US 20090042224A1

(19) **United States**

(12) **Patent Application Publication**

Hellstrom et al.

(10) **Pub. No.: US 2009/0042224 A1**

(43) **Pub. Date: Feb. 12, 2009**

(54) **METHODS FOR DIAGNOSIS AND/OR PROGNOSIS OF OVARIAN CANCER**

(75) Inventors: **Ingegerd Hellstrom**, Seattle, WA (US); **Karl Erik Hellstrom**, Seattle, WA (US); **Yi Yang**, Lynnwood, WA (US)

Correspondence Address:
CHRISTENSEN, O'CONNOR, JOHNSON, KINDNESS, PLLC
1420 FIFTH AVENUE, SUITE 2800
SEATTLE, WA 98101-2347 (US)

(73) Assignee: **WASHINGTON, UNIVERSITY OF**, Seattle, WA (US)

(21) Appl. No.: **12/134,093**

(22) Filed: **Jun. 5, 2008**

Related U.S. Application Data

(60) Provisional application No. 60/942,102, filed on Jun. 5, 2007.

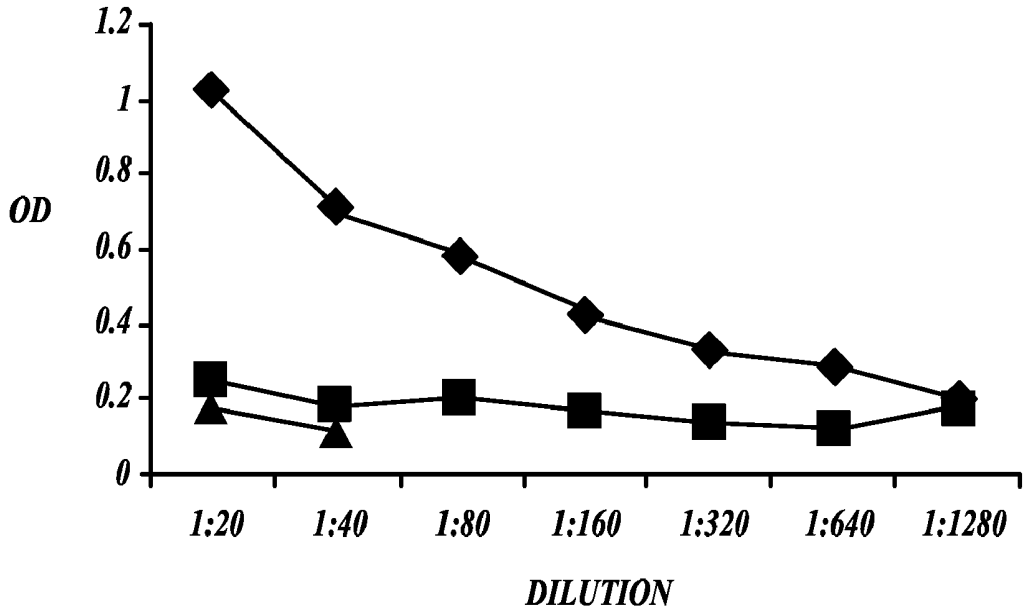
Publication Classification

(51) **Int. Cl.**
G01N 33/53 (2006.01)
G01N 33/566 (2006.01)

(52) **U.S. Cl.** **435/7.92**; 436/501; 435/7.1

(57) **ABSTRACT**

In one embodiment, methods are provided for assessing the presence of mesothelin-expressing tumor cells in a human subject. In another embodiment, methods are provided for monitoring the efficacy of treatment of a human cancer patient diagnosed with a mesothelin-expressing tumor.



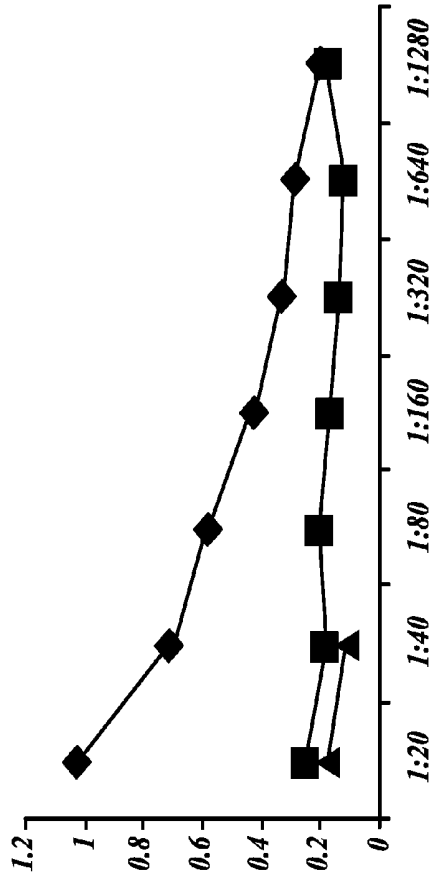

1.2
1
0.8
0.6
0.4
0.2
0
OD
1:20
1:40
1:80
1:160
1:320
1:640
1:1280
DILUTION

Fig.1.

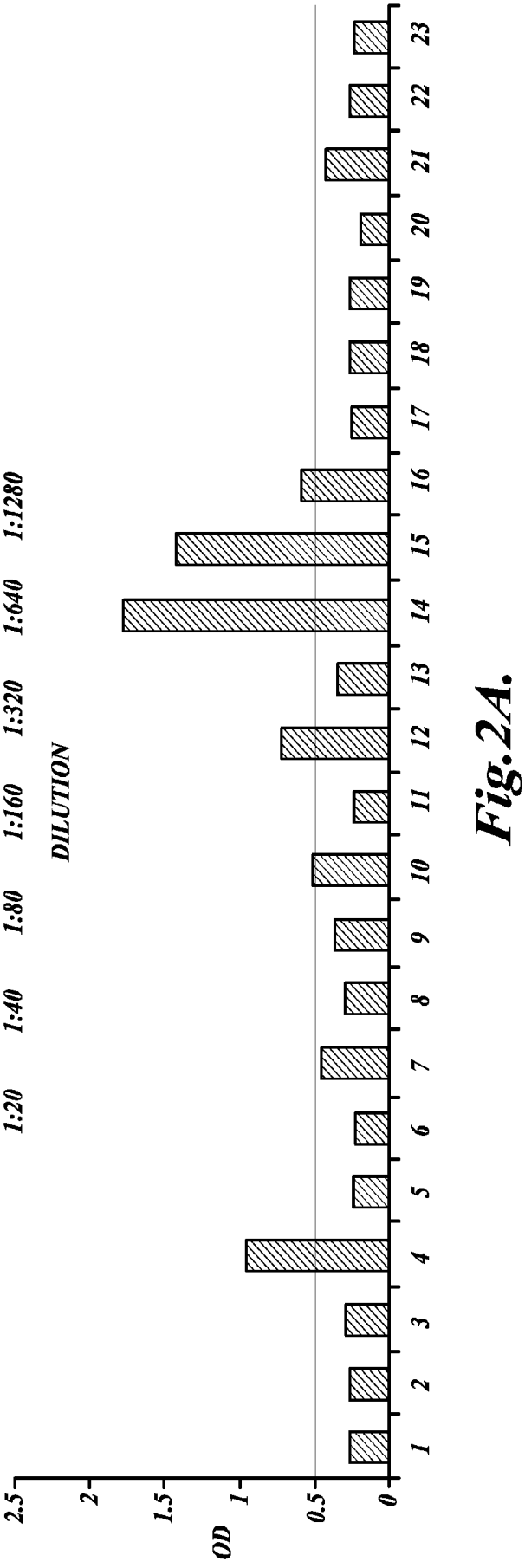

2.5
2
1.5
1
0.5
0
OD
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23

Fig.2A.

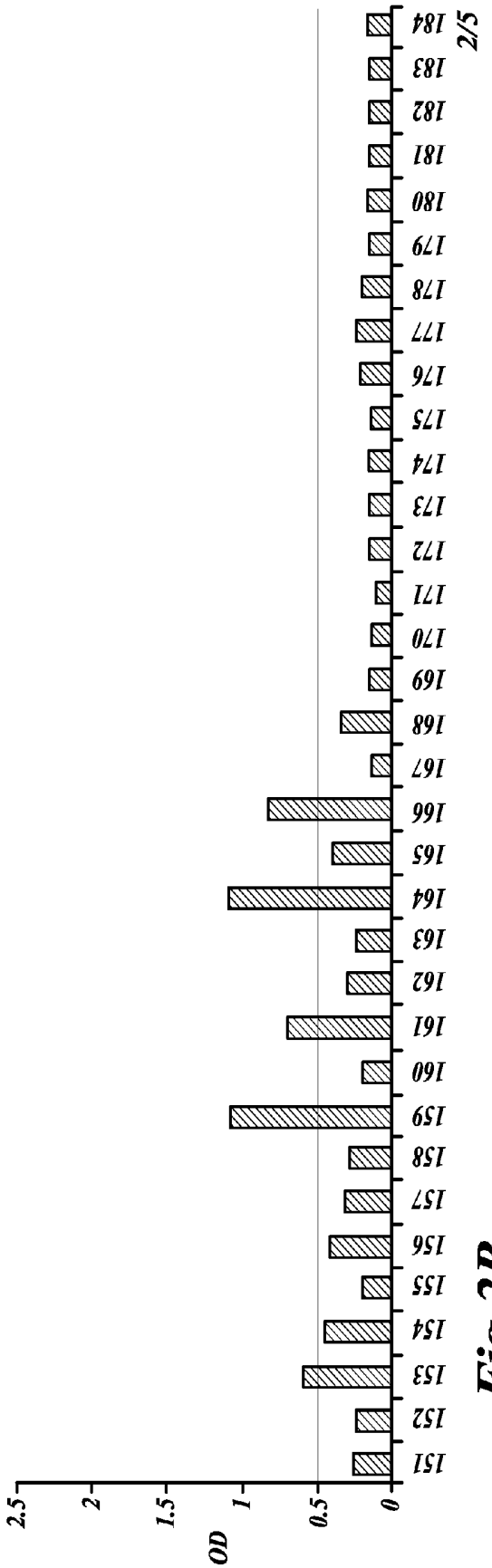

OD
2.5
2
1.5
1
0.5
0
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
2/5

Fig.2B.

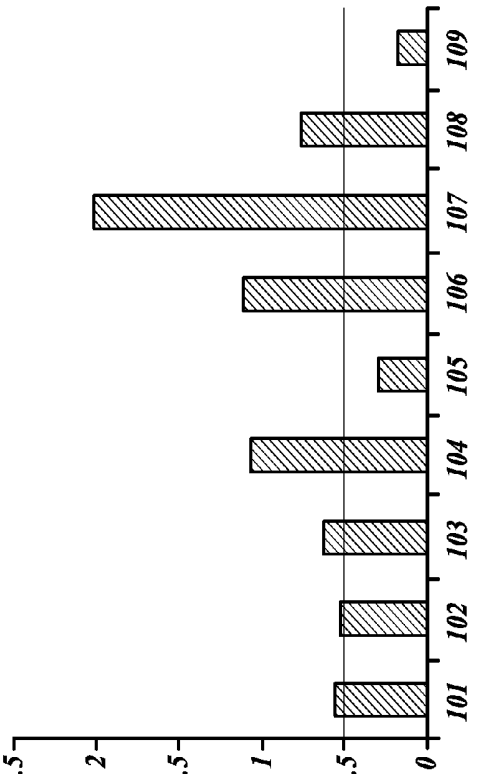

OD
2.5
2
1.5
1
0.5
0
101
102
103
104
105
106
107
108
109

Fig.2C.

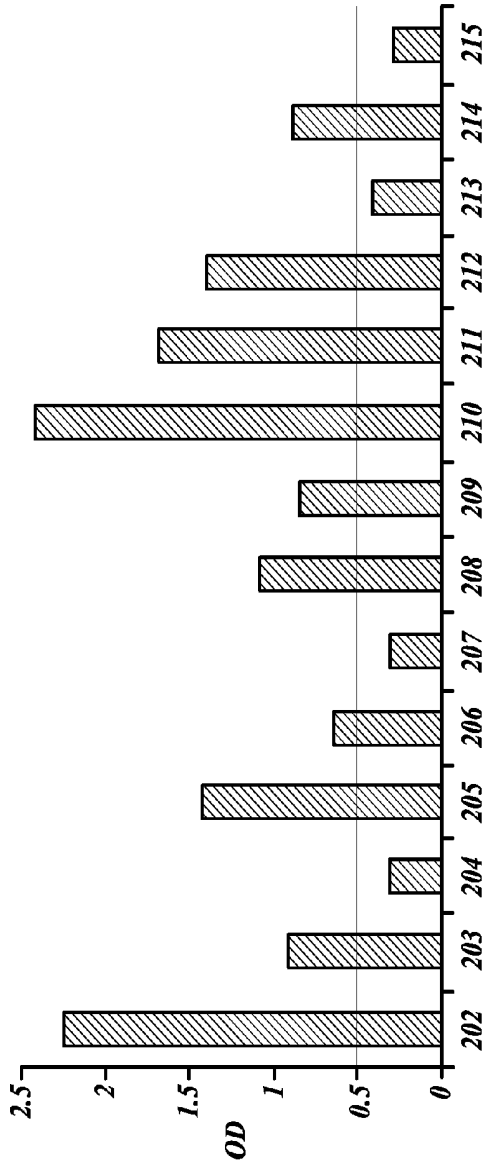

OD
2.5
2
1.5
1
0.5
0
202
203
204
205
206
207
208
209
210
211
212
213
214
215

Fig.3A.

OD
2.5
2
1.5
1
0.5
0
-0.5
202
203
204
205
206
207
208
209
210
211
212
213
214
215

Fig.3B.

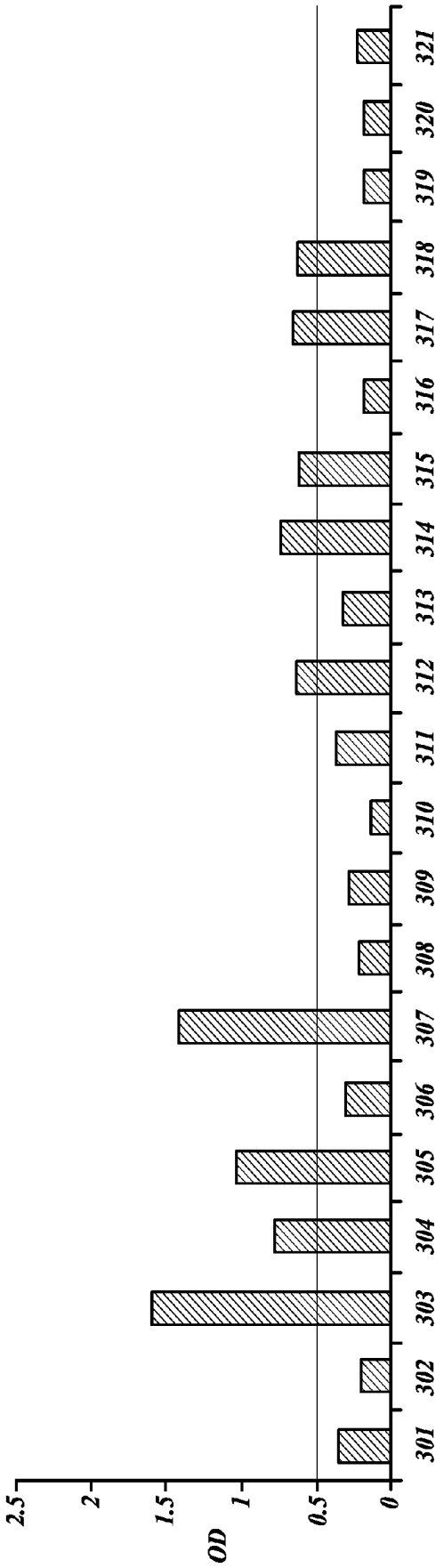

OD
2.5
2
1.5
1
0.5
0
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321

Fig. 4A.

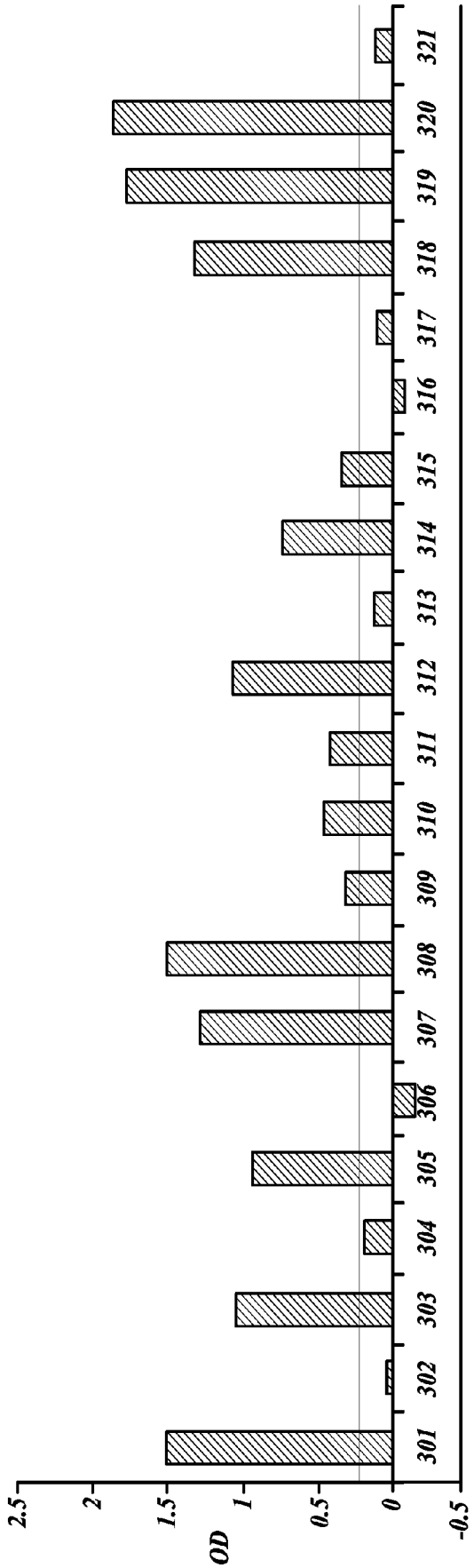

OD
2.5
2
1.5
1
0.5
0
-0.5
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321

Fig.4B.

METHODS FOR DIAGNOSIS AND/OR PROGNOSIS OF OVARIAN CANCER

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of Provisional Application No. 60/942,102 filed Jun. 5, 2007.

FIELD OF THE INVENTION

[0002] The present invention relates to the diagnosis and/or prognosis of subjects suffering from cancer associated with mesothelin-expressing tumors and, in particular, to methods of measuring anti-mesothelin antibodies for use as an indicator of the presence of mesothelin expressing tumors and/or the clinical status of a patient undergoing treatment for a cancer associated with one or more mesothelin-expressing tumors.

BACKGROUND OF THE INVENTION

[0003] Ovarian carcinoma (OvC) is the second most frequent and the most lethal gynecologic malignancy in the western world. Most cases are diagnosed at an advanced stage and this is reflected by a poor prognosis with the overall five-year survival rate not exceeding 35%. Ovarian carcinoma is disproportionately deadly because symptoms are vague and non-specific. Ovarian cancers shed malignant cells into the naturally occurring fluid within the abdominal cavity. These cells then have the potential to float in this fluid and frequently implant on other abdominal (peritoneal) structures including the uterus, urinary bladder, bowel and lining of the bowel wall (omentum). These cells can begin forming new tumor growths before cancer is even suspected. More than 60% of patients presenting with this disease already have stage III or stage IV disease, when it has already spread beyond the ovaries, and more than 75% of these patients die from disease, in spite of recent improvements of chemotherapy for ovarian cancer. However, if diagnosis is made early in the disease, five-year survival rates can reach 90% to 98%.

[0004] One marker for ovarian cancer that is used in serum assays for ovarian cancer is CA125 (Bast, R. C., et al., *Gynecol. Oncol.* 22:115-120 (1985); Einhorn, N., et al., *Obstet. Gynaecol.* 67:414-416 (1986); Einhorn, et al., *Obstet. Gynecol.* 80:14-18 (1992); Jacobs, I. J., et al., *Br. Med. J.* 313:1355-1358 (1996)). However, CA125 is also elevated in several non-malignant conditions (Fung, M. F., et al., *J. Obstet. Gynaecol. Can.,* 26:717-728 (2004); Mas, M. R., et al., *Dig. Liver Dis.* 32:595-597 (2000); Malkasian, G. D., et al., *Am. J. Obstet. Gynecol.* 159:341-346 (1988)), which can lead to a false positive result.

[0005] Mesothelin is highly expressed on the surface of pancreatic cancers, ovarian cancers, mesothelioma, lung cancers, and some other cancers. See, e.g., Scholler, et al., *Proc. Natl. Acad. Sci. USA* 96:11531-11536 (1999); Cao, et al., *Mod. Pathol.* 14 (2005); Hassan, et al., *Clin. Cancer Res.* 10:3937-42 (2004).

[0006] Thus, there is a need to develop more effective tools for detecting potentially curable, early stage ovarian carcinoma and other cancers that have tumors that express mesothelin. There is also a need to identify patients afflicted with ovarian cancer and other cancer cells that express mesothelin who are most likely to have tumor recurrence following therapy, and to have effective tools for detecting recurrence as early as possible.

SUMMARY OF THE INVENTION

[0007] In accordance with the foregoing, in one aspect, a method is provided for detecting the presence of mesothelin-expressing tumor cells in a human subject comprising determining the presence or amount of anti-mesothelin antibodies in a biological sample obtained from the human subject, wherein the presence or amount of anti-mesothelin antibodies in the biological sample is indicative of the presence of mesothelin-expressing tumor cells in the human subject.

[0008] In another aspect, a method is provided for monitoring the efficacy of treatment of a human cancer patient undergoing therapeutic treatment for a mesothelin-expressing tumor. The method comprises: (a) providing a biological sample from a human patient undergoing therapeutic treatment for a cancer associated with a mesothelin-expressing tumor; (b) determining the presence or amount of anti-mesothelin antibodies in the biological sample by contacting the biological sample with a polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence at least 90% identical to a sequence comprising at least 20 contiguous nucleotides of SEQ ID NO: 1; and (c) comparing the presence or amount of anti-mesothelin antibodies determined in step (b) to an antibody reference value, wherein an amount of anti-mesothelin antibody greater than the antibody reference value is indicative of a positive response to the therapeutic treatment for the cancer.

[0009] In another aspect, a kit is provided for detecting the presence of mesothelin-expressing tumor cells in a human subject comprising reagents specific for detection of the presence or amount of anti-mesothelin antibodies in a biological sample obtained from a human subject and printed instructions for comparison of the detected presence or amount of anti-mesothelin antibodies with a reference standard.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

[0011] FIG. **1** graphically illustrates the titration of sera using an ELISA to detect anti-mesothelin antibodies from one ovarian carcinoma patient with no evidence of disease ("NED") and one healthy donor as described in Example 1, wherein the diamond symbols represent NED patient number 208, the squares represent healthy control subject number 8, and the triangles represent the negative (BSA) control;

[0012] FIG. **2**A graphically illustrates the results of an anti-mesothelin antibody ELISA assay demonstrating the concentration level of antibodies to mesothelin in sera, diluted 1:20, obtained from apparently healthy women, as described in Example 2;

[0013] FIG. **2**B graphically illustrates the results of an anti-mesothelin antibody ELISA assay demonstrating the concentration level of antibodies to mesothelin in sera obtained from women with non-malignant gynecological conditions excluding pelvic inflammatory disease, as described in Example 2;

[0014] FIG. **2**C graphically illustrates the results of an anti-mesothelin antibody ELISA assay demonstrating the concen-

tration level of antibodies to mesothelin in sera obtained from women with pelvic inflammatory disease, as described in Example 2;

[0015] FIG. **3**A graphically illustrates the results of an anti-mesothelin antibody ELISA assay demonstrating the concentration level of antibodies to mesothelin in sera obtained from ovarian cancer patients exhibiting no clinical evidence of disease after treatment for ovarian cancer, as described in Example 2;

[0016] FIG. **3**B graphically illustrates the results of an anti-soluble mesothelin-related protein (SMRP) ELISA assay demonstrating the concentration level of circulating SMRP in sera obtained from the same panel of ovarian cancer patients shown in FIG. **3**A, as described in Example 3;

[0017] FIG. **4**A graphically illustrates the results of an anti-mesothelin antibody ELISA assay demonstrating the concentration level of antibodies to mesothelin in sera obtained from ovarian cancer patients exhibiting clinical evidence of disease, as described in Example 2; and

[0018] FIG. **4**B graphically illustrates the results of an anti-soluble mesothelin-related protein (SMRP) ELISA assay demonstrating the concentration level of circulating SMRP in sera obtained from the same panel of ovarian cancer patients shown in FIG. **4**A, as described in Example 3.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0019] Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention.

[0020] The terms "percent identity" or "percent identical," as applied to polypeptide sequences, such as the mesothelin polypeptide, or a portion thereof, is defined as the percentage of amino acid residues in a candidate protein sequence that are identical with the subject protein sequence (such as the amino acid sequence set forth in SEQ ID NO:2, or a portion thereof comprising at least 10 consecutive amino acid residues) after aligning the candidate and subject sequences to achieve the maximum percent identity. For example, percentage identity between two protein sequences can be determined by pairwise comparison of the two sequences using the bl2seq interface at the Web site of the National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, Md. 20894, U.S. A. The bl2seq interface permits sequence alignment using the BLAST tool described by Tatiana, A., et al., "Blast 2 Sequences—A New Tool for Comparing Protein and Nucleotide Sequences," *FEMS Microbiol. Lett.* 174:247-250 (1999). The following alignment parameters are used: Matrix=BLOSUM62; Gap open penalty=11; Gap extension penalty=1; Gap x_dropff=50; Expect=10.0; Word size=3; and Filter=off.

[0021] The terms "percent identity" or "percent identical," as applied to nucleic acid molecules, is the percentage of nucleotides in a candidate nucleic acid sequence that are identical with a subject nucleic acid molecule sequence (such as the nucleic acid molecule sequence set forth in SEQ ID NO: 1, or a portion thereof comprising at least 20 consecutive nucleotides) after aligning the sequences to achieve the maximum percent identity, and not considering any nucleic acid residue substitutions as part of the sequence identity. No gaps are introduced into the candidate nucleic acid sequence in order to achieve the best alignment. Nucleic acid sequence identity can be determined in the following manner. The subject polynucleotide molecule sequence is used to search a nucleic acid sequence database, such as the Genbank database, using the program BLASTN version 2.1 (based on Altschul, et al., *Nucleic Acids Research* 25:3389-3402 (1997)). The program is used in the ungapped mode. Default filtering is used to remove sequence homologies due to regions of low complexity as defined in Wootton, J. C., and S. Federhen, *Methods in Enzymology* 266:554-571 (1996). The default parameters of BLASTN are utilized.

[0022] As used herein, the term "healthy human subject" refers to an individual who is known not to suffer from cancer, such knowledge being derived from clinical data on the individual including, but not limited to, a different cancer assay to that described herein. The healthy individual is also preferably asymptomatic with respect to the early symptoms associated with mesothelin-expressing tumors such as ovarian cancer, which include, for example, rectal pressure, abdominal bloating, and swelling.

[0023] As used herein, the term "mesothelin-expressing tumor" refers to any type of cancer cells and/or tumors that are identified as having a neoplastic condition associated with an increased expression of mesothelin as compared to normal tissues, including but not limited to, ovarian cancer, mesothelioma, pancreatic carcinoma, or lung carcinoma. See, e.g., Scholler, et al., *Proc. Natl. Acad. Sci. USA* 96:11531-11536 (1999).

[0024] As used herein, the term "ovarian cancer" refers to any type of ovarian cancer including, but not limited to, serous ovarian cancer, non-invasive ovarian cancer, mixed phenotype ovarian cancer, mucinous ovarian cancer, endometrioid ovarian cancer, clear cell ovarian cancer, papillary serous ovarian cancer, Brenner cell, and undifferentiated adenocarcinoma.

[0025] As used herein, the term "recurrence of a tumor expressing mesothelin" refers to clinical evidence of cancer related to cells expressing mesothelin, for example, ovarian cancer, mesothelioma, pancreatic carcinoma, or lung carcinoma, or tumor cells derived therefrom based upon clinical data on the individual including, but not limited to, a different cancer assay to that described herein.

[0026] As used herein, the term "good prognosis" in the context of cancer associated with one or more mesothelin-expressing tumors (e.g., ovarian cancer) refers to patients who are likely to be cured from their disease, or to have at least a five-year tumor-free survival period following the initial diagnosis.

[0027] As used herein, the term "poor prognosis" in the context of cancer associated with one or more mesothelin-expressing tumors (e.g., ovarian cancer) refers to patients who are likely to die from their disease within a five-year period following the initial diagnosis.

[0028] In one aspect, a method is provided for detecting the presence of mesothelin-expressing tumor cells in a human subject. The method comprises determining the presence or amount of anti-mesothelin antibodies in a biological sample obtained from the human subject, wherein the presence or amount of anti-mesothelin antibodies in the biological sample indicates the presence of mesothelin-expressing tumor cells in the human subject. In one embodiment, the presence or amount of anti-mesothelin antibodies in the biological sample is determined by contacting the biological sample with a polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence at least 80% identical, or at least 90% identical to a sequence comprising at least 20

contiguous nucleotides of SEQ ID NO: 1. In one embodiment, the presence or amount of anti-mesothelin antibodies in comparison to a reference standard (e.g., a negative control) is indicative of the presence of mesothelin-expressing cells, such as tumor cells in the human subject. In another embodiment, the amount of anti-mesothelin antibodies over a predetermined threshold amount is indicative of the presence of mesothelin-expressing tumor cells in a human subject.

[0029] A wide variety of biological samples may be used in the methods of the invention, including biological fluids. Non-limiting examples of biological fluids include blood, plasma, serum, ascitic fluid, urine, saliva, tears, pleural fluid, sputum, vaginal fluid (discharge), and washings obtained during a medical procedure (e.g., pelvic or other washings obtained during biopsy, endoscopy or surgery).

[0030] The methods of this aspect of the invention may be used as a diagnostic tool to distinguish between a subject suffering from a disease associated with the expression of mesothelin and a disease or disorder not associated with the expression of mesothelin. Examples of diseases associated with the expression of mesothelin include ovarian cancer, mesothelioma, pancreatic cancer, lung carcinoma, and pelvic inflammatory disease. In some embodiments, the methods of the invention may be used as a diagnostic tool to distinguish between a subject suffering from a disease related to a mesothelin-expressing tumor and a disease or disorder unrelated to the presence of mesothelin-expressing cancer cells. In such embodiments, a biological sample is obtained from a human subject suffering from at least one symptom associated with a mesothelin-expressing tumor (e.g., ovarian cancer, mesothelioma, pancreatic cancer or lung carcinoma) and assayed for the presence or amount of anti-mesothelin antibodies, wherein the presence or amount of anti-mesothelin antibodies is indicative of the presence of mesothelin-expressing tumor cells in the subject, and the absence of anti-mesothelin antibodies is indicative of a disease or disorder unrelated to the presence of mesothelin expressing cancer cells.

[0031] In one embodiment, the method of this aspect of the invention further comprises determining if the human subject having anti-mesothelin antibodies has pelvic inflammatory disease.

[0032] In another embodiment, the methods of the invention may be used as a diagnostic tool to distinguish between a subject suffering from pelvic inflammatory disease and a subject suffering from a non-malignant (benign) gynecological condition. In one embodiment of this aspect of the invention, the presence of anti-mesothelin antibodies indicates the subject is suffering from pelvic inflammatory disease, whereas the absence of anti-mesothelin antibodies indicates the subject is suffering from a non-malignant gynecological condition.

[0033] In one embodiment of the method, a biological sample is obtained from a human subject suffering from at least one symptom associated with ovarian cancer. Symptoms associated with ovarian cancer are known to those of skill in the field of medicine. Non-limiting examples of such symptoms include abdominal swelling/bloating; abdominal/pelvic pain or pressure; gastrointestinal symptoms (e.g., gas, indigestion, nausea, or changes in bowel movements); vaginal bleeding or discharge; urinary problems (e.g., urgency, burning or spasms); fatigue; fever; back pain; difficulty breathing. In some embodiments, the methods of this aspect of the invention further comprise performing at least one additional diagnostic assay for ovarian cancer on the subject, such as, for example, detecting the presence of CA125 in a biological sample, ultrasound, CT scan, MRI scan, biopsy, aspirate, and the like.

[0034] In one embodiment of the method, the presence of anti-mesothelin antibodies in a sample obtained from a human subject suffering from ovarian cancer that does not have pelvic inflammatory disease is indicative of the presence of mesothelin-expressing tumor cells in the subject.

[0035] In another embodiment of the method, a biological sample is obtained from a human subject suffering from at least one symptom associated with mesothelioma. Symptoms associated with mesothelioma are known to those of skill in the in the field of medicine. Non-limiting examples of symptoms associated with pleural mesothelioma may include difficulty in breathing, chest pain, weight loss, fever, night sweats, cough. Non-limiting examples of symptoms associated with peritoneal mesothelioma may include swelling, pain due to accumulation of fluid in the abdominal cavity, weight loss, mass in the abdomen, bowel obstruction, blood clotting abnormalities, anemia and/or fever. In some embodiments, the methods of this aspect of the invention further comprise performing at least one additional diagnostic assay for mesothelioma on the subject, such as, for example, chest x-ray, ultrasound, CT scan, MRI scan, biopsy, aspirate, and the like.

[0036] In another embodiment of the method, a biological sample is obtained from a human subject suffering from at least one symptom associated with pancreatic cancer. Symptoms associated with pancreatic cancer are well known to those of skill in the field of medicine and include, but are not limited to weight loss, loss of appetite, discomfort or pain around the stomach area, back pain and/or jaundice. In some embodiments, the methods of this aspect of the invention further comprise performing at least one additional diagnostic assay for pancreatic cancer on the subject such as, for example, ultrasound, CT scan, MRI scan, biopsy, aspirate, and the like.

[0037] In another embodiment of the method, a biological sample is obtained from a human subject suffering from at least one symptom associated with lung carcinoma. Symptoms associated with lung carcinoma are well known to those of skill in the field of medicine and include, but are not limited to coughing, hoarseness, hemoptysis, dyspnea, noncardiac chest pain, extrathoracic pain, neurologic symptoms, weight loss, and weakness/fatigue. In some embodiments, the methods of this aspect of the invention further comprise performing at least one additional diagnostic assay for lung carcinoma on the subject, such as, for example, chest x-ray, CT scan, MRI scan, biopsy, aspirate, and the like.

[0038] In another embodiment of the method, a biological sample is obtained from a human subject suffering from at least one symptom associated with pelvic inflammatory disease (PID). As used herein, PID refers to infection and inflammation of the female reproductive organs, including the uterus, fallopian tubes, ovaries, and other reproductive organs. Symptoms associated with pelvic inflammatory disease are well known to those of skill in the field of medicine and include, but are not limited to lower abdominal pain, fever, fatigue, diarrhea or vomiting, vaginal discharge with or without an unpleasant odor, pain during sexual intercourse, painful or difficult urination, irregular menstrual bleeding, and low back pain. In another embodiment, a biological sample is obtained from a human subject who is not experi-

encing any symptoms of PID, but who may be at risk of developing PID. Subjects at risk of developing PID are well known to those of skill in the field of medicine and include, but are not limited to subjects with sexually transmitted diseases, such as gonorrhea and/or Chlamydia, sexually active women in their childbearing years, in particular those under age 25 years, subjects with multiple sexual partners and/or whose partners have more than one sexual partner, and subjects who douche or use an intrauterine device. In some embodiments, the methods of this aspect of the invention further comprise performing at least one additional diagnostic assay for PID on the subject, such as, for example, a pelvic exam, analysis of vaginal discharge, cervical cultures, pelvic ultrasound and pelvic laparoscopy.

[0039] In one embodiment, the method of this aspect of the invention further comprises determining the presence or amount of soluble mesothelin-related peptides (SMRP) in a biological sample obtained from the human subject. The amount of SMRP detected in the biological sample may be compared to a reference standard such as an antigen reference value, wherein detection of an increased amount of SMRP in the sample as compared to the reference standard is indicative of the presence of mesothelin-expressing tumor cells in the human subject.

[0040] In another embodiment, a method is provided for monitoring the efficacy of treatment of a human cancer patient undergoing therapeutic treatment for a mesothelin-expressing tumor. The method comprises: (a) providing a biological sample from a human patient undergoing therapeutic treatment for a cancer associated with a mesothelin-expressing tumor; (b) determining the presence or amount of anti-mesothelin antibodies in the biological sample by contacting the biological sample with a polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence at least 90% identical to a sequence comprising at least 20 contiguous nucleotides of SEQ ID NO: 1; and (c) comparing the determined presence or amount of anti-mesothelin antibodies to an antibody reference value wherein an amount of anti-mesothelin antibody greater than the antibody reference value is indicative of a positive response to the therapeutic treatment for the cancer.

[0041] In another embodiment, a method is provided for determining the likelihood of recurrence of a mesothelin-expressing tumor in a human patient undergoing therapeutic treatment for a cancer associated with a mesothelin-expressing tumor. The method comprises: (a) providing a biological sample from a human patient undergoing therapeutic treatment for a cancer associated with a mesothelin-expressing tumor; (b) determining the presence or amount of anti-mesothelin antibodies in the biological sample by contacting the biological sample with a polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence at least 80%, such as at least 90% identical to a sequence comprising at least 20 contiguous nucleotides of SEQ ID NO:1; and (c) comparing the presence or amount of anti-mesothelin antibodies determined in step (b) to an antibody reference value, wherein an amount of anti-mesothelin antibody greater than the antibody reference value is indicative of a lower risk of mesothelin-expressing tumor recurrence and wherein an amount of anti-mesothelin antibody lower than the reference value is indicative of greater risk of mesothelin-expressing tumor recurrence in the human patient.

[0042] In accordance with various embodiments of the methods of the invention, the present inventors have generated a reproducible assay for detecting antibodies to native mesothelin (SEQ ID NO:2) and applied it to discriminate between women with clinical evidence of ovarian cancer, referred to as "alive with disease" or "AWD," women with no clinical evidence of disease following therapy for ovarian cancer, referred to as "no clinical evidence of disease" or "NED," and healthy women. As described in more detail herein, the methods of the invention that include the detection of antibodies to native mesothelin may be used, and optionally combined with an assay to detect SMRP, in order to detect the presence of mesothelin-expressing tumor cells, to determine the presence or likelihood of recurrence of a cancer associated with a mesothelin-expressing tumor, such as ovarian cancer, to assess the clinical status and/or prognosis of a patient suffering from a cancer associated with mesothelin-expressing tumors, and/or to monitor the efficacy of treatment of cancer in a patient.

[0043] As used herein, the term "mesothelin" protein refers to native human mesothelin, such as is isolated from body fluids from patients with ovarian carcinoma (e.g., ascites, pleural fluid, or urine), or isolated from cultured cells making mesothelin (e.g., cultured mesothelium or ovarian carcinoma cells), or made by recombinant DNA technology (e.g., in eukaryotic expression systems (e.g., COS cells)), in yeast, insert, or in bacterial expression systems. Mesothelin is a 40 kDa glycoprotein that is publicly available in the GenBank database under the accession number AAV87530 set forth as SEQ ID NO:2, which is encoded by the cDNA sequence set forth as SEQ ID NO: 1 (Genbank accession number AY743922.1), and mammalian homologs or a fragment thereof comprising at least ten consecutive residues of the protein (SEQ ID NO:2), or at least 20 consecutive nucleotides of the cDNA (SEQ ID NO:1).

[0044] It has been determined that there are at least three mesothelin variants, variant 1 (SEQ ID NO:4, encoded by SEQ ID NO:3, Genbank reference NP_005814); variant 2, which has a 24-bp insert (Genbank reference NP_037536), the sequence of which is hereby incorporated by reference; and variant 3, which has an 82-bp insert (Genbank reference AF180951), the sequence of which is hereby incorporated by reference. See Muminova, Z.E., et al., *BMC Cancer* 4:19-29 (2004); Hassan, R., et al., *Clin. Cancer Res.* 10:8751-3 (2004).

[0045] A recent study has shown that mesothelin variant 1, with a molecular weight of approximately 40 kDa, was found to be the form predominately expressed at the surface of cells on certain tumors and to be released into body fluids, whereas mesothelin variants 2 and 3 were found to be expressed and released less frequently. Hellstrom, I., et al., *Cancer Epidemiol. Biomarkers Prev.* 15(5):1014-1020 (2006).

[0046] As shown below in TABLE 1, the cDNA sequences encoding mesothelin and variants 1 and 2 are highly conserved. As shown below in TABLE 2, the mesothelin and variant 1 and variant 2 proteins are also highly conserved.

TABLE 1

Mesothelin cDNA Sequence Homology

Sequence Name	Genbank Reference	% Identity to Mesothelin cDNA
human mesothelin (SEQ ID NO: 1)	AY743922.1	100%
human mesothelin variant 1 (SEQ ID NO: 3)	NM_005823.4	99%
human mesothelin variant 2	NM_013404.3	98%

TABLE 2

Mesothelin Protein Sequence Homology

Sequence Name	Genbank Reference	% Identity to Mesothelin Protein
human mesothelin (SEQ ID NO: 2)	AAV87530	100%
human mesothelin variant 1 (SEQ ID NO: 4)	NP_005814	99%
human mesothelin variant 2	NP_037536	98%

[0047] Mesothelin has been shown to be attached to the cell surface by phosphatidylinositol and is thought to have a role in cell adhesion and possibly in cell-to-cell recognition and signaling. Robinson, B., et al., *The Lancet* 362:1612-1616 (2003); Chang, K., et al., *Cancer Res.* 52:181-86 (1992). It has been shown that mesothelin can specifically bind to CA125 at the tumor cell surface and mediate heterotypic cell adhesion, suggesting that it is involved in OvC pathogenesis and progression (Rump, A., et al., *J. Biol. Chem.* 279:9190-9198 (2004)). This binding can be inhibited by antibodies to mesothelin. Id.

[0048] The detection of soluble mesothelin-related peptides (SMRP) has been reported to aid the diagnosis of mesothelioma (Robinson, B., et al., *The Lancet* 362:1612-1616 (2003)) and ovarian carcinoma (Scholler, N., et al., *Proc. Natl. Acad. Sci. USA* 96:11531-11536 (1999)). An ELISA assay (Scholler, et al., *Proc. Natl. Acad. Sci. USA* 96:11531-11536 (1999)) has been recently developed that measures circulating mesothelin variant 1 molecules (SEQ ID NO:4), (Hellstrom, et al., *Cancer Epidemiol.* 15:1014-1020 (2006)) in serum and other body fluids, often referred to as "SMRP." Such an ELISA assay provides a diagnostic tool which favorably complements CA125 for the diagnosis and prognosis of ovarian carcinoma (McIntosh, et al., *Gynecol. Oncology* 95:9-15 (2004)), and which also may be used in the diagnosis/prognosis of mesothelioma (Robinson, et al., *Lancet* 362:1612-1616 (2003)). The measurement of antibodies to mesothelin that are present in a patient, in accordance with the methods of the present invention, may be used alone or in combination with the above-referenced assays to improve detection of mesothelin-expressing tumors in patients, such as patients suffering from ovarian carcinoma, mesothelioma, or other cancers.

[0049] In accordance with one embodiment of the methods of the invention, a human patient undergoing therapeutic treatment for a cancer associated with a mesothelin-expressing tumor is assessed for their clinical status and likelihood of recurrence of cancer. The methods in accordance with this embodiment may be practiced with patients previously diagnosed and treated for a mesothelin-expressing tumor, such as ovarian cancer, mesothelioma, pancreatic cancer or lung carcinoma (e.g., treated with surgery and/or previously or currently undergoing therapeutic treatment, such as chemotherapy, radiation therapy, protein therapeutics (e.g., antibodies, gene therapy, cancer vaccine therapy, stem cell transplant, or other therapy). Recurrence of ovarian cancer is a clinical recurrence as determined by the presence of one or more clinical symptoms of an ovarian cancer, such as, for example, a metastases, or alternatively, as determined in a biochemical test, immunological test, or serological test such as, for example, a cross-reactivity in a biological sample to a CA125 antibody, or other diagnostic test. Preferably, the recurrence of ovarian cancer is capable of being detected at least about 2 years from treatment, more preferably about 2-3 years from treatment, and even more preferably, about 4 or 5 or 10 years from treatment.

[0050] A 1-4 staging system is used for ovarian cancer, as described by the International Federation of Gynecology and Obstetrics ("FIGO") staging system, which uses information obtained after surgery, which can include a total abdominal hysterectomy, removal of one or both ovaries and fallopian tubes, the omentum, and/or pelvic washings for cytology.

[0051] Stage I—limited to one or both ovaries

[0052] IA—involves one ovary; capsule intact; no tumor on ovarian surface; no malignant cells in ascites or peritoneal washings

[0053] IB—involves both ovaries; capsule intact; no tumor on ovarian surface; negative washings

[0054] IC—tumor limited to ovaries with any of the following: capsule ruptured, tumor on ovarian surface, positive washings

[0055] Stage II—pelvic extension or implants

[0056] IIA—extension or implants onto uterus or fallopian tube; negative washings

[0057] IIB—extension or implants onto other pelvic structures; negative washings

[0058] IIC—pelvic extension or implants with positive peritoneal washings

[0059] Stage III—microscopic peritoneal implants outside of the pelvis; or limited to the pelvis with extension to the small bowel or omentum

[0060] IIIA—microscopic peritoneal metastases beyond pelvis

[0061] IIIB—macroscopic peritoneal metastases beyond pelvis less than 2 cm in size

[0062] IIIC—peritoneal metastases beyond pelvis >2 cm or lymph node metastases, note: para-aortic lymph node metastases are considered regional lymph nodes

[0063] Stage IV—distant metastases—in the liver, or outside the peritoneal cavity

[0064] In accordance with some embodiments of the invention, a biological sample is obtained from a human patient (previously diagnosed with and previously treated for ovarian cancer, or currently undergoing treatment for ovarian cancer) which is assayed for the presence or concentration of anti-mesothelin antibodies. Biological samples for use in the methods of the invention include biological fluids. Non-limiting examples of biological fluids include blood, plasma, serum, ascitic fluid, urine, saliva, tears, pleural fluid, sputum, vaginal fluid (discharge) and washings obtained during a medical procedure (e.g., pelvic or other washings obtained during biopsy, endoscopy or surgery). The ability to use a sample of biological fluid to assess the clinical status of a subject with regard to a mesothelin-expressing tumor (such as ovarian cancer or other mesothelin-expressing tumors), pro-

vides relative ease as compared to obtaining a tissue biopsy sample of a tumor. Moreover, it enables monitoring of a patient during and/or post-treatment and, importantly, allows for earlier detection of recurrence and/or progression of ovarian cancer (or other mesothelin-expressing tumors).

[0065] In some embodiments of the invention, a biological sample is obtained from a human patient diagnosed with or at risk of developing pelvic inflammatory disease.

[0066] In accordance with the methods of this aspect of the invention, the concentration of anti-mesothelin antibody is measured in a biological sample obtained from a human patient. Any immunoassay may be used to measure the concentration of anti-mesothelin antibody, for example, enzyme linked immunosorbent assays (ELISA) and radioimmunoassays (RIA), western blotting, FACS analysis, and the like. More preferably, the assay will be capable of generating quantitative results. The biological sample may be diluted in a suitable buffer prior to analysis, for example, the sample may be diluted by a factor of at least 1:2, 1:5, 1:10, 1:20, 1:30, 1:40, 1:50, 1:80, 1:100, 1:200 or greater.

[0067] In one embodiment, the presence or amount of anti-mesothelin antibody in the biological sample is determined by contacting the biological sample with an SMRP polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence at least 80% identical (e.g., at least 85% identical, or at least 90% identical, or at least 95% identical, or at least 99% identical) to SEQ ID NO: 1, or a fragment thereof comprising at least 20 consecutive nucleotides, (or at least 25 or 30, or at least 40, 60, or 80 consecutive nucleotides) of SEQ ID NO: 1.

[0068] In another embodiment, the presence or amount of anti-mesothelin antibody in the biological sample is determined by contacting the biological sample with an SMRP polypeptide at least 80% identical (e.g., at least 85% identical, or at least 90% identical, or at least 95% identical, or at least 99% identical) to the human soluble mesothelin-related protein provided as SEQ ID NO:2, or a fragment thereof comprising at least 10 consecutive amino acid residues, (or at least 20 or at least 30, such as at least 50 consecutive amino acid residues) of SEQ ID NO:2.

[0069] In one embodiment, the anti-mesothelin antibody presence or amount is measured in the biological sample through the use of an ELISA assay. Standard solid phase ELISA formats are particularly useful in determining the concentration of a protein or antibody from a variety of biological samples, such as serum. In one form, such an assay involves immobilizing an SMRP polypeptide or fragment thereof onto a solid matrix, such as, for example, a polystyrene or polycarbonate microwell or dipstick, a membrane, or a glass support (e.g., a glass slide). For example, an SMRP-coated well of an ELISA plate may be utilized. The biological sample is contacted with the SMRP-coated well and the anti-mesothelin antibody in the sample is bound and captured. After binding and washing to remove non-specifically bound immune complexes, the antibody-antigen complex is detected. Detection may be carried out with any suitable method, such as the addition of a second antibody linked to a label.

[0070] In accordance with various embodiments of the methods of this aspect of the invention, an anti-mesothelin antibody reference value may be obtained from a control group of apparently healthy subjects, for example, as described in Examples 1 and 2. In some embodiments, the antibody reference value is determined in an ELISA assay using serum obtained from healthy subjects diluted at least 1:20. In another embodiment, the antibody reference value is determined using serum obtained from patients with pelvic inflammatory disease. In one embodiment, the antibody reference value is determined using serum obtained from patients diagnosed with and/or previously treated for a cancer comprising mesothelin-expressing tumor cells. An exemplary ELISA assay for detecting anti-mesothelin antibody levels in blood samples is described in Example 1.

[0071] In accordance with the prognostic applications of the invention, in one embodiment the level of anti-mesothelin antibody in a biological sample obtained from an ovarian cancer patient is then compared to the antibody reference value. If the antibody concentration in the patient tested is higher than the reference value, such as at least 1.5 fold, more preferably at least two-fold or higher, with a P value of less than 0.05, and the patient has previously undergone treatment for ovarian cancer, then the patient has a reduced likelihood of recurrence of ovarian cancer. If the antibody concentration in an ovarian cancer patient is lower than the reference value, such as at least 1.5 fold or two-fold or lower, with a P value of less than 0.05, and the patient has previously undergone treatment for ovarian cancer, then the patient has an increased likelihood of recurrence of ovarian cancer. In another embodiment, the presence of anti-mesothelin antibody is determined by comparison to a negative antibody control sample and optionally also to a positive antibody control sample.

[0072] In another aspect, the invention provides a method of assessing the prognosis of a human cancer patient suffering from a mesothelin-expressing tumor. The method comprises: (a) determining the presence or amount of anti-mesothelin antibodies in a biological sample from a human patient suffering from a mesothelin-expressing tumor by contacting the biological sample with a polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence that is at least 80% identical, such as at least 90% identical to a sequence comprising at least 20 contiguous nucleotides of SEQ ID NO:1; (b) determining the presence or amount of soluble mesothelin-related peptides (SMRP) encoded by a polynucleotide that selectively hybridizes to a sequence at least 80%, such as at least 90% identical to a sequence comprising at least 20 contiguous nucleotides of SEQ ID NO:1 in a biological sample from the human patient tested in step (a); and (c) comparing the amount of anti-mesothelin antibodies determined in step (a) to an antibody reference level, and comparing the amount of SMRP determined in step (b) to an antigen reference level, wherein the detection of SMRP in the sample at a lower amount than the antigen reference level, in combination with the detection of anti-mesothelin antibodies in the sample at a higher amount than the antibody reference level, is indicative of a good prognosis for the patient.

[0073] In accordance with this aspect of the invention, the method comprises the step of determining the presence and/or amount of SMRP in a biological sample obtained from a patient suffering from a mesothelin-expressing tumor, such as an ovarian cancer patient. As described above, SMRP is a soluble protein that has been found in the circulation of both healthy and cancer patients. The presence or amount of SMRP may be determined using any assay capable of detecting and/or measuring the amount of SMRP polypeptide.

[0074] In one embodiment, the concentration of an SMRP polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence at least 80% identical (e.g., at least

85% identical, or at least 90% identical, or at least 95% identical, or at least 99% identical) to SEQ ID NO:1, or a fragment thereof comprising at least 20 consecutive nucleotides, (or at least 25 or 30, or at least 40, 60, or 80 consecutive nucleotides) of SEQ ID NO: 1 is measured in the biological sample.

[0075] In another embodiment, the amount of an SMRP polypeptide at least 80% identical (e.g., at least 85% identical, or at least 90% identical, or at least 95% identical, or at least 99% identical) to the human soluble mesothelin-related protein provided as SEQ ID NO:2, or a fragment thereof comprising at least 10 consecutive amino acid residues (or at least 20 or at least 30, such as at least 50 consecutive amino acid residues) of SEQ ID NO:2 is measured in the biological sample.

[0076] The concentration and/or relative amount, or detection of soluble mesothelin-related protein (SMRP) present in a biological fluid sample may be determined using any convenient method for measuring SMRP including, but not limited to, ELISA, radioimmunoassay, chemiluminescence assay, immunofluorescence staining and the like that include an antibody that specifically binds to SMRP. Other protein detection methods may also be used to measure SMRP, including mass spectroscopy, western blot, FACS, and the like. Suitable biological samples include a biological fluid selected from the group consisting of blood, plasma, serum, ascitic fluid, and urine.

[0077] Specific antibodies, including monoclonal antibodies directed against SMRP and variants thereof, can be readily prepared using conventional techniques, and may be used in such methods. Examples of suitable antibodies are shown below in TABLE 3. For example, a double determinant ("sandwich") ELISA assay using two mAbs 569 and 4H3 (which recognize two different epitopes on the same antigen) may be used to detect SMRP in sera, as described in Scholler, N., et al., *Proc. Natl. Acad. Sci. USA* 96:11531-6 (1999). Other ELISA assays may be used to detect one or more variants of mesothelin using antibodies described in TABLE 3, or other antibodies against mesothelin.

TABLE 3

mAbs to Mesothelin Variants That Are Publically Available

Name	Mice Immunized Against (All mAbs are Mouse IgG1)	Binding Specificity	Reference
569	Human ovarian cancer cells	mesothelin variants 1 and 3	Scholler, N., et al., PNAS 96: 11531-6 (1999)
4H3	Antigen purified from ascites via immunoadsorption	mesothelin variants 1, 2, and 3	Scholler, N., et al., PNAS 96: 11531-6 (1999)
1A6-10	Antigen purified from ascites via immunoadsorption	mesothelin variant 1	Hellstrom, I., et al., Cancer Epidemiol Biomarkers Prev 15(5): 1014-1020 (2006)
2B10	Mesothelin variant 2 fusion protein	mesothelin variant 2	Hellstrom, I., et al., Cancer Epidemiol Biomarkers Prev 15(5): 1014-1020 (2006)
8C8	Mesothelin variant 3 fusion protein	mesothelin variant 3	Hellstrom, I., et al., Cancer Epidemiol Biomarkers Prev 15(5): 1014-1020 (2006)
4A10	Mesothelin variant 3 fusion protein	mesothelin variant 3	Hellstrom, I., et al., Cancer Epidemiol Biomarkers Prev 15(5): 1014-1020 (2006)
13H5	Mesothelin variant 3 fusion protein	mesothelin variant 3	Hellstrom, I., et al., Cancer Epidemiol Biomarkers Prev 15(5): 1014-1020 (2006)

[0078] In accordance with various embodiments of the methods of this aspect of the invention, an SMRP antigen reference value may be obtained from a control group of apparently healthy subjects, for example, as described in Example 3. In some embodiments, the antigen reference value is determined in an ELISA assay using serum obtained from healthy subjects. For example, in a serum sample, the serum may be diluted 1:40 and measured in an ELISA assay, where a negative control obtained from a healthy subject gives an absorbance value of zero at a dilution of 1:40 and a positive control obtained from a ovarian cancer patient gives an absorbance value of >0.2 at a dilution of 1:1,280. See Hellstrom, I., et al. *Cancer Epidemiol Biomarkers Prev* 15(5): 1014-1020 (2006). Absorbance values may be determined by any method known in the art. For example, absorbance of light at 450 nanometers, often referred to as the optical density (OD), is commonly used. In one embodiment, the antigen reference value is determined using serum obtained from patients diagnosed with and/or previously treated for a cancer comprising mesothelin-expressing tumor cells.

[0079] In another embodiment, the SMRP in a biological sample is detected using mass spectrometry. For example, the technologies of electrospray isonisation mass spectrometry, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS), surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI-TOFMS), and microcapillary reverse-phase HPLC nano-electrospray tandem mass spectrometry (μLC/MS/MS), which are commonly used in proteomic methods, are capable of analyzing small molecular weight proteins, such as SMRP, present in complex biological fluids such as serum, plasma or ascites.

[0080] It has been determined that the presence of a higher amount of SMRP in biological fluid samples obtained from an ovarian cancer patient in comparison to a control antigen reference value, in combination with the finding of a lower amount, or absence, of anti-mesothelin antibodies in a biological sample obtained from the patient is indicative of a high risk of recurrence of ovarian cancer, and an indicator of a poor prognosis with shorter survival rates, as described in Examples 2-3. Conversely, it has also been determined that the presence of a lower amount of SMRP in a biological fluid sample obtained from an ovarian cancer patient in comparison to a control antigen reference value, in combination with a finding of a higher amount of anti-mesothelin antibodies in

a biological sample obtained from the patient, as compared to a control antibody reference value, is indicative of a lower risk of recurrence of ovarian cancer, and correlates with a good prognosis and longer survival rates, as described in Examples 2-3.

[0081] In some embodiments, the methods of the invention further comprise the step of determining levels of another ovarian cancer marker, such as integrin-linked kinase (INK), CA125, TADG-12, kallikrein 10, prostasin, osteopontin, creatine kinase beta, serotransferrin, neutrophil-gelatinase associated lipocalin (NGAL), CD163, or Gc-globulin in a biological sample obtained from the subject. The second marker may be detected at the DNA, RNA or protein level using conventional methods known in the art.

[0082] In another aspect, the invention provides a method of monitoring the efficacy of treatment of a human patient diagnosed with a mesothelin-expressing tumor. The method comprises: (a) determining a first concentration of anti-mesothelin antibodies in a first biological sample taken from a human patient diagnosed with a mesothelin-expressing tumor prior to initiation of treatment for cancer; (b) determining a second concentration of anti-mesothelin antibodies in a second biological sample from the human patient taken after initiation of treatment for cancer; and (c) comparing the first and second concentrations of anti-mesothelin antibodies, wherein an increase in the second concentration of anti-mesothelin antibodies as compared to the first concentration of anti-mesothelin antibodies measured in the first biological sample indicates a positive response to the treatment for cancer.

[0083] In accordance with the method of this aspect of the invention, a first biological sample is taken from a cancer patient before initiation of treatment and a second biological sample is taken from the patient at least one time after initiation of treatment. In some embodiments, plural treated biological samples from the subject (e.g., a subject in a preclinical trial) are taken over periodic intervals of time after initiation of treatment.

[0084] As used herein, the term "treatment" refers to surgical intervention or to the administration of one or more cancer inhibitory agents for the alleviation of symptoms associated with cancer, or halt of further progression or worsening of the symptoms. For example, successful treatment may include a removal of a tumor, such as a mesothelin-expressing tumor; an alleviation of symptoms or halting the progression of the disease, as measured by a reduction in the growth rate of a tumor, a halt in the growth of a tumor, a reduction in size of the tumor; partial or complete remission of the cancer; or increased survival or clinical benefit. For example, treatment of a subject suffering from a mesothelin-expressing tumor may include one or more of the following: surgery to remove one or more tumors and/or administration of a therapeutic agent, such as chemotherapy, radiation therapy, protein therapeutics (e.g., antibodies, gene therapy, cancer vaccine therapy, stem cell transplant, or other therapy).

[0085] For example, with regard to treatment for ovarian cancer, surgery is a preferred treatment. The type of surgery depends upon how widespread the cancer is when diagnosed (the cancer stage), as well as the type and grade of cancer. The surgeon may remove one (unilateral oophorectomy) or both ovaries (bilateral oophorectomy), the fallopian tubes (salpingectomy), and the uterus (hysterectomy). For some very early tumors (stage 1, low grade or low-risk disease), only the involved ovary and fallopian tube will be removed (called a "unilateral salpingo-oophorectomy," USO), especially in young females who wish to preserve their fertility. In advanced stages of disease, as much tumor as possible is removed (debulking surgery). In cases where this type of surgery is successful, the prognosis is improved compared to patients where large tumor masses (more than 1 cm in diameter) are left behind. Chemotherapy is typically used after surgery to treat any residual disease. Chemotherapeutic agents, such as a platinum derivative (e.g., taxane) may be administered systemically, or may be administered intra-peritoneally via direct infusion into the abdominal cavity. Other examples of therapeutic agents for use in treatment of ovarian cancer include, but are not limited to protein therapeutics (e.g., antibodies), gene therapy, cancer vaccine therapy, and stem cell transplants. The methods of this aspect of the invention may also be used to measure the efficacy of candidate therapeutic agents for treatment of ovarian cancer.

[0086] The methods of this aspect of the invention may also be used to determine the clinical status of a patient after undergoing a treatment, such as surgery to remove a tumor. In accordance with this embodiment, the level of anti-mesothelin antibody in a biological sample obtained from a cancer patient that has been treated for a mesothelin-expressing tumor is then compared to the antibody reference value. If the antibody concentration in the patient tested is higher than the reference value, such as at least 1.5 fold, more preferably at least two-fold or higher, with a P value of less than 0.05, then the patient's clinical status was improved with the treatment (i.e. the patient has a reduced likelihood of recurrence of ovarian cancer). If the antibody concentration in the treated cancer patient is lower than the reference value, such as at least 1.5 fold or two-fold or lower, with a P value of less than 0.05, then the patient's clinical status was not improved with the treatment (i.e. the patient has an increased likelihood of recurrence of ovarian cancer).

[0087] In another aspect, a kit is provided for detecting the presence of mesothelin-expressing tumor cells in a human subject. The kit comprises reagents specific for detection of anti-mesothelin antibodies in a biological sample obtained from a human subject and printed instructions for comparison of the detected presence or amount of anti-mesothelin antibodies with a reference standard. The methods for detection of anti-mesothelin antibodies described herein may be performed using the kits of the invention. In one embodiment, the kit comprises a detection reagent for detecting anti-mesothelin antibodies comprising a polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence that is at least 80% identical to a sequence comprising at least 20 contiguous nucleotides of SEQ ID NO: 1.

[0088] In some embodiments, the kit further comprises a reference standard selected from the group consisting of a specific numerical threshold; a negative control sample for concurrent evaluation, or statistical information correlating the amount of anti-mesothelin antibodies detected with the likelihood of the presence of mesothelin-expressing cancer cells in the subject. In some embodiments, the reference standard is a negative control sample, and wherein the negative control sample is included in the kit.

[0089] In preferred embodiments, the methods and kits of the invention are capable of use at a point-of-care location, such as a medical clinic (e.g., doctor's office), or hospital, in order to rapidly obtain test results. Point-of-care testing (POCT) refers to any hospital or medical clinic (doctor's office) employee performing any type of laboratory test out-

side of the central laboratory. POCT has revolutionized the continuum of patient care process by providing laboratory results efficiently at the patient's bedside for various tests such as HIV testing, urine dipstick, etc. For example, rapid tests to detect HIV antibodies have been developed that demonstrate sensitivities and specificities comparable to those of enzyme immunoassays without the need for sophisticated laboratory equipment and highly-trained technicians. POCT can be used with unprocessed whole blood or oral fluid specimens. See Branson, B. M., *J. Lab Medicine* 27(7/8):288-295 (2003). POCT assays may be in any assay format that allows for rapid testing, such as particle agglutination, immunoconcentration and immunochromatography.

[0090] For example, particle agglutination POCT assays for detecting anti-mesothelin antibodies may be carried out by mixing a patient specimen containing anti-mesothelin antibodies with latex particles coated with mesothelin polypeptide (antigen), and if anti-mesothelin antibody is present, cross-linking occurs within 10 to 60 minutes and results in agglutination, with results interpreted visually.

[0091] In another example of a POCT assay format for detecting anti-mesothelin antibodies, an immunoconcentration device (flow through) may be used which employs solid-phase capture technology, which involves the immobilization of mesothelin polypeptides (antigen) on a porous membrane. The patient specimen flows through the membrane and is absorbed into an absorbent pad. If anti-mesothelin antibodies are present in the specimen a dot or a line visibly forms on the membrane when developed with a signal reagent (e.g., a colloidal gold or selenium conjugate). A procedural control may also be included on the membrane.

[0092] In yet another example of a POCT assay format to detect anti-mesothelin antibodies, immunochromatographic (lateral flow) strips may be used that incorporate both antigen (mesothelin) and signal reagent into a nitrocellulose strip. The patient specimen is applied to an absorbent pad, or the specimen may be diluted in a vial of buffer into which the test device is inserted. The specimen migrates through the strip and combines with the signal reagent. A positive reaction results in a visual line on the membrane where the mesothelin antigen has been applied. A procedural control line may be applied to the strip beyond the mesothelin antigen line.

[0093] The following examples merely illustrate the best mode now contemplated for practicing the invention, but should not be construed to limit the invention.

Example 1

[0094] This Example describes the development of an ELISA assay to measure antibodies to native mesothelin.

[0095] Materials and Methods

[0096] Assay for Anti-Mesothelin Antibodies

[0097] Native mesothelin was isolated from samples of urine of patients with metastatic ovarian cancer using Sepharose 4B conjugated with monoclonal antibody mAb 569 (Scholler, N., et al., *Proc. Natl. Acad. Sci. USA* 96:11531-11536 (1999)). The Sepharose 4B-mAb 569 conjugate was generated as follows. The mAb 569 was dissolved in 0.1M $NaHCO_3$ buffer containing 0.5 M NaCl (pH 8.5). Cyanogen-bromide activated Sepharose 4B (Sigma, St. Louis, Mo.) was washed and swelled in cold 1 mM HCl for 30 minutes and then washed with 10 volumes of water followed by 0.1M $NaHCO_3$/0.5M NaCl buffer. Immediately thereafter, mAb 569 was added to the washed resin at a concentration of 10 mg antibody per ml resin. Following 2 hours incubation at room temperature, unbound antibody was removed by washing with $NaHCO_3$/NaCl buffer, and unreacted groups were blocked by incubation with 0.2M Glycine, pH 6.0, overnight at 4° C.

[0098] Urine samples obtained from women with metastatic ovarian cancer were pretested to confirm the presence of a high level of SMRP. The pH of the urine was adjusted by addition of 1 M $NaHCO_3$ until it was >8.0, after which the sample was filtered. Sepharose 4B that had been conjugated with mAb 569 was washed with 10 volumes of PBS and the urine sample was added, followed by washing with 10 volumes of PBS. Subsequently, native mesothelin was eluted with 0.1M Glycine-HCl pH 4.5, after which the pH was neutralized by adding 2M Tris and the preparation dialyzed against PBS.

[0099] As an alternative source of mesothelin, the mesothelioma cell line Meso, established in the laboratory of the inventors, was adapted to grow in Iscove's modified Dulbecco's medium (IMDM) without serum. Culture supernatant was collected every fifth day during 4-12 weeks of culture, and the supernatant was frozen until use. After pooling culture supernatants and adjusting pH with $NaHCO_3$, the supernatants were filtered and run through a Sepharose 4B column conjugated with Mab 569. After washing the column with 10 volumes of PBS, mesothelin antigen was eluted from the column with Glycine-HCl pH.2.7.

[0100] ELISA assays were performed to confirm that the material isolated as described above from either urine or culture supernatants was mesothelin (Scholler N. et al., "Soluble member(s) of the mesothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian carcinoma," Proc Natl Acad Sci USA 96:11531-11536, 1999). Protein sequencing was performed to confirm that the purified material represented mesothelin, as described below.

[0101] The purified mesothelin was diluted in Carbonate-Bicarbonate buffer at 5 ug/mL and incubated overnight to coat the wells of a 96-well ELISA plate. After blocking for 2 hours with 3% bovine serum albumin (BSA), the plate was washed with PBS-1% Tween 20. Serum samples at dilutions 1:20 and 1:80 were added to each well and incubated at room temperature for 1 hour. 3% BSA was added in some wells as a negative control. After washing the plate with PBS-Tween 20, 1:1000 diluted HRP-conjugated mouse anti-human IgG antibody (Invitrogen, Carlsbad, Calif.) was added to each well and incubated for 1 hour at room temperature. After washing the plate with PBS-Tween, SureBlue™ TMB Microwell Peroxidase Substrate (KPL, Gaithersburg, Md.) was added to each well and incubated for 15 minutes at room temperature before the interaction was terminated by adding the TMB stop solution (KPL). Optical density (OD) at 450 nanometers was measured with a DynaTech MR5000 plate reader (DynaTech Laboratories Inc., Chantilly, Va.).

[0102] Validation Testing and Stability of the Anti-Mesothelin Antibody Assay

[0103] Antibody tests were carried out on serum samples obtained from three patients, two tumor-bearing (AWD), and one with no clinical evidence of disease (NED). Ten ml of venous blood was withdrawn from each participant, and serum was separated using an established protocol (Zhang, P., et al., *Electrophoresis* 25:1823-1828 (2004)).

[0104] To determine the reproducibility of the anti-mesothelin antibody assay, repeated tests of the same sera were

performed, and the mesothelin antibody assay gave results (measured in OD) that varied less than 10% (data not shown).

[0105] To determine the longitudinal stability of the assay, serial samples of sera were harvested from the same three OvC patients within a 4-month interval, during which time there was no detectable change in the patients' clinical status. As illustrated in TABLE 4, the ODs of individual serum samples from the same patient displayed very little variation.

TABLE 4

Mesothelin Antibody Tests on Serum Samples Harvested From the Same Patients Within a 4 Month Period. The Patient's Clinical Status Remained the Same Over the Course of the Study.

Patient Identifier	Clinical Status	Serum Sample (dil 1:20)	OD
310	Tumor-bearing (AWD)	Sample 1	0.148
310	Tumor bearing (AWD)	Sample 2	0.171
310	Tumor-bearing (AWD)	Sample 3	0.023
305	Tumor-bearing (AWD)	Sample 1	1.147
305	Tumor-bearing (AWD)	Sample 2	1.259
305	Tumor-bearing (AWD)	Sample 3	1.103
208	No clinical evidence of disease after treatment (NED)	Sample 1	1.110
208	NED	Sample 2	1.079
208	NED	Sample 3	1.062

[0106] As shown by the results in TABLE 4, the ELISA assay may be used to reproducibly measure antibodies to native mesothelin.

[0107] The data was further evaluated at several cut-offs for the OD (0.2, 0.5 or 1.0) and at different dilutions of serum. Unless otherwise indicated, sera tested for antibodies were diluted 1:20, and an OD of 0.5 was used as the cut-off for positive serum. All tests were performed on coded samples. Data were statistically evaluated using the Student's t test and chi square assays.

[0108] Antibody levels were measured in the same sera against mesothelin that had been purified from either urine or culture supernatants, with the same source of antigen being used in each experiment with various sources of sera. There were no significant differences between the ODs obtained when the same sera were tested against antigens from urine or supernatant (data not shown). The data was not expressed as quantitative protein units but as ODs, as done in studies by others (Cramer D W, et al. "Conditions associated with antibodies against the tumor-associated antigen MUC1 and their relationship to risk for ovarian cancer." Cancer Epidemiol Biomarkers Prev 2005; 14: 1125-31; Ho MH, et al. "Humoral immune response to mesothelin in mesothelioma and ovarian cancer patients." Clin Cancer Res 2005; 11: 3814-20.)

[0109] Characterization of Mesothelin Isolated from Urine or Culture Medium

[0110] Samples were purified by immunoaffinity chromatography using Mab 569 (Scholler N. et al., 1999). The purified material was sequenced to confirm that the purified material represented mesothelin. Sequence analysis was performed at the Harvard Microchemistry and Proteomics Analysis Facility by microcapillary reverse-phase high-performance liquid chromatography (HPLC) nano-electrospray tandem mass spectrometry (μLC/MS/MS) on a Thermo LTQ-Orbitrap mass spectrometer. Tandem mass spectrometry spectra were correlated with known sequences using the algorithm Sequest developed at the University of Washington (Eng, K, et al., "An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database," J. Am. Soc. Mass Spectrom 5:976-989, 1994) and programs developed by Chittum et al, ("Rabbit betaglobin is extended beyond its UGA stop codon by multiple suppressions and translational reading gaps," Biochemistry 37:10866-10870, 1998). Tandem mass spectrometry peptide sequences were reviewed for consensus with known proteins and the results manually confirmed for fidelity.

[0111] Peptides recovered from liquid chromatography-mass spectrometry were examined for unique features to identify the different mesothelin isoforms. Variant 2 contains an 8 amino acid insertion as published previously (Hellstrom, I. et al., 2006). Variants 1 and 3 contain an Asp to Asn amino acid change and additional C-terminal sequence extensions, respectively. Both antigen sources were found to contain all three mesothelin variants based on the presence of the Asp-Asn replacement (data not shown). However, a lack of peptide resolution at the C-terminal end, which was also observed previously (Hellstrom, I. et al., 2006), remained a problem for detailed sequence analysis of variants 1 and 3. It should be noted, that published sequence data may not be entirely reliable, and it is conceivable that the Asp-Asn replacement does not represent an actual protein peptide difference but rather an error in the initial sequence data retrieved from data banks and sequencing projects.

[0112] Pilot Experiments Titrating Sera from NED Patients and Healthy Controls

[0113] The amount of anti-mesothelin antibodies in sera from OvC patients with NED following therapy as well as from healthy control women was titrated. One such experiment is presented in FIG. **1**. While the serum from the healthy subject #8 (square symbols) gave no higher OD at any dilution than the negative control (BSA) (triangles), the OD from patient #208 (diamonds) was higher than that of BSA at dilution 1:640, and repeat tests of the same sera gave ODs that varied with <10%. In other cases, sera from healthy controls were as reactive at dilution 1:20 as were sera from NED patient #208 and gave as high titers (data not shown). To conserve the amount of available, purified antigen, subsequent tests for antibodies (except when otherwise stated) used sera diluted 1:20 and an OD of 0.5 as the cut-off for positive serum.

[0114] In summary, this Example describes the successful development of an ELISA assay that is useful to measure antibodies to native mesothelin in serum. Further, this Example shows that the ELISA assay is reproducible and shows longitudinal stability.

Example 2

[0115] This Example describes a retrospective study of samples obtained from ovarian cancer patients using the anti-mesothelin antibody to compare antibody levels in serum from healthy women, women with benign gynecological conditions, women with pelvic inflammatory disease (PID), ovarian cancer patients with no evidence of disease after treatment (NED), and ovarian cancer patients with clinical evidence of disease (AWD).

[0116] Patients

[0117] A retrospective study was done with serum obtained from 35 ovarian cancer patients, all Jewish Israeli women that were diagnosed and treated for OvC at the Gynecology-Oncology Department, Sheba Medical Center, from Jan. 1, 2000, to Jan. 31, 2003. All patients were routinely examined at the outpatient clinic of the Sheba Medical Center, and sera

were harvested over a 12-month period beginning Feb. 1, 2003, with >75% of the patients providing at least 3 serial samples. The final evaluation of the patients' health status was carried out in February 2005. The diagnosis of OvC was confirmed by histopathology in all patients. The clinical details were extracted from the medical records and, when needed, via a telephone interview with the patient. Patients were followed every 2-3 months for the first year after completion of first line chemotherapy and every 3-4 months over the subsequent 2-4 years. All patients were treated with 6-8 cycles of standard platinum and taxane-based regimens. The study was approved by the institutional review board (IRB), using criteria similar to those in the United States, and each patient signed a written consent form. For follow up, blood was withdrawn at the time of visit, as part of a routine management scheme.

[0118] The status of the 35 cancer patients was defined as no evidence of disease (NED; n=11) or alive with disease (AWD, n=21), 14 of which died of disease during the observation period. Sera were also harvested from 34 age-matched control women who had been in- or out-patients for diseases other than cancer (benign diseases of the ovary), including 9 women who were diagnosed with pelvic inflammatory disease (PID), 14 women with endometriosis, and 7 women with ovarian cysts. In addition, sera were tested from an age-matched control group of 23 U.S. women who had no known diseases and specifically no gynecological symptoms.

[0119] All 35 OvC patients were Jewish Israeli women, as were the 34 age-matched controls with benign gynecological disease, except for 10 of the 14 women with endometriosis who were patients at the University of Washington. Age at diagnosis was 56+/−13 years (range 28-84 years). Twenty-six of the cancer patients had serous OvC, 3 had adenocarcinoma, 4 had endometrioid type carcinoma, and 2 had mucinous OvC. At the time of diagnosis, 2 patients were stage I, 1 patient was stage II, 30 were stage III, and two patients had metastatic stage IV disease.

[0120] Comparison of Anti-Mesothelin Antibodies in Sera From Study Participants. The mesothelin antibody assay described in Example 1 was applied to test one serum sample from each of 23 healthy women, one serum sample from each woman with a benign disease of the ovary (including PID), 46 serial samples from 14 OvC patients with NED, 77 serial samples from 21 OvC patients who had clinical evidence of disease (AWD). The combined data for each category was as follows:

TABLE 5

Anti-Mesothelin Antibody Assay (Serum Diluted 1:20)

Subject Category	Number of Samples	Mean OD +/− S.D.
Healthy women (n = 23)	23	0.47 +/− 0.40
Women with benign disease (excluding PID) (n = 34)	34	0.305 +/− 0.26
Women with PID (n = 9)	9	0.785 +/− 0.55
Women with NED (n = 14)	46	1.08 +/− 0.63
Women AWD (n = 21)	77	0.54 +/− 0.46

[0121] Comparing the combined data from all tested sera, shown above in TABLE 5, the difference in mean OD between 23 sera from healthy women (0.47+/−0.40) and 46 sera from women with NED (1.08+/−0.63) was statistically significant ($p<0.0001$), as was the difference ($p<0.0000009$) between the 46 sera from patients with NED and 77 sera from AWD patients with clinical evidence of tumor (0.54+/−0.46). The p value for the difference in mean OD between the sera from healthy women and women AWD was <0.37. The p value for the difference in mean OD between the sera from healthy women and women with PID was <0.085. The difference in mean OD between the sera from women with benign gynecological disease and women with PID was statistically significant ($p<0.001$).

[0122] FIG. **2**A shows the anti-mesothelin antibody levels in serum (diluted 1:20) obtained from 23 healthy women. FIG. **2**B shows the anti-mesothelin antibody levels in serum (diluted 1:20) obtained from 34 women with non-malignant gynecological conditions, excluding PID. FIG. **2**C shows the anti-mesothelin antibody levels in serum (diluted 1:20) obtained from 9 women with PID. FIG. **3**A shows the anti-mesothelin antibody levels in serum (diluted 1:20) obtained from 14 ovarian cancer patients who had NED. FIG. **4**A shows the anti-mesothelin antibody levels in serum (diluted 1:20) obtained from 21 ovarian cancer patients with clinical evidence of disease (AWD).

[0123] As shown in FIG. **2**A, sera from 6 of the 23 healthy women (26%) had an OD>0.5, with 2 donors (9%) having an OD>1.0. The mean OD for serum diluted 1:20 was 0.474+/−0.399 (Table 5). None of the healthy women had detectable levels of circulating mesothelin antigen when tested at a dilution of 1:40 and using OD 0.2 as cut-off (data not shown).

[0124] As shown in FIG. **2**B, 5 of 34 women with non-malignant gynecological conditions, but excluding PID, had sera with an OD>0.5 at a dilution of 1:20 for a mean reactivity of 0.305+/−0.262 (Table 5). Three of the 5 women whose sera were positive with the criteria applied had ovarian cysts. All 14 women with endometriosis were negative for anti-mesothelin antibodies, including 4 from Israel (#171-174) and 10 from the United States (#175-184).

[0125] Serum samples were also tested from 9 women with PID. As shown in FIG. **2**C, sera from 7 (78%) of these donors had an OD>0.5, and 3 (33%) had an OD>1.0. The mean OD was 0.785+/−0.549 (Table 5). The difference in mean OD for sera from women with PID was different from that of sera from women with other benign gynecological diseases ($p<0.001$); the p value in comparison with sera from healthy women was <0.085.

[0126] As shown in FIG. **3**A, 10 of 14 NED patients (71%) had an OD>0.5 and 6 NED patients (43%) had an OD>1.0. As shown in FIG. **4**A, the anti-mesothelin antibody levels in AWD patients fell between the healthy and NED patient groups, with 9 of 21 patients (43%) having an OD>0.5 and 3 patients (14%) having an OD>1.0. These data are summarized below in TABLE 6. TABLE 7 summarizes the data from sera diluted 1:80, with a cutoff value of OD 0.5 or 0.2. Note that women with benign (non-malignant) disease were not tested at the 1:80 dilution.

TABLE 6

Relationship Between Clinical Status and Anti-Mesothelin Antibodies in Serum Diluted 1:20, at Two Different Cut-Offs (OD > 0.5 and OD > 1.0)

Subject Category	Number of Subjects	OD > 0.5 (dil 1:20)	OD > 0.1 (dil 1:20)
Healthy	23	6 (26%)	2 (9%)
Women with benign disease (excluding PID)	34	5 (15%)	2 (7%)
NED	14	10 (71%)*,#	6 (43%)**
AWD	21	9 (43%)	3 (14%)

*p < 0.01 in comparison with serum from healthy women
**p < 0.025 in comparison with serum from healthy women
#p < 0.001 in comparison with serum from women with benign disease excluding PID

TABLE 7

Relationship Between Clinical Status and Anti-Mesothelin Antibodies in Serum Diluted 1:80, at Two Different Cut-Offs (OD > 0.5 and OD > 1.0)

Subject Category	Number of Subjects	OD > 0.5 (dil 1:80)	OD > 0.2 (dil 1:80)
Healthy	23	2 (9%)	7 (30%)
NED	14	7 (50%)*	11 (79%)*
AWD	21	4 (19%)**	13 (62%)***

*p < 0.01 in comparison with serum from healthy women
**p < 0.05 in comparison with serum from patients with NED
***p < 0.025 in comparison with serum from healthy women

[0127] As shown above in TABLE 6 and TABLE 7, the same relative differences were maintained in sera from healthy women, ovarian cancer patients with NED and patients AWD. In agreement with previously published data by Ho, et al., the results presented above show that many OvC patients make antibodies to mesothelin which, like most other tumor-associated antigens, can induce an immune response in the tumor-bearing host. Ho, et al., *Clin. Cancer Res.* 11:3814-3820 (2005).

[0128] However, in contrast to the results published in the Ho, et al. study, anti-mesothelin antibodies were detected in a substantial fraction of healthy individuals. It is likely that the detection of anti-mesothelin antibodies in healthy individuals was observed due to the use of a lesser dilution (1:20) of the sera in the present study as compared to the Ho, et al. study. In the present study a significant difference in anti-mesothelin antibody concentration was observed between sera from patients with OvC and healthy women, with the difference being most pronounced when testing sera from the NED group.

[0129] Seven of the 9 women diagnosed with inflammatory pelvic disease (78%) had high antibody levels, as compared to a much lower percentage of women with other benign gynecological diseases (15%) or healthy women (26%).

[0130] While not wishing to be bound by theory, the observed protective effect of anti-mesothelin antibodies with regard to the development and progression of OvC may be due to the fact that anti-mesothelin antibodies have been shown to prevent the binding of mesothelin to CA125 and thereby impact cellular adhesion, as demonstrated in vitro. Rump, A., et al., *J. Biol. Chem.* 279:9190-9198 (2004). Furthermore, it is known that antibodies can be cytotoxic in the presence of complement, can mediate antibody-dependent cellular cytotoxicity in the presence of NK cells or macrophages, and can also have an impact of the generation and expansion of T cell responses to tumor antigens. Hellstrom, K. E., et al., *Expert Rev. Vaccines* 2:517-532 (2003). Therefore, it is likely that the presence of both mesothelin antigen and anti-mesothelin antibodies will result in the formation of immune complexes of various sizes.

[0131] It is known that immune complexes can be preferentially taken up by the Fc receptors of APC, and their amounts and relative composition may determine whether this will lead to the generation/expansion of a potentially tumor-destructive Th1 type immune response or inhibit it, e.g., by stimulating the formation of suppressor/Treg cells. Gershon, R. K., et al., *Nature* 250:594-596 (1974). Anti-cancer therapy is likely to influence antibody formation, both by decreasing the number of tumor cells releasing antigen and by acting directly on antibody forming cells, as in the case of cytotoxic drugs. Therefore, changes in antibody levels are likely to influence the amount of SMRP that is detectable by ELISA.

Example 3

[0132] This Example describes the assessment of serum SMRP levels in study participants described in Example 2.

[0133] Elisa for Serum SMRP Levels

[0134] Sera obtained from each participant described in Example 2 was diluted 1:40 with PBS containing 3% BSA. Serum SMRP levels were determined by a sandwich ELISA using 2 mAbs (OV569 and 4H3), which bind to different SMRP epitopes (Scholler, N., et al., *Proc. Natl. Acad. Sci. USA* 96:11531-11536 (1999); Hellstrom, I., et al., *Cancer Epidemiol. Biomarkers Prev.* 15:1014-1020 (2006)). SMRP levels were determined as optical density (OD) according to absorbance measurement by an ELISA plate reader at 450 nm (Scholler, N., et al., *Proc. Natl. Acad. Sci. USA* 96:11531-11536 (1999)). A serum is classified as positive for SMRP when the OD at dilution 1:40 is above the commonly accepted cut-off of 0.20 OD (Scholler, et al., *Proc. Natl. Acad. Sci. USA*96:11531-11536 (1999); Robinson, B., et al., *Lancet*362: 1612-1616 (2003); McIntosh, M., et al., *Gynecologic Oncology* 95:9-15 (2004)), which corresponds to 3 standard deviations (SD) above the mean absorbance measurement at 460 nm as previously determined with a group of >100 healthy controls (I. Hellstrom, unpublished findings).

[0135] Results

[0136] FIG. **3**B shows the SMRP peptide levels measured in serum (diluted 1:40) obtained from 14 ovarian cancer patients who had NED. FIG. **4**B shows the SMRP peptide levels measured in serum (diluted 1:40) obtained from 21 ovarian cancer patients with clinical evidence of disease (AWD). As shown in FIG. **3**B, in the NED group, 3 of 14 patients (21%) had sera that were positive for SMRP when tested at a dilution of 1:40. Eleven of 14 patients (79%) had ODs less than the 0.20 cut-off level. Nine of those patients (64%) tested positive for antibodies to mesothelin (as discussed in Example 2), while two patients had neither detectable antibodies to mesothelin nor detectable SMRP. One patient was positive for both anti-mesothelin antibodies and SMRP, and two patients were positive for SMRP and negative for anti-mesothelin antibodies. In contrast, as shown in FIG. **4**B, sera from 15 of 21 AWD patients (71%) were positive for SMRP, a result which is significantly different from the NED group ($p<0.005$). Only one of 21 patients (5%) in the AWD group had anti-mesothelin antibodies and no detectable SMRP, 7 patients (33%) had SMRP and no anti-mesothelin antibodies, and 8 patients (38%) had both anti-mesothelin

antibodies and SMRP. These results are summarized below in TABLE 8.

TABLE 8

Relationship Between Clinical Status, SMRP In Serum, and Anti-Mesothelin Antibodies in Patients With OvC

SMRP Antigen/ Antibody Status	Number of OvC Patients with NED	Number of OvC Patients with Tumor (AWD)
Ag+Ab+	1	8 ($p < 0.05$)
Ag+Ab−	2	7
Ag−Ab+	9 ($p < 0.0002$)	1
Ag−Ab−	2	5

[0137] These results demonstrate that the majority of ovarian cancer patients who are clinically tumor free following therapy (NED) have antibodies to native mesothelin and do not have detectable circulating SMRP. In contrast, patients with clinical evidence of tumors (AWD) have circulating SMRP and either do, or do not have antibodies to mesothelin. Therefore, the presence of antibodies in the absence of circulating antigen correlates with a low, clinically undetectable tumor load (NED).

[0138] While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 4

<210> SEQ ID NO 1
<211> LENGTH: 2187
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (169)..(2037)

<400> SEQUENCE: 1

```
tgccaggctc tccaccccca cttcccaatt gaggaaaccg aggcagagga ggctcagcgc     60

cacgcactcc tctttctgcc tggccggcca ctcccgtctg ctgtgacgcg cggacagaga    120

gctaccggtg gacccacggt gcctccctcc ctgggatcta cacagacc atg gcc ttg     177
                                                    Met Ala Leu
                                                    1

cca acg gct cga ccc ctg ttg ggg tcc tgt ggg acc ccc gcc ctc ggc      225
Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr Pro Ala Leu Gly
        5                  10                  15

agc ctc ctg ttc ctg ctc ttc agc ctc gga tgg gtg cag ccc tcg agg      273
Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp Val Gln Pro Ser Arg
20                  25                  30                  35

acc ctg gct gga gag aca ggg cag gag gct gcg ccc ctg gac gga gtc      321
Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu Asp Gly Val
                40                  45                  50

ctg gcc aac cca cct aac att tcc agc ctc tcc cct cgc caa ctc ctt      369
Leu Ala Asn Pro Pro Asn Ile Ser Ser Leu Ser Pro Arg Gln Leu Leu
            55                  60                  65

ggc ttc ccg tgt gcg gag gtg tcc ggc ctg agc acg gag cgt gtc cgg      417
Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu Arg Val Arg
        70                  75                  80

gag ctg gct gtg gcc ttg gca cag aag aat gtc aag ctc tca aca gag      465
Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu Ser Thr Glu
    85                  90                  95

cag ctg cgc tgt ctg gct cac cgg ctc tct gag ccc ccc gag gac ctg      513
Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro Glu Asp Leu
100                 105                 110                 115

gac gcc ctc cca ttg gac ctg ctg cta ttc ctc aac cca gat gcg ttc      561
Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro Asp Ala Phe
                120                 125                 130

tcg ggg ccc cag gcc tgc acc cat ttc ttc tcc cgc atc acg aaa gcc      609
Ser Gly Pro Gln Ala Cys Thr His Phe Phe Ser Arg Ile Thr Lys Ala
```

-continued

```
           135                 140                 145

aat gtg gac ctg ctc ccg agg ggg gct ccc gag cga cag cgg ctg ctg      657
Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln Arg Leu Leu
        150                 155                 160

cct gcg gct ctg gcc tgc tgg ggt gtg cgg ggg tct ctg ctg agc gag      705
Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu Leu Ser Glu
    165                 170                 175

gct gat gtg cgg gct ctg gga ggc ctg gct tgc gac ctg cct ggg cgc      753
Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu Pro Gly Arg
180                 185                 190                 195

ttt gtg gcc gag tcg gcc gaa gtg ctg cta ccc cgg ctg gtg agc tgc      801
Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu Val Ser Cys
                200                 205                 210

ccg gga ccc ctg gac cag gac cag cag gag gca gcc agg gcg gct ctg      849
Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg Ala Ala Leu
            215                 220                 225

cag ggc ggg gga ccc ccc tac ggc ccc ccg tcg aca tgg tct gtc tcc      897
Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp Ser Val Ser
        230                 235                 240

acg atg gac gct ctg cgg ggc ctg ctg ccc gtg ctg ggc cag ccc atc      945
Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly Gln Pro Ile
    245                 250                 255

atc cgc agc atc ccg cag ggc atc gtg gcc gcg tgg cgg caa cgc tcc      993
Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg Gln Arg Ser
260                 265                 270                 275

tct cgg gac cca tcc tgg cgg cag cct gaa cgg acc atc ctc cgg ccg     1041
Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile Leu Arg Pro
                280                 285                 290

cgg ttc cgg cgg gaa gtg gag aag aca gcc tgt cct tca ggc aag aag     1089
Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser Gly Lys Lys
            295                 300                 305

gcc cgc gag ata gac gag agc ctc atc ttc tac aag aag tgg gag ctg     1137
Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys Trp Glu Leu
        310                 315                 320

gaa gcc tgc gtg gat gcg gcc ctg ctg gcc acc cag atg gac cgc gtg     1185
Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg Val
    325                 330                 335

aac gcc atc ccc ttc acc tac gag cag ctg gac gtc cta aag cat aaa     1233
Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu Lys His Lys
340                 345                 350                 355

ctg gat gag ctc tac cca caa ggt tac ccc gag tct gtg atc cag cac     1281
Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln His
                360                 365                 370

ctg ggc tac ctc ttc ctc aag atg agc cct gag gac att cgc aag tgg     1329
Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile Arg Lys Trp
            375                 380                 385

aat gtg acg tcc ctg gag acc ctg aag gct ttg ctt gaa gtc aac aaa     1377
Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu Val Asn Lys
        390                 395                 400

ggg cac gaa atg agt cct cag gtg gcc acc ctg atc gac cgc ttt gtg     1425
Gly His Glu Met Ser Pro Gln Val Ala Thr Leu Ile Asp Arg Phe Val
    405                 410                 415

aag gga agg ggc cag cta gac aaa gac acc cta gac acc ctg acc gcc     1473
Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp Thr Leu Thr Ala
420                 425                 430                 435

ttc tac cct ggg tac ctg tgc tcc ctc agc ccc gag gag ctg agc tcc     1521
Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu Glu Leu Ser Ser
```

-continued

```
               440                 445                 450

gtg ccc ccc agc agc atc tgg gcg gtc agg ccc cag gac ctg gac acg     1569
Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln Asp Leu Asp Thr
            455                 460                 465

tgt gac cca agg cag ctg gac gtc ctc tat ccc aag gcc cgc ctt gct     1617
Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys Ala Arg Leu Ala
        470                 475                 480

ttc cag aac atg aac ggg tcc gaa tac ttc gtg aag atc cag tcc ttc     1665
Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys Ile Gln Ser Phe
    485                 490                 495

ctg ggt ggg gcc ccc acg gag gat ttg aag gcg ctt agt cag cag aat     1713
Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu Ser Gln Gln Asn
500                 505                 510                 515

gtg agc atg gac ttg gcc acg ttc atg aag ctg cgg acg gat gcg gtg     1761
Val Ser Met Asp Leu Ala Thr Phe Met Lys Leu Arg Thr Asp Ala Val
                520                 525                 530

ctg ccg ttg act gtg gct gag gtg cag aaa ctt ctg gga ccc cac gtg     1809
Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu Gly Pro His Val
            535                 540                 545

gag ggc ctg aag gcg gag gag cgg cac cgc ccg gtg cgg gac tgg atc     1857
Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val Arg Asp Trp Ile
        550                 555                 560

cta cgg cag cgg cag gac gac ctg gac acg ctg ggg ctg ggg cta cag     1905
Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly Leu Gly Leu Gln
    565                 570                 575

ggc ggc atc ccc aac ggc tac ctg gtc cta gac ctc agc gtg caa gag     1953
Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp Leu Ser Val Gln Glu
580                 585                 590                 595

gcc ctc tcg ggg acg ccc tgc ctc cta gga cct gga cct gtt ctc acc     2001
Ala Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly Pro Val Leu Thr
                600                 605                 610

gtc ctg gca ctg ctc cta gcc tcc acc ctg gcc tga gggccccact          2047
Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
            615                 620

cccttgctgg ccccagccct gctggggatc cccgcctggc caggagcagg cacgggtggt   2107

ccctgttcca ccccaagaga actcgcgctc agtaaacggg aacatgcccc ctgcaaaaaa   2167

aaaaaaaaaa aaaaaaaaaa                                              2187


<210> SEQ ID NO 2
<211> LENGTH: 622
<212> TYPE: PRT
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 2

Met Ala Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr Pro
1               5                   10                  15

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
```

-continued

```
                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 His 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
```

-continued

```
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

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

Val Leu Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
    610                 615                 620


<210> SEQ ID NO 3
<211> LENGTH: 2052
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (52)..(1920)

<400> SEQUENCE: 3

agagctaccg gtggacccac ggtgcctccc tccctgggat ctacacagac c atg gcc      57
                                                          Met Ala
                                                          1

ttg cca acg gct cga ccc ctg ttg ggg tcc tgt ggg acc ccc gcc ctc      105
Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr Pro Ala Leu
        5                   10                  15

ggc agc ctc ctg ttc ctg ctc ttc agc ctc gga tgg gtg cag ccc tcg      153
Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp Val Gln Pro Ser
    20                  25                  30

agg acc ctg gct gga gag aca ggg cag gag gct gcg ccc ctg gac gga      201
Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu Asp Gly
35                  40                  45                  50

gtc ctg gcc aac cca cct aac att tcc agc ctc tcc cct cgc caa ctc      249
Val Leu Ala Asn Pro Pro Asn Ile Ser Ser Leu Ser Pro Arg Gln Leu
                55                  60                  65

ctt ggc ttc ccg tgt gcg gag gtg tcc ggc ctg agc acg gag cgt gtc      297
Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu Arg Val
            70                  75                  80

cgg gag ctg gct gtg gcc ttg gca cag aag aat gtc aag ctc tca aca      345
Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu Ser Thr
        85                  90                  95

gag cag ctg cgc tgt ctg gct cac cgg ctc tct gag ccc ccc gag gac      393
Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro Glu Asp
    100                 105                 110

ctg gac gcc ctc cca ttg gac ctg ctg cta ttc ctc aac cca gat gcg      441
Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro Asp Ala
115                 120                 125                 130

ttc tcg ggg ccc cag gcc tgc acc cgt ttc ttc tcc cgc atc acg aag      489
Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile Thr Lys
                135                 140                 145
```

-continued

```
gcc aat gtg gac ctg ctc ccg agg ggg gct ccc gag cga cag cgg ctg      537
Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln Arg Leu
            150                 155                 160

ctg cct gcg gct ctg gcc tgc tgg ggt gtg cgg ggg tct ctg ctg agc      585
Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu Leu Ser
        165                 170                 175

gag gct gat gtg cgg gct ctg gga ggc ctg gct tgc gac ctg cct ggg      633
Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu Pro Gly
    180                 185                 190

cgc ttt gtg gcc gag tcg gcc gaa gtg ctg cta ccc cgg ctg gtg agc      681
Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu Val Ser
195                 200                 205                 210

tgc ccg gga ccc ctg gac cag gac cag cag gag gca gcc agg gcg gct      729
Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg Ala Ala
                215                 220                 225

ctg cag ggc ggg gga ccc ccc tac ggc ccc ccg tcg aca tgg tct gtc      777
Leu Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp Ser Val
            230                 235                 240

tcc acg atg gac gct ctg cgg ggc ctg ctg ccc gtg ctg ggc cag ccc      825
Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly Gln Pro
        245                 250                 255

atc atc cgc agc atc ccg cag ggc atc gtg gcc gcg tgg cgg caa cgc      873
Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg Gln Arg
    260                 265                 270

tcc tct cgg gac cca tcc tgg cgg cag cct gaa cgg acc atc ctc cgg      921
Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile Leu Arg
275                 280                 285                 290

ccg cgg ttc cgg cgg gaa gtg gag aag aca gcc tgt cct tca ggc aag      969
Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser Gly Lys
                295                 300                 305

aag gcc cgc gag ata gac gag agc ctc atc ttc tac aag aag tgg gag     1017
Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys Trp Glu
            310                 315                 320

ctg gaa gcc tgc gtg gat gcg gcc ctg ctg gcc acc cag atg gac cgc     1065
Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg
        325                 330                 335

gtg aac gcc atc ccc ttc acc tac gag cag ctg gac gtc cta aag cat     1113
Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu Lys His
    340                 345                 350

aaa ctg gat gag ctc tac cca caa ggt tac ccc gag tct gtg atc cag     1161
Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln
355                 360                 365                 370

cac ctg ggc tac ctc ttc ctc aag atg agc cct gag gac att cgc aag     1209
His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile Arg Lys
                375                 380                 385

tgg aat gtg acg tcc ctg gag acc ctg aag gct ttg ctt gaa gtc aac     1257
Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu Val Asn
            390                 395                 400

aaa ggg cac gaa atg agt cct cag gtg gcc acc ctg atc gac cgc ttt     1305
Lys Gly His Glu Met Ser Pro Gln Val Ala Thr Leu Ile Asp Arg Phe
        405                 410                 415

gtg aag gga agg ggc cag cta gac aaa gac acc cta gac acc ctg acc     1353
Val Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp Thr Leu Thr
    420                 425                 430

gcc ttc tac cct ggg tac ctg tgc tcc ctc agc ccc gag gag ctg agc     1401
Ala Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu Glu Leu Ser
435                 440                 445                 450
```

-continued

```
tcc gtg ccc ccc agc agc atc tgg gcg gtc agg ccc cag gac ctg gac     1449
Ser Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln Asp Leu Asp
                455                 460                 465

acg tgt gac cca agg cag ctg gac gtc ctc tat ccc aag gcc cgc ctt     1497
Thr Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys Ala Arg Leu
            470                 475                 480

gct ttc cag aac atg aac ggg tcc gaa tac ttc gtg aag atc cag tcc     1545
Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys Ile Gln Ser
        485                 490                 495

ttc ctg ggt ggg gcc ccc acg gag gat ttg aag gcg ctc agt cag cag     1593
Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu Ser Gln Gln
    500                 505                 510

aat gtg agc atg gac ttg gcc acg ttc atg aag ctg cgg acg gat gcg     1641
Asn Val Ser Met Asp Leu Ala Thr Phe Met Lys Leu Arg Thr Asp Ala
515                 520                 525                 530

gtg ctg ccg ttg act gtg gct gag gtg cag aaa ctt ctg gga ccc cac     1689
Val Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu Gly Pro His
                535                 540                 545

gtg gag ggc ctg aag gcg gag gag cgg cac cgc ccg gtg cgg gac tgg     1737
Val Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val Arg Asp Trp
            550                 555                 560

atc cta cgg cag cgg cag gac gac ctg gac acg ctg ggg ctg ggg cta     1785
Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly Leu Gly Leu
        565                 570                 575

cag ggc ggc atc ccc aac ggc tac ctg gtc cta gac ctc agc atg caa     1833
Gln Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp Leu Ser Met Gln
    580                 585                 590

gag gcc ctc tcg ggg acg ccc tgc ctc cta gga cct gga cct gtt ctc     1881
Glu Ala Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly Pro Val Leu
595                 600                 605                 610

acc gtc ctg gca ctg ctc cta gcc tcc acc ctg gcc tga gggccccact      1930
Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
                615                 620

cccttgctgg ccccagccct gctggggatc cccgcctggc caggagcagg cacgggtggt   1990

ccccgttcca ccccaagaga actcgcgctc agtaaacggg aacatgcccc ctgcagacac   2050

gt                                                                  2052


<210> SEQ ID NO 4
<211> LENGTH: 622
<212> TYPE: PRT
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 4

Met Ala Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr Pro
1               5                   10                  15

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
```

-continued

```
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
```

-continued

```
            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

Met Gln Glu Ala Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly Pro
        595                 600                 605

Val Leu Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
    610                 615                 620
```

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method for detecting the presence of mesothelin-expressing tumor cells in a human subject comprising determining the presence or amount of anti-mesothelin antibodies in a biological sample obtained from the human subject, wherein the presence or amount of anti-mesothelin antibodies in the biological sample is indicative of the presence of mesothelin-expressing tumor cells in the human subject.

2. The method of claim **1**, wherein the presence or amount of anti-mesothelin antibodies in the biological sample is determined by contacting the biological sample with a polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence at least 90% identical to a sequence comprising at least 20 contiguous nucleotides of SEQ ID NO: 1.

3. The method of claim **1**, further comprising comparing the determined amount of anti-mesothelin antibodies to a reference standard, wherein an amount of anti-mesothelin antibody detected greater than the reference standard is indicative of the presence of mesothelin-expressing tumor cells in the human subject.

4. The method of claim **1**, further comprising determining if the human subject with anti-mesothelin antibodies is suffering from a disease selected from the group consisting of ovarian cancer, mesothelioma, pancreatic cancer, and lung carcinoma.

5. The method of claim **1**, further comprising performing at least one diagnostic assay to determine if the human subject with anti-mesothelin antibodies has pelvic inflammatory disease.

6. The method of claim **5**, wherein the presence of anti-mesothelin antibodies in the sample obtained from the human subject and the absence of pelvic inflammatory disease in the subject is indicative of the presence of mesothelin-expressing tumor cells in the subject.

7. The method of claim **1** wherein the human subject is undergoing therapeutic treatment for a cancer associated with a mesothelin-expressing tumor.

8. The method of claim **1**, wherein the biological sample is a biological fluid selected from the group consisting of blood, plasma, serum, ascitic fluid, urine, saliva, tears, pleural fluid, sputum, vaginal fluid, and washings obtained during a medical procedure.

9. A method of monitoring the efficacy of treatment of a human cancer patient undergoing therapeutic treatment for a mesothelin-expressing tumor, the method comprising:

(a) providing a biological sample from a human patient undergoing therapeutic treatment for a cancer associated with a mesothelin-expressing tumor;

(b) determining the presence or amount of anti-mesothelin antibodies in the biological sample by contacting the biological sample with a polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence at least 90% identical to a sequence comprising at least 20 contiguous nucleotides of SEQ ID NO: 1; and

(c) comparing the determined presence or amount of anti-mesothelin antibodies to an antibody reference value wherein an amount of anti-mesothelin antibody greater than the antibody reference value is indicative of a positive response to the therapeutic treatment for the cancer.

10. The method of claim **9**, wherein the antibody reference value is determined from a biological sample obtained from healthy control subjects.

11. The method of claim **9**, wherein the antibody reference value is determined from a biological sample obtained from the patient prior to treatment for cancer.

12. The method of claim **9**, further comprising determining if the human patient that has anti-mesothelin antibodies has pelvic inflammatory disease.

13. The method of claim **9**, wherein the antibody specifically binds to a polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence at least 95% identical to a sequence comprising at least 20 contiguous nucleotides of SEQ ID NO:3.

14. The method of claim **9**, wherein the antibody specifically binds to a polypeptide comprising a sequence at least 95% identical to a sequence comprising at least 10 contiguous amino acids of SEQ ID NO:4.

15. The method of claim **9**, wherein the biological sample is a biological fluid selected from the group consisting of

blood, plasma, serum, ascitic fluid, urine, saliva, tears, pleural fluid, sputum, vaginal fluid, and washings obtained during a medical procedure.

16. The method of claim **9**, wherein the biological sample is serum.

17. The method of claim **9**, wherein the amount of anti-mesothelin antibodies in the serum is determined using an ELISA assay.

18. The method of claim **9**, further comprising the step of determining the presence or amount of soluble mesothelin-related peptides (SMRP) encoded by a polynucleotide that selectively hybridizes to a sequence at least 90% identical to a sequence comprising at least 20 contiguous nucleotides of SEQ ID NO: 1 in the biological sample, and comparing the determined amount of the polypeptide to an antigen reference value, wherein the detection of a lower amount of the polypeptide in the sample as compared to the antigen reference value in combination with the detection of an increased amount of anti-mesothelin antibodies in the sample, as compared to the antibody reference value, is indicative of a positive response to the treatment for cancer.

19. The method of claim **18**, wherein the antigen reference value is determined from a biological sample obtained from healthy control subjects.

20. The method of claim **18**, wherein the antigen reference value is determined from a biological sample obtained from the human patient prior to treatment for cancer.

21. The method of claim **9**, wherein the human patient is suffering from a disease selected from the group consisting of ovarian cancer, mesothelioma, pancreatic carcinoma, and lung carcinoma.

22. The method of claim **9**, wherein the treatment includes administration of at least one of a chemotherapeutic agent, radiation treatment, antibody therapy, cancer vaccine therapy, gene therapy, or stem cell transplant.

23. The method of claim **9**, wherein the treatment includes surgery to remove at least a portion of a tumor-expressing mesothelin.

24. A kit for detecting the presence of mesothelin-expressing tumor cells in a human subject, the kit comprising reagents specific for detection of the presence or amount of anti-mesothelin antibodies in a biological sample obtained from a human subject and printed instructions for comparison of the detected presence or amount of anti-mesothelin antibodies with a reference standard.

25. The kit of claim **24**, wherein the reference standard is selected from the group consisting of a specific numerical threshold, a negative control sample for concurrent evaluation, or statistical information correlating the amount of anti-mesothelin antibodies detected with the likelihood of the presence of mesothelin-expressing tumor cells in the subject.

26. The kit of claim **24**, wherein the reference standard is a negative control sample, and wherein the negative control sample is included in the kit.

27. The kit of claim **26**, wherein the reagents specific for detection of anti-mesothelin antibodies comprise a polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence that is at least 80% identical to a sequence comprising at least 20 contiguous nucleotides of SEQ ID NO: 1.

28. The kit of claim **24**, further comprising at least one reagent for detecting the presence of pelvic inflammatory disease in the human subject.

* * * * *

专利名称(译)	用于诊断和/或预测卵巢癌的方法		
公开(公告)号	US20090042224A1	公开(公告)日	2009-02-12
申请号	US12/134093	申请日	2008-06-05
[标]申请(专利权)人(译)	华盛顿大学		
申请(专利权)人(译)	华盛顿大学学报		
当前申请(专利权)人(译)	华盛顿大学学报		
[标]发明人	HELLSTROM INGEGERD HELLSTROM KARL ERIK YANG YI		
发明人	HELLSTROM INGEGERD HELLSTROM KARL ERIK YANG YI		
IPC分类号	G01N33/53 G01N33/566		
CPC分类号	G01N33/57449 G01N2800/50 G01N33/6854		
优先权	60/942102 2007-06-05 US		
外部链接	Espacenet USPTO		

摘要(译)

在一个实施方案中，提供了用于评估人受试者中表达间皮素的肿瘤细胞的存在的方法。在另一个实施方案中，提供了用于监测诊断患有间皮素的肿瘤的人癌症患者的治疗功效的方法。