregation, and surface plasmon resonance formats. The kit can include an MMPFF peptide for use as a positive control. Of course, the kit can also include reagents for assessing other known markers of ovarian cancer.

BRIEF SUMMARY OF THE SEVERAL VIEWS OF THE DRAWINGS

15   [0022]   In all figures comprising amino acid sequences, standard single-letter amino acid codes are used to represent amino acid residues.

[0023]   Figure 1 is the amino acid sequence (SEQ ID NO: 1) of mesothelin precursor protein. This sequence is the same as that listed in GENBANK® accession number 2207386A and reported in Chang et al., 1996, Proc. Natl. Acad. Sci. USA 93(1):136-140.

20   [0024]   Figure 2 is the amino acid sequence (SEQ ID NO: 2) of megakaryocyte potentiating factor (MPF) precursor protein. This sequence is the same as that listed in GENBANK® accession number NP_005814 and reported in Yamaguchi et al., 1994, J. Biol. Chem. 269(2):805-808.

[0025]   Figure 3 is a partial amino acid sequence (SEQ ID NO: 3) of a soluble variant

25   form of MPF precursor protein listed in GENBANK® accession number AF180951 and reported in Scholler et al., 1999, Proc. Natl. Acad. Sci. USA 96(20):11531-11536.

[0026]   Figure 4, comprising Figures 4A and 4B, is a comparison of residues 294-628 of SEQ ID NO: 1 and a nearly identical portion of SEQ ID NO: 2. Residue numbers are listed in the right margin. "MST" means mesothelin. "MPF" means megakaryocyte potentiating

30   factor. In this alignment, identical amino acid residues are indicated by a vertical line, a conservative amino acid substitution is indicated by a cross (+), and dashes (-) indicate a gap inserted into SEQ ID NO: 2 for the purpose of aligning the sequences.

[0027]   Figure 5, comprising Figures 5A and 5B, is a comparison of similar portions of

SEQ ID NOs: 1-3. Residue numbers are listed in the right margin. "MST" means

mesothelin. "MPF" means megakaryocyte potentiating factor. "SMR" means the soluble

variant form of MPF for which the sequence is listed in Fig. 3. In this alignment, residues

5   that are identical in all three sequences are represented by upper case single-letter codes,

residues that are not identical in all three sequences are represented by lower case single-

letter codes, and gaps inserted for the purpose of aligning the sequences are represented by

dashes (-).

[0028]   Figure 6 comprises Figures 6A and 6B. Figure 6A is an amino acid sequence

10   (SEQ ID NO: 4) that includes preferred epitopes for generation of antibodies described

herein. Figure 6B is another amino acid sequence (SEQ ID NO: 5) that includes preferred

epitopes for generation of antibodies described herein. In each of SEQ ID NOs: 4 and 5, the

underlined, bold X represents any amino acid residue, preferably being either aspartate or

asparagine. Other underlined, bold residues in Figure 6A represent residues that are not

15   present in the sequence shown in Figure 6B.

[0029]   Figure 7 is a bar graph that indicates serum levels of CA125II antigen (bars with

stripes slanting downward from left to right) and urine levels of an MMPFF peptide (bars

with stripes slanting upward from left to right), assayed as disclosed herein. Assays were

performed for four patients, designated 1-4, both at the time of ovarian cancer diagnosis

20   ("a") and approximately one month after the onset of therapy ("b").

DETAILED DESCRIPTION OF THE INVENTION

[0030]   The invention relates to the discovery that one or more polypeptides having

significant amino acid sequence similarity with a family of proteins that includes

25   mesothelin, megakaryocyte potentiating factor (MPF), and a soluble variant of MPF occurs

in the urine of women afflicted with ovarian cancer. These urine polypeptides bind

specifically with antibodies that are raised against proteins of that family. Occurrence of

these polypeptides in a patient's urine is diagnostic that the patient is afflicted with ovarian

cancer. The amount of the polypeptides decreases with effective treatment of the ovarian

30   cancer. Thus, the urine polypeptides can also be used to assess the efficacy of anti-ovarian

cancer therapy.

[0031]   Definitions

[0032]   As used herein, each of the following terms has the meaning associated with it in this section.

[0033]   A mesothelin/megakaryocyte potentiating factor family peptide ("MMPFF

5   peptide") is a polypeptide that has an amino acid sequence that either (i) comprises at least 10 consecutive residues of a sequence selected from the group consisting of SEQ ID NOs: 1-5 or (ii) comprises a portion of at least 20 consecutive amino acid residues wherein the amino acid sequence of the portion is at least 90% (preferably at least 95% or 100%) identical to 20 consecutive residues of a sequence selected from the group consisting of

10   SEQ ID NOs: 1-5.

[0034]   An "antibody" refers collectively and individually to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds an MMPFF peptide. A molecule which specifically binds with an MMPFF peptide is a molecule which binds the peptide, but

15   does not substantially bind other molecules in a sample (e.g., a urine sample) which naturally contains the polypeptide. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')2 fragments which can be generated by treating the antibody with an enzyme such as papain or pepsin, respectively. Either or both of polyclonal and monoclonal antibodies can be used in the methods and kits described

20   herein.

[0035]   The term "monoclonal antibody" refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope.

[0036]   The term "labeled," with regard to an antibody or a peptide, includes direct

25   labeling of the peptide or antibody by coupling (i.e., physically linking) a detectable substance to the peptide or antibody, as well as indirect labeling of the peptide or antibody by coupling it with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a peptide with biotin such that it can be detected with fluorescently

30   labeled streptavidin.

[0037]   An "instructional material" is a publication, a recording, a diagram, or any other medium of expression which can be used to communicate how to use a kit described herein,

information for interpreting occurrence of an MMPFF in urine as it relates to an ovarian

cancer, or both. The instructional material of the kit of the invention can, for example, be

affixed to a container which contains a kit of the invention or be shipped together with a

container which contains the kit. Alternatively, the instructional material can be shipped

5   separately from the container with the intention that the instructional material and the kit be

used cooperatively by the recipient.

[0038]   Detailed Description

[0039]   It has been discovered that one or more polypeptides having significant amino

10   acid sequence similarity with a family of proteins (the mesothelin/megakaryocyte

potentiating factor family; "MMPFF") occurs in the urine of women afflicted with ovarian

cancer. These urine peptides can be detected, for example with an antibody raised against a

protein of the MMPFF. Occurrence of such peptides in a patient's urine is an indication that

the patient is afflicted with ovarian cancer. Owing to the variety of ovarian cancer types,

15   the genetic variability among individuals, and other factors, MMPFF peptides will likely not

appear in the urine of all patients afflicted with ovarian cancer, and the peptides will likely

appear in differing amounts in individuals afflicted with ovarian cancers of substantially the

same type. Even taking these variations into account, occurrence of one or more MMPFF

peptides in the urine of a woman is an indication that the woman is afflicted with ovarian

20   cancer.

[0040]   Advantageously, occurrence of MMPFF peptides in the urine of women afflicted

with ovarian cancer does not appear to depend on the stage by which the ovarian cancer is

characterized. For this reason, urinary occurrence of MMPFF peptides can be used to

diagnose ovarian cancer at an early stage, facilitating efficacious treatment of the cancer

25   before it has reached a phase of the disease at which therapy is futile or nearly so.

[0041]   Another advantage of the kits and methods disclosed herein is that the amount

and concentration of MMPFF peptide in the urine of an ovarian cancer patient is indicative

of the state of the tumor. For this reason, assessment of urinary MMPFF peptide occurrence

can be used to assess the efficacy of an ovarian cancer therapeutic regimen. To the extent

30   the regimen induces tumor shrinkage or regression, urinary concentration of MMPFF

peptide can be expected to decrease. Similarly, gradual or unimpeded increase in urinary

MMPFF peptide concentration during ovarian cancer therapy is indicative of a therapeutic regimen that is not inhibiting tumor growth.

[0042]   The kits and methods described herein can be used to assess occurrence or progression of various ovarian cancer types. In an important embodiment, the kits and

5   methods are used to assess occurrence or progression of an epithelial ovarian cancer, which are among the most common ovarian cancers. In other embodiments, the kits and methods are used to assess occurrence of progression of stromal ovarian tumors or germ cell ovarian tumors.

10   [0043]   Diagnostic Methods

[0044]   The invention includes a method of diagnosing an ovarian cancer in a woman. The method comprises assessing occurrence of a mesothelin/megakaryocyte potentiating factor family (MMPFF) peptide in urine obtained from the woman. Occurrence of the MMPFF peptide in the urine is an indication that the woman is afflicted with an ovarian

15   cancer. Ovarian cancers of various type (e.g., epithelial ovarian cancer), grade, and stage can be diagnosed using this method. The method can also be used to diagnose ovarian cancers characterized by various cell types (e.g., serous, mucinous, endometrioid, clear, and non-differentiated ovarian tumors).

[0045]   The method used to assess occurrence of the MMPFF peptide in the woman's

20   urine is not critical. Any method that can be used to assess whether the peptide is present or absent is suitable. Methods capable of assessing the quantity (e.g., concentration) of the MMPFF peptide in the urine are preferable in some embodiments, particularly when assessments made over a period of time are to be compared (e.g., as when assessing the efficacy of a regimen of anti-cancer therapy).

25   [0046]   In one embodiment, occurrence of the MMPFF peptide in the patient's urine is assessed by contacting the urine with an antibody that binds specifically with the MMPFF peptide and assessing whether the MMPFF peptide has bound with the first antibody.

[0047]   Numerous MMPFF-binding antibodies are known in the art. For example, the antibody described in U.S. Patent No. 5,320,956 and secreted by the hybridoma deposited

30   with the ATCC as accession no. HB 10570 can be used. That antibody binds specifically with an approximately 40 kilodalton portion of mesothelin protein, as described by Chang et al. (1996, Proc. Natl. Acad. Sci. USA 93(1):136-140). Other suitable antibodies have been

described by Scholler et al. (1999, Proc. Natl., Acad. Sci. USA 96(20):11531-11536) and in PCT publication number WO 00/50900, wherein such antibodies are designated OV569, 4H3, 3G3, and 1A6.

[0048]   In diagnostic kits and methods that use two anti-MMPFF peptide antibodies, the

5   two antibodies preferably do not compete to bind the same epitope. By way of example, the antibody designated OV569 by Scholler et al. binds at an epitope which is independent of the epitopes bound by the antibodies designated 4H3, 3G3, and 1A6. Thus, for example, OV569 can be bound to a substrate and used as a 'capture' antibody to bind MMPFF peptide present in a patient's urine under appropriate antibody-binding conditions, and one of

10   antibodies 4H3, 3G3, and 1A6 can be detectably labeled and contacted with the 'capture' antibody after contacting the 'capture' antibody with the patient's urine. Binding of the detectably labeled antibody with the 'capture' antibody indicates that the MMPFF peptide was present in the patient's urine. Analysis of the amount of detectable label co-localized with the 'capture' antibody can indicate the quantity of the peptide that was present.

15   [0049]   As described herein, portions of MMPFF proteins share significant amino acid sequence homology. Figs. 1-3 show at least portions of the amino acid sequences of three MMPFF proteins. Figure 4 and 5 show alignments of those sequences, indicating the regions of greatest sequence homology among these proteins. From these figures, it is apparent that the sequences shown in Figs. 6A and 6B (SEQ ID NOs: 4 and 5, respectively)

20   represent useful portions of MMPFF peptides. Antibodies raised against all or a portion (e.g., 10, 20, 50, or 200 consecutive residues) of either of these sequences can be expected to bind specifically with a broad range of MMPFF peptides, including those that occur in the urine of ovarian cancer patients.

[0050]   An MMPFF (e.g., one described herein or in the prior art), or a fragment thereof,

25   can be used as an immunogen to generate antibodies using standard techniques for polyclonal and monoclonal antibody preparation. A fragment of a full-length MMPFF, when used as an immunogen, should comprise at least 10 (preferably 12, 15, 20, 50, 100, or 20 or more) amino acid residues of the amino acid sequence of any of SEQ ID NOs: 1-5 or the amino acid sequence of another MMPFF member.

30   [0051]   Appropriate MMPFF peptides for generation of antibodies useful in the kits and methods described herein comprise a portion of at least 20 consecutive amino acid residues that is at least 90% (preferably at least 95% or 100%) identical to 20 consecutive residues of

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a sequence selected from the group consisting of SEQ ID NOs: 1-5 (preferably either SEQ ID NO: 4 or SEQ ID NO: 5).

[0052]   Methods of generating antibodies are well known and only briefly summarized here. An immunogen typically is used to prepare antibodies by immunizing a suitable (i.e., immunocompetent) subject such as a rabbit, goat, mouse, or other mammal or vertebrate. An appropriate immunogenic preparation can contain, for example, recombinantly-expressed or chemically-synthesized polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or a similar immunostimulatory agent.

[0053]   Polyclonal antibodies can be prepared as described above by immunizing a suitable subject with a polypeptide of the invention as an immunogen. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized polypeptide. If desired, the antibody molecules can be harvested or isolated from the subject (e.g., from the blood or serum of the subject) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction.

[0054]   At an appropriate time after immunization, e.g., when the specific antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) Nature 256:495-497, the human B cell hybridoma technique (Kozbor et al. (1983) Immunol. Today 4:72), the EBV-hybridoma technique (Cole et al. (1985), Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing hybridomas is well known (see generally Current Protocols in Immunology (1994) Coligan et al. (Eds.) John Wiley & Sons, Inc., New York, NY). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind the polypeptide of interest, e.g., using a standard ELISA assay.

[0055]   Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody directed against a polypeptide of the invention can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with the polypeptide of interest. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia

Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene

SURFZAP™ Phage Display Kit, Catalog No. 240612). Additionally, examples of methods

and reagents particularly amenable for use in generating and screening antibody display

library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No. WO

5   92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT

Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No.

WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809;

Fuchs et al. (1991) Bio/Technology 9:1370-1372; Hay et al. (1992) Hum. Antibod.

Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; Griffiths et al. (1993)

10   EMBO J. 12:725-734.

[0056]   Recombinant antibodies, such as chimeric and humanized monoclonal

antibodies, comprising both human and non-human portions, which can be made using

standard recombinant DNA techniques, are within the scope of the invention. Such

chimeric and humanized monoclonal antibodies can be produced by recombinant DNA

15   techniques known in the art, for example using methods described in PCT Publication No.

WO 87/02671; European Patent Application 184,187; European Patent Application

171,496; European Patent Application 173,494; PCT Publication No. WO 86/01533; U.S.

Patent No. 4,816,567; European Patent Application 125,023; Better et al. (1988) Science

240:1041-1043; Liu et al. (1987) Proc. Natl. Acad. Sci. USA 84:3439-3443; Liu et al.

20   (1987) J. Immunol. 139:3521-3526; Sun et al. (1987) Proc. Natl. Acad. Sci. USA 84:214-

218; Nishimura et al. (1987) Cancer Res. 47:999-1005; Wood et al. (1985) Nature 314:446-

449; and Shaw et al. (1988) J. Natl. Cancer Inst. 80:1553-1559); Morrison (1985) Science

229:1202-1207; Oi et al. (1986) Bio/Techniques 4:214; U.S. Patent 5,225,539; Jones et al.

(1986) Nature 321:552-525; Verhoeyan et al. (1988) Science 239:1534; and Beidler et al.

25   (1988) J. Immunol. 141:4053-4060.

[0057]   In embodiments in which an anti-MMPFF peptide antibody is contacted with

urine obtained from a patient, the urine can be used in its naturally-expressed state or it can

be partially purified or clarified prior to use. For example, urine can be centrifuged using

standard methods to substantially remove any sediment therefrom prior to contacting the

30   urine with the antibody. Alternatively, the urine can be filtered or ultrafiltered to reduce its

volume or to remove large contaminants. Because MMPFF peptides that occur in urine can

be expected to be polypeptides having molecular weights in the range 10,000 - 100,000

Daltons, selection of an appropriate filtration/ultrafiltration is necessary to prevent removal of the MMPFF peptide prior to its detection. By way of example, a relatively large pore filter (e.g., one that permits passage of globular particles having molecular weights of 250,000 to 1 million Daltons can be used to remove particulate material from urine and an

5   ultrafiltration device equipped with a membrane that excludes passage of proteins having molecular weights greater than about 5,000 Daltons can be used to concentrate the filtrate. In this example, occurrence of the MMPFF can be assessed in the retentate in the ultrafiltration device.

[0058]   Because MMPFF peptides are often relatively abundant in urine of ovarian

10   cancer patients and because many of the methods (e.g., the immunological methods) described herein are relatively sensitive, concentration of urine is usually not necessary to detect occurrence of MMPFF peptides in urine of ovarian cancer patients. In situations in which clarification of urine is desired, centrifugation can be preferable, since the potential that MMPFF peptides present in the urine will become bound with or enmeshed within the

15   filter medium is not present.

[0059]   One preferred method for assessing occurrence of an MMPFF peptide in urine of a woman is the procedure commonly known as a "sandwich ELISA" assay. (ELISA is an abbreviation for enzyme-linked immunosorbent assay.) In this method, an antibody is bound to a substrate, such as a glass bead or the bottom surface of a plastic multi-well assay

20   plate. This antibody is designated a 'capture' antibody. Raw, clarified, or purified urine from the woman is contacted with the substrate under conditions (e.g., low concentrations of salt and detergents and non-protein-denaturing conditions), so that any MMPFF peptide present in the urine binds specifically with the capture antibody. The substrate is optionally rinsed with a fluid that does not comprise the MMPFF peptide to remove residual urine and

25   any non-bound MMPFF. The substrate is then contacted with a second antibody that specifically binds with the MMPFF peptide at an epitope independent of the epitope at which the capture antibody binds. The second antibody is either detectably labeled or linked with either a ligand or a receptor of the ligand. Either way, binding of the second antibody is detected (and, optionally, quantitated) after rinsing the substrate with a fluid that

30   does not comprise the second antibody. Detection of the second antibody is indicative of the presence in the urine of the MMPFF peptide. If the amount of the second antibody is quantitated, then the amount of the MMPFF peptide in the urine can be quantitated, for

example, by comparison of the amount of second antibody detected with measurements made using control samples containing known amounts of the MMPFF.

[0060] Detection an antibody can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups,

5 fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin / biotin and avidin / biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate,

10 rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

[0061] Use of standardized assay apparatus permits automation of the method described

15 herein. For example, many different standardized assay containers are known, such as 24-, 48-, 96-, and 384-well plastic plates. Where these containers are adapted to fit a robotic apparatus, automated analysis of samples can be achieved, permitting high-throughput screening of many samples in a relatively short time. Automated assay apparatus and containers adapted for their use in computer-controlled assays are known in the art and

20 readily adapted for the kits and methods described herein.

[0062] Another type of immunological assay that can be used to assess occurrence of an MMPFF peptide in urine obtained from a woman is commonly designated a 'competition' assay. In this assay, an antibody that binds specifically with the MMPFF peptide is fixed to a substrate. Raw, clarified, or purified urine is contacted with the substrate (preferably for a

25 period of minutes or hours), so that any MMPFF peptide present in the urine binds with the antibody. A labeled ligand (e.g., a labeled version of the same MMPFF peptide or another MMPFF peptide) that binds specifically with the antibody is then contacted with the substrate (preferably for a period of minutes or hours). The amount of labeled ligand bound with the substrate is assessed after rinsing the substrate with a fluid that does not comprise

30 the label or the labeled ligand. The amount of label bound with the substrate is compared with the amount of label bound with an otherwise identically treated substrate that is not contacted with the patient's urine. The difference between the two amounts of bound label

represents the amount of MMPFF peptide bound to the substrate, and is indicative of the amount of the MMPFF peptide that was present in the urine sample applied to the substrate.

[0063]   When an immunological competition assay is used, the labeled ligand of the antibody bound with the substrate should be as nearly identical to the MMPFF peptide

5   known or expected to be present in the patient's urine as possible. For example, the ligand and the MMPFF peptide should have or comprise the same amino acid sequence. Similarly, if the MMPFF peptide that may occur in the patient's urine is expected to by glycosylated, then the ligand should be glycosylated in the same way, at the same position, and to the same extent. In this way, affinity differences of the antibody for the ligand and for the

10   MMPFF peptide can be minimized and the accuracy of the competition assay can be improved.

[0064]   The kits and methods described herein can be used alone to assess occurrence of an MMPFF peptide in urine of a human patient, and such occurrence is indicative that the woman is afflicted with ovarian cancer. However, a greater number of ovarian cancers can

15   be detected and greater confidence in the diagnosis of ovarian cancer can be achieved if occurrence of more than one marker of ovarian cancer is assessed in the woman. Any additional ovarian cancer marker(s) can be a marker normally found in urine, a marker normally found in blood, or a marker normally found in both urine an blood. By way of example, MMPFF peptides are normally found in the serum of patients afflicted with

20   ovarian cancer (Scholler et al., 1999, Proc. Natl. Acad. Sci. USA 96(20):11531-11536). Thus, the kits and methods described herein for assessing occurrence of MMPFF peptides in urine can be used in conjunction with kits and methods of detecting the same or other MMPFF peptides in serum.

[0065]   Other ovarian cancer markers known to occur in urine include urinary

25   gonadotropin peptide (UGP; Schutter et al., 1999, Anticancer Res. 19(6C):5551-5557), cysteine proteinase (UCP; Gao et al., 1996, Hua Xi Yi Ke Da Xue Xue Bao 27(3):291-294), neopterin (Szarka et al., 1988, Acta Chir. Hung. 29(4):359-364), and tumor-associated trypsin inhibitor (TATI; Medl et al., 1995, Br. J. Cancer 71(5):1051-1054; Halila et al., 1988, Br. J. Cancer 57(3):304-307).

30   [0066]   Other ovarian cancer markers known to occur in serum include CA 125 (Halila et al., 1987, Br. J. Cancer 56(2):153-156), carcinoembryonic antigen (CEA; Juweid et al., 1997, Gynecol. Oncol. 67(3):259-271), vascular endothelial growth factor (VEGF; Candido

Dos Reis et al., 2002, Gynecol. Obstet. Invest. 54(3):132-136), tumor-associated trypsin

inhibitor (TATI; Medl et al., 1995, Br. J. Cancer 71(5):1051-1054; Halila et al., 1988, Br. J.

Cancer 57(3):304-307), CD44 splice variants (Uhl-Steidl et al., 1995, Oncology 52(5):400-

406), anti-malignin antibody (Bogoch et al., 1991, Lancet 337:927), lactate dehydrogenase

5   (LDH), alpha-hydroxybutyrate dehydrogenase (aHBDH), CA19-9, tissue polypeptide

antigen (TPA), and sialyl TN (STN; Kudoh et al., 1999, Gynecol. Obstet. Invest. 47(1);52-

57). This invention includes kits and methods for assessing any combination of these

known markers in blood and/or urine, so long as the kits and methods include assessing

occurrence of at least MMPFF peptide in urine.

10   [0067]   The kits and methods described herein for assessing occurrence of a MMPFF

peptide in urine obtained from a woman can be used to assess the likelihood that the woman

will develop an ovarian cancer. Detection of occurrence of an MMPFF peptide in a

woman's urine is an indication that the woman is more likely to develop an ovarian cancer

than an otherwise identical woman in whose urine the MMPFF does not occur. It is

15   recognized that the difference between being afflicted with an ovarian tumor, the earliest

stages of tumor growth, the onset of tumorigenesis, and enhanced susceptibility to ovarian

tumorigenesis may be substantially indistinguishable. Nonetheless, the outcome of each of

these processes is development of an ovarian tumor to the detriment of the woman's health.

For this reason, assessment of any of these states using the kits and methods disclosed

20   herein is useful.

[0068]   Anti-MMPFF peptide antibodies can be used diagnostically or prognostically to

monitor MMPFF levels in urine as part of a clinical testing procedure, e.g., to, for example,

determine the efficacy of a given ovarian cancer treatment regimen.

[0069]   The kits and methods described herein can be used to determine whether an

25   agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small

molecule, or other drug candidate) can be administered to a subject in order to alleviate,

inhibit, reverse, or prevent ovarian cancer. For example, such methods can be used to

determine whether an ovarian cancer patient can be effectively treated using a specific

agent or class of agents.

30

[0070]   Kits for Assessing Occurrence of Urinary MMPFF Peptides

[0071]   The invention also encompasses kits for detecting occurrence of an MMPFF in a

urine sample obtained from a mammal (e.g., a woman). The kit comprises a first agent for

binding the MMPFF peptide, a second agent for assessing binding of the MMPFF peptide

5   with the agent, and an instructional material that describes contacting urine with the first

agent. These kits can be used to determine if a subject is suffering from or is at increased

risk of developing ovarian cancer. For example, the kit can comprise a labeled compound

or agent capable of detecting the MMPFF peptide in a urine sample. The kit can also, or

alternatively, contain means for determining the amount of the MMPFF peptide in the

10   sample. Kits can include instructions for assessing whether the tested subject is suffering

from or is at risk of developing ovarian cancer if the amount of the MMPFF peptide is

above or below a normal level (e.g., an absorbance measurement of at least 0.2 at 450

nanometers using the methodology set forth in Example 1).

[0072]   For antibody-based kits, the kit can comprise, for example: (1) a first antibody

15   (e.g., attached to a solid support) which specifically binds with an MMPFF peptide and,

optionally, (2) a second, different antibody which specifically binds with either the

MMPFF peptide (e.g., at a epitope distinct from the epitope at which the first antibody

binds) or the first antibody and is conjugated with a detectable agent. Alternatively, the

kit can comprise the first antibody and a labeled ligand of the first antibody. After

20   contacting the first antibody with a woman's urine sample, binding of the labeled ligand

with the first antibody can be assessed in a competition-type assay, as described herein.

[0073]   The kits can further comprise reagents and/or instructions for assessing

occurrence in urine or serum of the woman another marker indicative of occurrence of

ovarian cancer in the woman.

25

[0074]   Examples

[0075]   The invention is now described with reference to the following Examples. These

Examples are provided for the purpose of illustration only, and the invention is not limited

to these Examples, but rather encompasses all variations which are evident as a result of the

30   teaching provided herein.

[0076]   Example 1

[0077]   Sandwich ELISA Assay for Assessing MMPFF in a Sample

[0078]   A typical assay used to detect an MMPFF in a urine sample is now described.

Assays were performed in a 96-well clear, flat-bottom microtiter plate that had been

5   previously coated with monoclonal antibody 4H3 (as described in Scholler et al., 1999,

Proc. Natl. Acad. Sci. USA 96(12):11531-11536 and in PCT publication WO 00/50900).

The bottom of the wells were coated with the antibody by adding to individual wells 50

microliters of a suspension comprising 3 micrograms of antibody per milliliter in carbonate-

bicarbonate buffer (pH 9.6). The plate was swirled to coat the bottom surface of the wells

10   and then permitted to dry overnight at 4°C. Each well was blocked by filling it with a 3%

(w/v) suspension of bovine serum albumin (BSA) and maintaining the filled wells for 2

hours at room temperature, after which time the BSA suspension was removed from the

wells.

[0079]   Standard curves were prepared using a fusion protein (as described in Scholler et

15   al., 1999, Proc. Natl. Acad. Sci. USA 96(20):11531-11536) comprising a portion of the

MPF amino acid sequence (i.e., the 44 kDa membrane-bound domain) fused to an

immunoglobulin (Ig) constant region using a described vector encoding a human Ig

sequence (as described in PCT publication number WO 00/50900 and in Hollenbaugh et al.,

1995, J. Immunol. Meth. 188:1-7). This fusion protein was designated hIgG. For the

20   standard curve, the fusion protein was assessed at concentrations of 1.0 microgram per

milliliter, 0.1 microgram per milliliter, and four serial 5-fold dilutions thereof (i.e., 1:5,

1:25, 1:125, and 1:625). The diluent used was a 7% (w/v) suspension of BSA.

[0080]   To perform the assay, 50 microliters of an hIgG fusion protein dilution, a

control, or a urine sample, or a diluted urine sample was added to individual wells. The

25   plate was incubated for 1 hour at room temperature and the wells were washed with tris-

buffered saline (pH 7.8 to 8) containing 0.05% (v/v) TWEEN® 20 surfactant. 50

microliters of a 200 nanogram per milliliter suspension of biotinylated monoclonal antibody

OV569 (as described in Scholler et al., 1999, Proc. Natl. Acad. Sci. USA 96(20):11531-

11536 and in PCT publication number WO 00/50900) was added to each well. After

30   incubating the plate for 1 hour at room temperature and thereafter washing each well with

tris-buffered saline (pH 7.8 to 8) containing 0.05% (v/v) TWEEN® 20 surfactant, 50

microliters of a 1:5000 dilution of Streptavidin-horseradish peroxidase (obtained from

Southern Biotechnology Associates, Inc., Birmingham, AL, catalog no. 7100-05) at was

added to each well, and the plate was incubated for 1 hour at room temperature. 50

Microliters of 3,3',5,5'-tetramethylbenzidine (TMB; a chromogenic substrate for horseradish

peroxidase; obtained from Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) was

5   added to each well to generate signal. The plate was incubated for 15 minutes at room

temperature before adding 50 microliters of TMB Stop solution (0.1 normal sulfuric acid;

obtained from Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) to halt the

peroxidase reaction. Blue color generated by the action of the peroxidase on TMB was

assessed using a Molecular Devices SpectraMax® Plus 384 spectrophotometric plate reader

10   (model number 02523) to measure the absorbance at 450 nanometers.

[0081]   Example 2

[0082]   Detection of an MMPFF in Urine Samples Obtained from Ovarian Cancer

Patients

15   [0083]   Urine samples were obtained from 25 patients known to be afflicted with ovarian

cancer. Individual urine samples were centrifuged for 15 minutes at 13,000 rpm (ca. 15,700

x g) to remove sediment. Individual 50 microliter aliquots of urine sample supernatants

were tested using the assay described in Example 1.

[0084]   Of the 25 samples that were tested, 22 of the samples registered an absorbance

20   value at 450 nanometers that was greater than 0.2. These data indicate that use of a

chromogenic sandwich ELISA-type assay for detecting an MMPFF in urine is a reliable

way of detecting occurrence of ovarian cancer in many patients.

[0085]   Example 3

25   [0086]   Confirmation of MMPFF Signal in Urine Samples Obtained from Ovarian

Cancer Patients

[0087]   In order to rule out non-specific binding that may have mimicked signal

corresponding to occurrence of MMPFF in a urine sample described in Example 2, urine

samples for which relatively high absorbance values were obtained in Example 2 were

30   selected. An assay plate was prepared as described in Example 1, except that the plate had

not been coated with antibody 4H3. Individual urine samples were prepared and added to

the plate as described in Example 2.

[0088]   No signal above the background level was detected in any of the samples tested in this Example.

[0089]   In a separate experiment, the urine samples described in Example 2 were tested using a plate prepared as described in Example 1. The 569 monoclonal antibody used in this experiment was not biotinylated, however. Otherwise, the assay was as described in Example 1. No signal above background level was detected for any of the samples tested.

[0090]   In another separate experiment, the assay was performed as described in Example 1, except that a biotinylated antibody that has no relation to the MMPFF was used in place of biotinylated monoclonal antibody 569. Assays of the urine samples described in Example 2 by this method yielded no signal above background when tested in this manner.

[0091]   These data indicate that the increased absorbance signal at 450 nanometers observed in the sandwich ELISA assay using urine obtained from ovarian cancer patients is a true response indicating the presence of the MMPFF peptide in the patients' urine.

[0092]   Example 4

[0093]   Urine samples obtained from 31 patients who had been diagnosed with bladder cancer, from 3 women who were afflicted with benign gynecological diseases, and from 22 urine samples obtained from normal, healthy adults were tested using the assay described in Example 1.

[0094]   The data demonstrate that urine obtained from normal, healthy patients has very low levels of MMPFF peptide. As noted in Example 2, urine from 22 of 25 ovarian cancer patients (88%) exhibited significantly elevated MMPFF peptide levels. Urine samples obtained from bladder cancer patients (i.e., another genitourinary disease) exhibited elevated levels of MMPFF peptide in only 8 of the 31 samples. Elevated MMPFF peptide levels were not detected in the 3 urine samples obtained from women who were afflicted with benign gynecological diseases. Taken together, urine samples obtained from only 8 of 56 non-ovarian cancer patients exhibited enhanced MMPFF peptide levels, while 22 of 25 urine samples from ovarian cancer patients did. These data indicate that the specificity of this assay method for ovarian caner is 86%.

[0095]   Example 5

[0096]   Correlation of Urine MMPFF Peptide Levels with Stage of Ovarian Cancer

[0097]   Information regarding the stage of their cancer was collected for each of the 25 ovarian cancer patients from whom urine samples were analyzed in Example 2. Patients having ovarian cancers for each of the four stages were represented. There was no apparent correlation between the stage of the cancer for the patient and the amount of MMPFF peptide detected in the patient's urine. An implication of this observation is that the urine MMPFF peptide assay method described herein can be used to assess occurrence of early stage ovarian cancer as effectively as late stage ovarian cancer.

[0098]   Example 6

[0099]   Effect of Ovarian Cancer Therapy on Urinary MMPFF Peptide Level

[0100]   The effect of ovarian cancer treatment on 4 of the 25 ovarian cancer patients described in Example 2 on MMPFF peptide levels were assessed and compared with serum levels of the ovarian cancer tumor marker designated CA 125. CA 125 was assessed in serum samples obtained from each of the four patients before and after treatment, using an immunoassay substantially similar to the CA125II™ (Fujirebio Diagnostics, Inc., Malvern, PA) assay. Urine MMPFF peptide levels were also assessed in each of these four patients before and after treatment, using the methods described in Examples 1 and 2.

[0101]   The change in urine MMPFF peptide level before and after treatment was approximately proportional to the change in serum CA 125 level in each of the four patients, as illustrated in Fig. 7. Because CA 125 is accepted in the art as a surrogate marker of ovarian cancer progression, these data demonstrate that a correlation exists between urinary MMPFF peptide levels and disease progression. These data confirm the clinical value of this test and its potential use as a non-invasive method of monitoring ovarian cancer therapy efficacy.

[0102]   Example 7

[0103]   Correlation Between Urinary and Serum Levels of MMPFF Peptide

[0104]   Blood was drawn from each of the 25 patients who provided the urine samples assayed in Example 2. Serum MMPFF peptide levels were determined for those blood

samples, using the protocol described in Example 1. Serum and urine samples were

collected from individual patients on the same day.

[0105]   MMPFF peptides could be detected in both urine and serum samples from all

patients, albeit at very low levels in some instances. Using a cut-off value of 0.2 absorbance

units (at 450 nanometers) for the test of urinary samples, 22 out of 25 urine samples (88%)

tested positive for MMPFF. Using a cut-off value of 0.1 absorbance units (at 450

nanometers) for the test of serum samples, only 16 of the 25 (64%) tested positive for

MMPFF. These data demonstrate that MMPFF peptide level is elevated in both the serum

and urine of ovarian cancer patients. However, detection of MMPFF peptide in urine

appears to be more sensitive and may therefore have greater diagnostic value.

[0106]   Example 8

[0107]   Assessment of Urine CA 125 Levels

[0108]   CA 125 level was assessed in each of the 25 urine samples described in Example

2 using the CA125II™ test kit. None of the urine samples yielded results above the assay's

background level. These results indicate that CA 125 is not present in the urine of these

patients. These data also confirm that serum tumor markers such as CA 125 are not

necessarily present in the urine of patients in whose serum they occur.

[0109]   The disclosure of every patent, patent application, and publication cited herein is

hereby incorporated herein by reference in its entirety.

[0110]   While this invention has been disclosed with reference to specific embodiments,

it is apparent that other embodiments and variations of this invention can be devised by

others skilled in the art without departing from the true spirit and scope of the invention.

The appended claims include all such embodiments and equivalent variations.

CLAIMS

What is claimed is:

1. A method of diagnosing an ovarian cancer in a woman, the method comprising assessing
occurrence of a mesothelin/megakaryocyte potentiating factor family (MMPFF) peptide in
urine obtained from the woman, whereby occurrence of the MMPFF peptide in the urine is
an indication that the woman is afflicted with an ovarian cancer.

2. The method of claim 1, wherein the ovarian cancer is an epithelial ovarian cancer.

3. The method of claim 1, wherein the ovarian cancer is a stromal ovarian cancer.

4. The method of claim 1, wherein the ovarian cancer is a stem cell ovarian cancer.

5. The method of claim 1, wherein occurrence of the MMPFF peptide in the urine is
assessed by contacting the urine with a first antibody that binds specifically with the
MMPFF peptide and assessing whether the MMPFF peptide has bound with the first
antibody.

6. The method of claim 5, wherein the urine is centrifuged to substantially remove any
sediment therefrom prior to contacting the urine with the first antibody.

7. The method of claim 5, wherein the first antibody is bound to a substrate.

8. The method of claim 7, wherein the substrate is a plastic container.

9. The method of claim 8, wherein the container is a multi-well plate.

10. The method of claim 9, wherein the plate is adapted for automated analysis by a robotic
apparatus.

11. The method of claim 5, wherein binding of the MMPFF peptide and the first antibody is assessed by contacting the first antibody with a second antibody that binds specifically with the MMPFF peptide after contacting the first antibody with the urine and assessing co-localization of the first and second antibodies.

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12. The method of claim 11, wherein the urine is contacted with the first antibody for at least 10 minutes before contacting the first antibody with the second antibody.

13. The method of claim 11, wherein the urine is contacted with the first antibody for at
10   least 60 minutes before contacting the first antibody with the second antibody.

14. The method of claim 11, wherein the second antibody is detectably labeled.

15. The method of claim 14, wherein the second antibody is linked with a compound
15   selected from the group consisting of an enzyme, a radionuclide, a fluorophore, and a chromophore.

16. The method of claim 11, wherein the second antibody is linked to a ligand and wherein co-localization of the first and second antibodies is assessed by contacting the second
20   antibody with a compound comprising (i) a receptor that binds with the ligand and (ii) a detectable label.

17. The method of claim 16, wherein the label is selected from the group consisting of an enzyme, a radionuclide, a fluorophore, and a chromophore.

25
18. The method of claim 16, wherein the ligand is biotin and the receptor is an avidin.

19. The method of claim 18, wherein the avidin is streptavidin.

30   20. The method of claim 19, wherein the label is an enzyme.

21. The method of claim 20, wherein the enzyme is horseradish peroxidase.

22. The method of claim 21, wherein co-localization of the first and second antibodies is assessed in the presence of 3,3',5,5'-tetramethylbenzidine.

5   23. The method of claim 5, wherein the first antibody is contacted with a labeled substrate of the first antibody after contacting the first antibody with the urine and comparing the amount of labeled ligand that binds with the first antibody that has been contacted with the urine and the amount of labeled ligand that binds with an equivalent amount of the first antibody that has not been contacted with the urine.

10

24. The method of claim 23, wherein the labeled ligand has the same amino acid sequence as the MMPFF peptide.

25. The method of claim 24, wherein the glycosylation state of the labeled ligand is the

15   same as the glycosylation state of the MMPFF peptide.

26. The method of claim 1, wherein the MMPFF peptide is glycosylated.

27. The method of claim 1, wherein the amino acid sequence of the MMPFF peptide

20   comprises at least 10 consecutive residues of a sequence selected from the group consisting of SEQ ID NOs: 1-5.

28. The method of claim 27, wherein the amino acid sequence of the MMPFF peptide comprises at least 20 consecutive residues of a sequence selected from the group consisting

25   of SEQ ID NOs: 1-5.

29. The method of claim 27, wherein the amino acid sequence of the MMPFF peptide comprises at least 50 consecutive residues of a sequence selected from the group consisting of SEQ ID NOs: 1-5.

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30. The method of claim 27, wherein the amino acid sequence of the MMPFF peptide comprises at least 200 consecutive residues of a sequence selected from the group consisting of SEQ ID NOs: 1-5.

5   31. The method of claim 27, wherein the amino acid sequence of the MMPFF peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 1-5.

32. The method of claim 27, wherein the amino acid sequence of the MMPFF peptide comprises at least 10 consecutive residues of SEQ ID NO: 4.

10

33. The method of claim 27, wherein the amino acid sequence of the MMPFF peptide comprises at least 10 consecutive residues of SEQ ID NO: 5.

34. The method of claim 1, wherein the MMPFF peptide comprises a portion of at least 20

15   consecutive amino acid residues and wherein the amino acid sequence of the portion is at least 90% identical to 20 consecutive residues of a sequence selected from the group consisting of SEQ ID NOs: 1-5.

35. The method of claim 34, wherein the MMPFF peptide comprises a portion of at least 20

20   consecutive amino acid residues and wherein the amino acid sequence of the portion is at least 90% identical to 20 consecutive residues of a sequence selected from the group consisting of SEQ ID NOs: 4 and 5.

36. The method of claim 1, further comprising assessing occurrence of a second ovarian

25   cancer marker in the urine of the woman, whereby occurrence of the second marker in the urine is also an indication that the woman is afflicted with an ovarian cancer.

37. The method of claim 36, wherein the second ovarian cancer marker is selected from the group consisting of urinary gonadotropin peptide, cysteine proteinase, neopterin, and tumor-

30   associated trypsin inhibitor.

38. The method of claim 1, further comprising assessing occurrence of a second ovarian cancer marker in the serum of the woman, whereby occurrence of the second marker in the serum is also an indication that the woman is afflicted with an ovarian cancer.

5　39. The method of claim 38, wherein the second ovarian cancer marker is selected from the group consisting of CA 125, carcinoembryonic antigen, vascular endothelial growth factor, tumor-associated trypsin inhibitor, a CD44 splice variant, anti-malignin antibody, lactate dehydrogenase, alpha-hydroxybutyrate dehydrogenase, CA19-9, tissue polypeptide antigen, and sialyl TN.

10

40. The method of claim 38, wherein the second ovarian cancer marker is CA 125.

41. The method of claim 1, further comprising assessing occurrence of the MMPFF peptide in the serum of the woman, whereby occurrence of the MMPFF peptide in the serum is also

15　an indication that the woman is afflicted with an ovarian cancer.

42. A method of assessing the likelihood that a woman will develop an ovarian cancer, the method comprising assessing occurrence of a mesothelin/megakaryocyte potentiating factor family (MMPFF) peptide in urine obtained from the woman, whereby occurrence of the

20　MMPFF peptide in the urine is an indication that the woman is more likely to develop an ovarian cancer than an otherwise identical woman in whose urine the MMPFF does not occur.

43. A method of assessing the efficacy of a therapy for an ovarian cancer in a woman, the

25　method comprising assessing the amount of a mesothelin/megakaryocyte potentiating factor family (MMPFF) peptide in urine obtained from the woman following the therapy and comparing that amount with the amount of the MMPFF in urine obtained from the woman before the therapy, whereby a lower amount of the MMPFF peptide in the urine following the therapy is an indication that the therapy is efficacious.

30

44. A kit for assessing occurrence of a mesothelin/megakaryocyte potentiating factor family (MMPFF) peptide in urine obtained from a woman, the kit comprising a first agent for

binding the MMPFF peptide and an instructional material that describes contacting urine with the first agent.

45. The kit of claim 44, wherein the first agent is an antibody.

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46. The kit of claim 45, wherein the antibody is bound to a thin metal film.

47. The kit of claim 46, wherein the film is adapted for analysis in a surface plasmon resonance apparatus.

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48. The kit of claim 44, further comprising a second agent for assessing binding of the MMPFF peptide with the first agent.

49. The kit of claim 48, wherein the second agent is a detectably labeled ligand of the first

15   agent.

50. The kit of claim 49, wherein the first agent is an antibody and the ligand is an MMPFF peptide.

20   51. The kit of claim 49, wherein the ligand is labeled with a compound selected from the group consisting of an enzyme, a radionuclide, a fluorophore, and a chromophore.

52. The kit of claim 48, wherein the second agent is a detectably labeled antibody.

25   53. The kit of claim 52, wherein the antibody is a biotinylated antibody.

54. The kit of claim 52, wherein the antibody is labeled with a compound selected from the group consisting of an enzyme, a radionuclide, a fluorophore, and a chromophore.

30   55. The kit of claim 48, wherein the first agent is an antibody that binds with an epitope of the MMPFF that is distinct from the epitope that binds with the detectably labeled antibody.

56. The kit of claim 44, further comprising a device for centrifuging a urine sample obtained from the woman.

57. The kit of claim 44, wherein the first agent is bound to a substrate.

5

58. The kit of claim 57, wherein the substrate is a plastic container.

59. The kit of claim 58, wherein the container is a multi-well plate.

10   60. The kit of claim 59, wherein the plate is adapted for automated analysis by a robotic apparatus.

61. The kit of claim 58, wherein the first agent is an antibody.

15   62. The kit of claim 61, wherein the second agent is second antibody.

63. The kit of claim 62, wherein the second antibody is linked to a ligand.

64. The kit of claim 63, further comprising a compound comprising (i) a receptor that binds
20   with the ligand and (ii) a detectable label.

65. The kit of claim 64, wherein the label is selected from the group consisting of an enzyme, a radionuclide, a fluorophore, and a chromophore.

25   66. The kit of claim 65, wherein the ligand is biotin and the receptor is an avidin.

67. The kit of claim 66, wherein the avidin is streptavidin.

68. The kit of claim 67, wherein the label is an enzyme.

30

69. The kit of claim 68, wherein the enzyme is horseradish peroxidase.

70. The kit of claim 69, further comprising 3,3',5,5'-tetramethylbenzidine.

71. The kit of claim 44, further comprising a control MMPFF peptide, wherein the amino acid sequence of the control peptide comprises at least 10 consecutive residues of a

5   sequence selected from the group consisting of SEQ ID NOs: 1-5.

72. The kit of claim 71, wherein the amino acid sequence of the control peptide comprises at least 20 consecutive residues of a sequence selected from the group consisting of SEQ ID NOs: 1-5.

10

73. The kit of claim 71, wherein the amino acid sequence of the control peptide comprises at least 50 consecutive residues of a sequence selected from the group consisting of SEQ ID NOs: 1-5.

15   74. The kit of claim 71, wherein the amino acid sequence of the control peptide comprises at least 200 consecutive residues of a sequence selected from the group consisting of SEQ ID NOs: 1-5.

75. The kit of claim 71, wherein the amino acid sequence of the control peptide comprises a

20   sequence selected from the group consisting of SEQ ID NOs: 1-5.

76. The kit of claim 71, wherein the amino acid sequence of the control peptide comprises at least 10 consecutive residues of SEQ ID NO: 4.

25   77. The kit of claim 71, wherein the amino acid sequence of the control peptide comprises at least 10 consecutive residues of SEQ ID NO: 5.

78. The kit of claim 44, further comprising a control MMPFF peptide comprises a portion of at least 20 consecutive amino acid residues and wherein the amino acid sequence of the

30   portion is at least 90% identical to 20 consecutive residues of a sequence selected from the group consisting of SEQ ID NOs: 1-5.

79. The kit of claim 78, wherein the control peptide comprises a portion of at least 20 consecutive amino acid residues and wherein the amino acid sequence of the portion is at least 90% identical to 20 consecutive residues of a sequence selected from the group consisting of SEQ ID NOs: 4 and 5.

5

80. The kit of claim 44, further comprising a reagent for assessing occurrence of a second ovarian cancer marker in the urine of the woman.

81. The kit of claim 80, wherein the second ovarian cancer marker is selected from the
10 group consisting of urinary gonadotropin peptide, cysteine proteinase, neopterin, and tumor-associated trypsin inhibitor.

82. The kit of claim 44, further comprising a reagent for assessing occurrence of a second ovarian cancer marker in the serum of the woman.

15

83. The kit of claim 82, wherein the second ovarian cancer marker is selected from the group consisting of CA 125, carcinoembryonic antigen, vascular endothelial growth factor, tumor-associated trypsin inhibitor, a CD44 splice variant, anti-malignin antibody, lactate dehydrogenase, alpha-hydroxybutyrate dehydrogenase, CA19-9, tissue polypeptide antigen,
20 and sialyl TN.

84. The kit of claim 83, wherein the second ovarian cancer marker is CA 125.

```
MALQRLDPCW SCGDRPGSLL FLLFSLGWVH PARTLAGETG TESAPLGGVL TTPHNISSLS   60
PRQLLGFPCA EVSGLSTERV RELAVALAQK NVKLSTEQLR CLAHRLSEPP EDLDALPLDL  120
LLFLNPDAFS GPQACTRFFS RITKANVDLL PRGAPERQRL LPAALACWGV RGSLLSEADV  180
RALGGLACDL PGRFVAESAE VLLPRLVSCP GPLDQDQQEA ARAALQGGGP PYGPPSTWSV  240
STMDALRGLL PVLGQPIIRS IPQGIVAAWR QRSSRDPSWR QPERTILRPR FRREVEKTAC  300
PSGKKAREID ESLIFYKKWE LEACVDAALL ATQMDRVNAI PFTYEQLDVL KHKLDELYPQ  360
GYPESVIQHL GYLFLKMSPE DIRKWNVTSL ETLKALLEVD KGHEMSPQAP RRPLPQVATL  420
IDRFVKGRGQ LDKDTLDTLT AFYPGYLCSL SPEELSSVPP SSIWAVRPQD LDTCDPRQLD  480
VLYPKARLAF QNMNGSEYFV KIQSFLGGAP TEDLKALSQQ NVSMDLATFM KLRTDAVLPL  540
TVAEVQKLLG PHVEGLKAEE RHRPVRDWIL RQRQDDLDTL GLGLQGGIPN GYLVLDLSVQ  600
ETLSGTPCLL GPGPVLTVLA LLLASTLA                                    628
```

Fig. 1

```
MALPTARPLL GSCGTPALGS LLFLLFSLGW VQPSRTLAGE TGQEAAPLDG VLANPPNISS  60
LSPRQLLGFP CAEVSGLSTE RVRELAVALA QKNVKLSTEQ LRCLAHRLSE PPEDLDALPL 120
DLLLFLNPDA FSGPQACTRF FSRITKANVD LLPRGAPERQ RLLPAALACW GVRGSLLSEA 180
DVRALGGLAC DLPGRFVAES AEVLLPRLVS CPGPLDQDQQ EAARAALQGG GPPYGPPSTW 240
SVSTMDALRG LLPVLGQPII RSIPQGIVAA WRQRSSRDPS WRQPERTILR PRFRREVEKT 300
ACPSGKKARE IDESLIFYKK WELEACVDAA LLATQMDRVN AIPFTYEQLD VLKHKLDELY 360
PQGYPESVIQ HLGYLFLKMS PEDIRKWNVT SLETLKALLE VNKGHEMSPQ VATLIDRFVK 420
GRGQLDKDTL DTLTAFYPGY LCSLSPEELS SVPPSSIWAV RPQDLDTCDP RQLDVLYPKA 480
RLAFQNMNGS EYFVKIQSFL GGAPTEDLKA LSQQNVSMDL ATFMKLRTDA VLPLTVAEVQ 540
KLLGPHVEGL KAEERHRPVR DWILRQRQDD LDTLGLGLQG GIPNGYLVLD LSVQEALSGT 600
PCLLGPGPVL TVLALLLAST LA                                         622
```

Fig. 2

```
VEKTACPSGK KAREIDESLI FYKKWELEAC VDAALLATQM DRVNAIPFTY EQLDVLKHKL  60
DELYPQGYPE SVIQHLGYLF LKMSPEDIRK WNVTSLETLK ALLEVNKGHE MSPQVATLID 120
RFVKGRGQLD KDTLDTLTAF YPGYLCSLSP EELSSVPPSS IWAVRPQDLD TCDPRQLDVL 180
YPKARLAFQN MNGSEYFVKI QSFLGGAPTE DLKALSQQNV SMDLATFMKL RTDAVLPLTV 240
AEVQKLLGPH VEGLKAEERH RPVRDWILRQ RQDDLDTLGL GLQGGIPNGY LVLDLSVQGG 300
RGGQARAGGR AGGVEVGALS HPSLCRGPLG DALPPRTWTC SHRPGTAPSL HPGLRAPLPC 360
WPQPCWGSPP GQEQARVIPV PPQENSRSVN GNMPPADT                         398
```

Fig. 3

```
MST                                                              EVEKTAC 300
                                                                 |||||||
MPF                                                              EVEKTAC 302

MST PSGKKAREID ESLIFYKKWE LEACVDAALL ATQMDRVNAI PFTYEQLDVL KHKLDELYPQ 360
    |||||||||| |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
MPF PSGKKAREID ESLIFYKKWE LEACVDAALL ATQMDRVNAI PFTYEQLDVL KHKLDELYPQ 362

MST GYPESVIQHL GYLFLKMSPE DIRKWNVTSL ETLKALLEVD KGHEMSPQAP RRPLPQVATL 420
    |||||||||| |||||||||| |||||||||| |||||||||+ |||||||||        ||||
MPF GYPESVIQHL GYLFLKMSPE DIRKWNVTSL ETLKALLEVN KGHEMSPQ-- -----VATL 414

MST IDRFVKGRGQ LDKDTLDTLT AFYPGYLCSL SPEELSSVPP SSIWAVRPQD LDTCDPRQLD 480
    |||||||||| |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
MPF IDRFVKGRGQ LDKDTLDTLT AFYPGYLCSL SPEELSSVPP SSIWAVRPQD LDTCDPRQLD 474

MST VLYPKARLAF QNMNGSEYFV KIQSFLGGAP TEDLKALSQQ NVSMDLATFM KLRTDAVLPL 540
    |||||||||| |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
MPF VLYPKARLAF QNMNGSEYFV KIQSFLGGAP TEDLKALSQQ NVSMDLATFM KLRTDAVLPL 534
```

Fig. 4A

```
MST  TVAEVQKLLG PHVEGLKAEE RHRPVRDWIL RQRQDDLDTL GLGLQGGIPN GYLVLDLSVQ 600
     |||||||||| |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
MPF  TVAEVQKLLG PHVEGLKAEE RHRPVRDWIL RQRQDDLDTL GLGLQGGIPN GYLVLDLSVQ 594

MST  ETLSGTPCLL GPGPVLTVLA LLLLASTLA                                   628
     | |||||||| |||||||||| ||||||||
MPF  EALSGTPCLL GPGPVLTVLA LLLLASTLA                                   622
```

Fig. 4B

```
MST                                                                eVEKT  298
MPF                                                                eVEKT  300
SMR                                                                -VEKT  300

MST   ACPSGKKARE IDESLIFYKK WELEACVDAA LLATQMDRVN AIPFTYEQLD VLKHKLDELY  358
MPF   ACPSGKKARE IDESLIFYKK WELEACVDAA LLATQMDRVN AIPFTYEQLD VLKHKLDELY  360
SMR   ACPSGKKARE IDESLIFYKK WELEACVDAA LLATQMDRVN AIPFTYEQLD VLKHKLDELY  360

MST   PQGYPESVIQ HLGYLFLKMS PEDIRKWNVT SLETLKALLE VdKGHEMSPQ APRRPLPQVA  418
MPF   PQGYPESVIQ HLGYLFLKMS PEDIRKWNVT SLETLKALLE VnKGHEMSPQ -------VA  412
SMR   PQGYPESVIQ HLGYLFLKMS PEDIRKWNVT SLETLKALLE VnKGHEMSPQ -------VA  412

MST   TLIDRFVKGR GQLDKDTLDT LTAFYPGYLC SLSPEELSSV PPSSIWAVRP QDLDTCDPRQ  478
MPF   TLIDRFVKGR GQLDKDTLDT LTAFYPGYLC SLSPEELSSV PPSSIWAVRP QDLDTCDPRQ  472
SMR   TLIDRFVKGR GQLDKDTLDT LTAFYPGYLC SLSPEELSSV PPSSIWAVRP QDLDTCDPRQ  472

MST   LDVLYPKARL AFQNMNGSEY FVKIQSFLGG APTEDLKALS QQNVSMDLAT FMKLRTDAVL  538
MPF   LDVLYPKARL AFQNMNGSEY FVKIQSFLGG APTEDLKALS QQNVSMDLAT FMKLRTDAVL  532
SMR   LDVLYPKARL AFQNMNGSEY FVKIQSFLGG APTEDLKALS QQNVSMDLAT FMKLRTDAVL  532

MST   PLTVAEVQKL LGPHVEGLKA EERHRPVRDW ILRQRQDDLD TLGLGLQGGI PNGYLVLDLS  598
MPF   PLTVAEVQKL LGPHVEGLKA EERHRPVRDW ILRQRQDDLD TLGLGLQGGI PNGYLVLDLS  592
SMR   PLTVAEVQKL LGPHVEGLKA EERHRPVRDW ILRQRQDDLD TLGLGLQGGI PNGYLVLDLS  592
```

Fig. 5A

```
MST  VQetlsgtpc  llgpgpvltv  lalllastla  ----------  ----------  ----------  -- 628
MPF  VQealsgtpc  llgpgpvltv  lalllastla  ----------  ----------  ----------  -- 622
SMR  VQggrgggqar aggraggvev  galshpslcr  gplgdalppr  twtcshrpgt  apslhpglra 652

MST  ----------  ----------  ----------  ----------  --
MPF  ----------  ----------  ----------  ----------  --
SMR  plpcwpqpcw  gsppgqeqar  vipvppqensr svngnmppa   dt            694
```

Fig. 5B

...

```
EVEKTACPSG KKAREIDESL IFYKKWELEA CVDAALLATQ MDRVNAIPFT YEQLDVLKHK LDELYPQGYP
ESVIQHLGYL FLKMSPEDIR KWNVTSLETL KALLEVXKGH EMSPQAPRRP LPQVATLIDR FVKGRGQLDK
DTLDTLTAFY PGYLCSLSPE ELSSVPPSSI WAVRPQDLDT CDPRQLDVLY PKARLAFQNM NGSEYFVKIQ
SFLGGAPTED LKALSQQNVS MDLATFMKLR TDAVLPLTVA EVQKLLGPHV EGLKAEERHR PVRDWILRQR
QDDLDTLGLG LQGGIPNGYL VLDLSVQ
```

Fig. 6A

```
EVEKTACPSG KKAREIDESL IFYKKWELEA CVDAALLATQ MDRVNAIPFT YEQLDVLKHK LDELYPQGYP
ESVIQHLGYL FLKMSPEDIR KWNVTSLETL KALLEVXKGH EMSPQVATLI DRFVKGRGQL DKDTLDTLTA
FYPGYLCSLS PEELSSVPPS SIWAVRPQDL DTCDPRQLDV LYPKARLAFQ NMNGSEYFVK IQSFLGGAPT
EDLKALSQQN VSMDLATFMK LRTDAVLPLT VAEVQKLLGP HVEGLKAEER HRPVRDWILR QRQDDLDTLG
LGLQGGIPNG YLVLDLSVQ
```

Fig. 6B
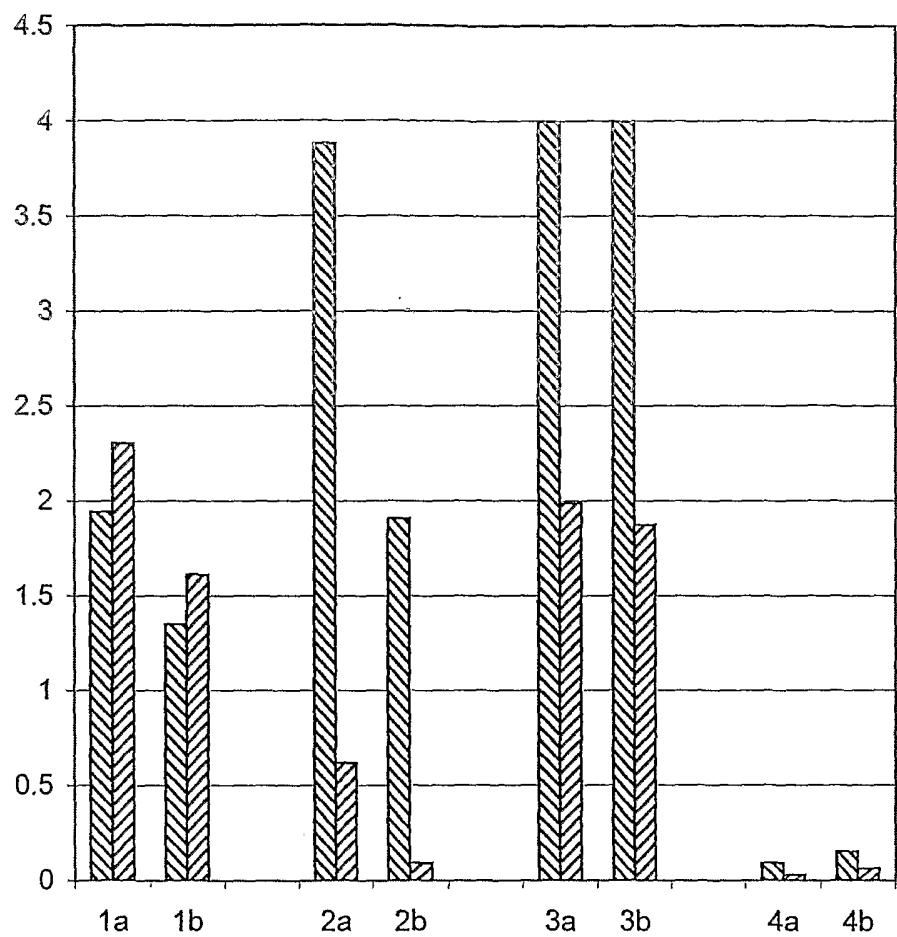
Fig. 7

SEQUENCE LISTING

<110> FUJIREBIO AMERICA, INC.
       O'SHANNESSY, Daniel J.
       SARDESAI, Niranjan Y
       BONES, Jennifer

<120> DETECTION OF URINARY MESOTHELIN-/MEGAKARYOCYTE POTENTIATING
       FACTOR-RELATED PEPTIDES FOR ASSESSMENT OF OVARIAN CANCER

<130> D4496-00021

<140> Not Yet Assigned
<141> 2004-03-15

<150> US 10/388,930
<151> 2003-03-13

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<170> PatentIn version 3.2

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<213> Homo sapiens

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            20   25   30

Arg Thr Leu Ala Gly Glu Thr Gly Thr Glu Ser Ala Pro Leu Gly Gly
        35   40   45

Val Leu Thr Thr Pro His Asn Ile Ser Ser Leu Ser Pro Arg Gln Leu
    50   55   60

Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu Arg Val
65   70   75   80

Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu Ser Thr
                85   90   95

Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro Glu Asp
            100   105   110

Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro Asp Ala
        115   120   125

Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile Thr Lys
        130   135   140

Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln Arg Leu
145   150   155   160

Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu Leu Ser
                165   170   175

Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu Pro Gly
            180   185   190

Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu Val Ser
        195   200   205

Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg Ala Ala
    210   215   220

Leu Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp Ser Val
225   230   235   240

Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly Gln Pro
                245   250   255

Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg Gln Arg
            260   265   270

Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile Leu Arg
        275   280   285

Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser Gly Lys
    290   295   300

Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys Trp Glu
305   310   315   320

Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg
            325   330   335

Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu Lys His

```
            340                    345                    350



Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln
        355                    360                    365


His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile Arg Lys
    370                    375                    380


Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu Val Asp
385                    390                    395                    400


Lys Gly His Glu Met Ser Pro Gln Ala Pro Arg Arg Pro Leu Pro Gln
                405                    410                    415


Val Ala Thr Leu Ile Asp Arg Phe Val Lys Gly Arg Gly Gln Leu Asp
            420                    425                    430


Lys Asp Thr Leu Asp Thr Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys
        435                    440                    445


Ser Leu Ser Pro Glu Glu Leu Ser Ser Val Pro Pro Ser Ser Ile Trp
    450                    455                    460


Ala Val Arg Pro Gln Asp Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp
465                    470                    475                    480


Val Leu Tyr Pro Lys Ala Arg Leu Ala Phe Gln Asn Met Asn Gly Ser
                485                    490                    495


Glu Tyr Phe Val Lys Ile Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu
            500                    505                    510


Asp Leu Lys Ala Leu Ser Gln Gln Asn Val Ser Met Asp Leu Ala Thr
        515                    520                    525


Phe Met Lys Leu Arg Thr Asp Ala Val Leu Pro Leu Thr Val Ala Glu
        530                    535                    540


Val Gln Lys Leu Leu Gly Pro His Val Glu Gly Leu Lys Ala Glu Glu
545                    550                    555                    560


Arg His Arg Pro Val Arg Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp
                565                    570                    575
```

Leu Asp Thr Leu Gly Leu Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr
            580   585   590

Leu Val Leu Asp Leu Ser Val Gln Glu Thr Leu Ser Gly Thr Pro Cys
            595   600   605

Leu Leu Gly Pro Gly Pro Val Leu Thr Val Leu Ala Leu Leu Leu Ala
        610   615   620

Ser Thr Leu Ala
625

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Ala Leu Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp Val Gln
            20   25   30

Pro Ser Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu
            35   40   45

Asp Gly Val Leu Ala Asn Pro Pro Asn Ile Ser Ser Leu Ser Pro Arg
        50   55   60

Gln Leu Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu
65   70   75   80

Arg Val Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu
                85   90   95

Ser Thr Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro
            100   105   110

Glu Asp Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro
            115   120   125

Asp Ala Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile
    130   135   140

Thr Lys Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln
145   150   155   160

Arg Leu Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu
                165   170   175

Leu Ser Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu
                180   185   190

Pro Gly Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu
            195   200   205

Val Ser Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg
    210   215   220

Ala Ala Leu Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp
225   230   235   240

Ser Val Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly
                245   250   255

Gln Pro Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg
                260   265   270

Gln Arg Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile
        275   280   285

Leu Arg Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser
    290   295   300

Gly Lys Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys
305   310   315   320

Trp Glu Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met
                325   330   335

Asp Arg Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu
            340   345   350

Lys His Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val

```
         355                    360                    365


Ile Gln His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile
    370                    375                380

Arg Lys Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu
385                    390                395                400

Val Asn Lys Gly His Glu Met Ser Pro Gln Val Ala Thr Leu Ile Asp
                405                410                415

Arg Phe Val Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp Thr
            420                425                430

Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu Glu
            435                440                445

Leu Ser Ser Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln Asp
    450                455                460

Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys Ala
465                470                475                480

Arg Leu Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys Ile
            485                490                495

Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu Ser
            500                505                510

Gln Gln Asn Val Ser Met Asp Leu Ala Thr Phe Met Lys Leu Arg Thr
            515                520                525

Asp Ala Val Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu Gly
    530                535                540

Pro His Val Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val Arg
545                550                555                560

Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly Leu
            565                570                575

Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp Leu Ser
            580                585                590
```

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Val Gln Glu Ala Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly Pro
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Val Leu Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
    610                 615             620


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1               5               10              15

Glu Ser Leu Ile Phe Tyr Lys Lys Trp Glu Leu Glu Ala Cys Val Asp
            20              25              30

Ala Ala Leu Leu Ala Thr Gln Met Asp Arg Val Asn Ala Ile Pro Phe
        35              40              45

Thr Tyr Glu Gln Leu Asp Val Leu Lys His Lys Leu Asp Glu Leu Tyr
    50              55              60

Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln His Leu Gly Tyr Leu Phe
65              70              75              80

Leu Lys Met Ser Pro Glu Asp Ile Arg Lys Trp Asn Val Thr Ser Leu
            85              90              95

Glu Thr Leu Lys Ala Leu Leu Glu Val Asn Lys Gly His Glu Met Ser
            100             105             110

Pro Gln Val Ala Thr Leu Ile Asp Arg Phe Val Lys Gly Arg Gly Gln
        115             120             125

Leu Asp Lys Asp Thr Leu Asp Thr Leu Thr Ala Phe Tyr Pro Gly Tyr
    130             135             140

Leu Cys Ser Leu Ser Pro Glu Glu Leu Ser Ser Val Pro Pro Ser Ser
145             150             155             160
```

Ile Trp Ala Val Arg Pro Gln Asp Leu Asp Thr Cys Asp Pro Arg Gln
                165   170   175

Leu Asp Val Leu Tyr Pro Lys Ala Arg Leu Ala Phe Gln Asn Met Asn
                180   185   190

Gly Ser Glu Tyr Phe Val Lys Ile Gln Ser Phe Leu Gly Gly Ala Pro
                195   200   205

Thr Glu Asp Leu Lys Ala Leu Ser Gln Gln Asn Val Ser Met Asp Leu
    210   215   220

Ala Thr Phe Met Lys Leu Arg Thr Asp Ala Val Leu Pro Leu Thr Val
225   230   235   240

Ala Glu Val Gln Lys Leu Leu Gly Pro His Val Glu Gly Leu Lys Ala
                245   250   255

Glu Glu Arg His Arg Pro Val Arg Asp Trp Ile Leu Arg Gln Arg Gln
                260   265   270

Asp Asp Leu Asp Thr Leu Gly Leu Gly Leu Gln Gly Gly Ile Pro Asn
                275   280   285

Gly Tyr Leu Val Leu Asp Leu Ser Val Gln Gly Gly Arg Gly Gly Gln
    290   295   300

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

His Pro Ser Leu Cys Arg Gly Pro Leu Gly Asp Ala Leu Pro Pro Arg
                325   330   335

Thr Trp Thr Cys Ser His Arg Pro Gly Thr Ala Pro Ser Leu His Pro
                340   345   350

Gly Leu Arg Ala Pro Leu Pro Cys Trp Pro Gln Pro Cys Trp Gly Ser
        355   360   365

Pro Pro Gly Gln Glu Gln Ala Arg Val Ile Pro Val Pro Pro Gln Glu
    370   375   380

Asn Ser Arg Ser Val Asn Gly Asn Met Pro Pro Ala Asp Thr

385   390   395

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Glu Val Glu Lys Thr Ala Cys Pro Ser Gly Lys Lys Ala Arg Glu Ile
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Asp Glu Ser Leu Ile Phe Tyr Lys Lys Trp Glu Leu Glu Ala Cys Val
            20   25   30

Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg Val Asn Ala Ile Pro
        35   40   45

Phe Thr Tyr Glu Gln Leu Asp Val Leu Lys His Lys Leu Asp Glu Leu
    50   55   60

Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln His Leu Gly Tyr Leu
65   70   75   80

Phe Leu Lys Met Ser Pro Glu Asp Ile Arg Lys Trp Asn Val Thr Ser
                85   90   95

Leu Glu Thr Leu Lys Ala Leu Leu Glu Val Xaa Lys Gly His Glu Met
            100   105   110

Ser Pro Gln Ala Pro Arg Arg Pro Leu Pro Gln Val Ala Thr Leu Ile
        115   120   125

Asp Arg Phe Val Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp

-9-

```
        130                  135                  140
```

Thr Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu
145   150   155   160

Glu Leu Ser Ser Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln
            165   170   175

Asp Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys
            180   185   190

Ala Arg Leu Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys
        195   200   205

Ile Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu
    210   215   220

Ser Gln Gln Asn Val Ser Met Asp Leu Ala Thr Phe Met Lys Leu Arg
225   230   235   240

Thr Asp Ala Val Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu
            245   250   255

Gly Pro His Val Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val
            260   265   270

Arg Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly
        275   280   285

Leu Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp Leu
    290   295   300

Ser Val Gln
305

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<212>   PRT
<213>   artificial

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<223>   Preferred epitopes for generation of antibodies

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<221>  VARIANT
<222>  (107)..(107)
<223>  X is any amino acid residue

<220>
<221>  misc_feature
<222>  (107)..(107)
<223>  Xaa can be any naturally occurring amino acid

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Glu Val Glu Lys Thr Ala Cys Pro Ser Gly Lys Lys Ala Arg Glu Ile
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Asp Glu Ser Leu Ile Phe Tyr Lys Lys Trp Glu Leu Glu Ala Cys Val
            20   25   30

Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg Val Asn Ala Ile Pro
        35   40   45

Phe Thr Tyr Glu Gln Leu Asp Val Leu Lys His Lys Leu Asp Glu Leu
    50   55   60

Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln His Leu Gly Tyr Leu
65   70   75   80

Phe Leu Lys Met Ser Pro Glu Asp Ile Arg Lys Trp Asn Val Thr Ser
                85   90   95

Leu Glu Thr Leu Lys Ala Leu Leu Glu Val Xaa Lys Gly His Glu Met
            100   105   110

Ser Pro Gln Val Ala Thr Leu Ile Asp Arg Phe Val Lys Gly Arg Gly
        115   120   125

Gln Leu Asp Lys Asp Thr Leu Asp Thr Leu Thr Ala Phe Tyr Pro Gly
        130   135   140

Tyr Leu Cys Ser Leu Ser Pro Glu Glu Leu Ser Ser Val Pro Pro Ser
145   150   155   160

Ser Ile Trp Ala Val Arg Pro Gln Asp Leu Asp Thr Cys Asp Pro Arg
                165   170   175

Gln Leu Asp Val Leu Tyr Pro Lys Ala Arg Leu Ala Phe Gln Asn Met
        180   185   190

Asn Gly Ser Glu Tyr Phe Val Lys Ile Gln Ser Phe Leu Gly Gly Ala
        195   200   205

Pro Thr Glu Asp Leu Lys Ala Leu Ser Gln Gln Asn Val Ser Met Asp
        210   215   220

Leu Ala Thr Phe Met Lys Leu Arg Thr Asp Ala Val Leu Pro Leu Thr
225   230   235   240

Val Ala Glu Val Gln Lys Leu Leu Gly Pro His Val Glu Gly Leu Lys
                245   250   255

Ala Glu Glu Arg His Arg Pro Val Arg Asp Trp Ile Leu Arg Gln Arg
            260   265   270

Gln Asp Asp Leu Asp Thr Leu Gly Leu Gly Leu Gln Gly Gly Ile Pro
        275   280   285

Asn Gly Tyr Leu Val Leu Asp Leu Ser Val Gln
    290   295