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(54) **PREDICTIVE BIOMARKERS FOR OVARIAN CANCER**

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(57) **ABSTRACT**

Methods are provided for predicting the presence, subtype and stage of ovarian cancer, as well as for assessing the therapeutic efficacy of a cancer treatment and determining whether a subject potentially is developing cancer. Associated test kits, computer and analytical systems as well as software and diagnostic models are also provided.

PREDICTIVE BIOMARKERS FOR OVARIAN CANCER

STATEMENT OF PRIORITY

[0001] This application claims priority under 35 USC Section 119 to Provisional Patent Applications Ser. Nos. 60/947,253 filed Jun. 29, 2007 and 61/037,946 filed Mar. 19, 2008, the disclosures of which are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

[0002] This invention provides methods for predicting and diagnosing ovarian cancer, particularly epithelial ovarian cancer, and it further provides associated analytical reagents, diagnostic models, test kits and clinical reports.

BACKGROUND

[0003] The American Cancer Society estimates that ovarian cancer will strike 22,430 women and take the lives of 15,280 women in 2007 in the United States. Ovarian cancer is not a single disease, however, and there are actually more than 30 types and subtypes of ovarian malignancies, each with its own pathology and clinical behavior. Most experts therefore group ovarian cancers within three major categories, according to the kind of cells from which they were formed: epithelial tumors arise from cells that line or cover the ovaries; germ cell tumors originate from cells that are destined to form eggs within the ovaries; and sex cord-stromal cell tumors begin in the connective cells that hold the ovaries together and produce female hormones.

[0004] Common epithelial tumors begin in the surface epithelium of the ovaries and account for about 90 per cent of all ovarian cancers in the U.S. (and the following percentages reflect U.S. prevalence of these cancers). They are further divided into a number of subtypes—including serous, endometrioid, mucinous, and clear cell tumors—that can be further subclassified as benign or malignant tumors. Serous tumors are the most widespread forms of ovarian cancer. They account for 40 per cent of common epithelial tumors. About 50 per cent of these serous tumors are malignant, 33 per cent are benign, and 17 per cent are of borderline malignancy. Serous tumors occur most often in women who are between 40 and 60 years of age.

[0005] Endometrioid tumors represent approximately 20 per cent of common epithelial tumors. In about 20 per cent of individuals, these cancers are associated with endometrial carcinoma (cancer of the womb lining). In 5 per cent of cases, they also are linked with endometriosis, an abnormal occurrence of endometrium (womb lining tissue) within the pelvic cavity. The majority (about 80 per cent) of these tumors are malignant, and the remainder (roughly 20 per cent) usually is borderline malignancies. Endometrioid tumors occur primarily in women who are between 50 and 70 years of age.

[0006] Clear cell tumors account for about 6 per cent of common epithelial tumors. Nearly all of these tumors are malignant. Approximately one-half of all clear cell tumors are associated with endometriosis. Most patients with clear cell tumors are between 40 and 80 years of age.

[0007] Mucinous tumors make up about 1 per cent of all common epithelial tumors. Most (approximately 80 per cent) of these tumors are benign, 15 per cent are of borderline malignancy, and only 5 per cent are malignant. Mucinous tumors appear most often in women between 30 to 50 years of age.

[0008] Ovarian cancer is by far the most deadly of gynecologic cancers, accounting for more than 55 percent of all gynecologic cancer deaths. But ovarian cancer is also among the most treatable—if it is caught early. When ovarian cancer is caught early and appropriately treated, the 5-year survival rate is 93 percent. See, for example, Luce et al, "Early Diagnosis Key to Epithelial Ovarian Cancer Detection," The Nurse Practitioner, December 2003 at p. 41. Extensive background information about ovarian cancer is readily available on the interne, for example, from the "Overview: Ovarian Cancer" of the Cancer Reference Information provided by the American Cancer Society (www.cancer.org) and the NCCN Clinical Practice Guidelines in Oncology™ Ovarian Cancer V.1.2007 (www.nccn.org).

[0009] The current reality for the diagnosis of ovarian cancer is that most cases—81 percent of all cases of ovarian cancer—are not caught in earliest stage. This is because early stage ovarian cancer is very difficult to diagnose. Its symptoms may not appear or be noticed at this point. Or, symptoms—such as bloating, indigestion, diarrhea, constipation and others—may be vague and associated with many common and less serious conditions. Most importantly, there has been no effective test for early detection. An effective tool for early and accurate detection of ovarian cancer is a critical unmet medical need.

Biomarkers for Ovarian Cancer

[0010] A variety of biomarkers to diagnose ovarian cancer have been proposed, and elucidated through a variety of technology platforms and data analysis tools. An interesting compilation of 1,261 potential protein biomarkers for various pathologies was presented by N. Leigh Anderson et al., "A Target List of Candidate Biomarkers for Targeted Proteomics," Biomarker Insights 2:1-48 (2006). A spreadsheet listing the markers discussed in this paper can be found at the website of the Plasma Proteome Institute (http://www.plasmaproteome.org). Several published studies are described immediately below and a number of other studies are listed as references at the end of this specification. All of these studies, all other documents cited in this specification, and related provisional patent applications Ser. Nos. 60/947,253 filed Jun. 29, 2007 and 61/037,946 filed Mar. 19, 2008, are hereby incorporated by reference in their entireties.

[0011] For example, Cole, "Methods for detecting the onset, progression and regression of gynecologic cancers." U.S. Pat. No. 5,356,817 (Oct. 18, 1994) described a method for detecting the presence of a gynecologic cancer in a female, said cancer selected from the group consisting of cervical cancer, ovarian cancer, endometrial cancer, uterine cancer and vulva cancer, the method comprising the steps of: (a) assaying a plasma or tissue sample from the patient for the presence of CA 125, and at or about the same time; and (b) assaying a bodily non-blood sample from said patient for the presence of human chorionic gonadotropin beta-subunit core fragment, wherein the detection of both CA 125 and human chorionic gonadotropin beta-subunit core fragment is an indication of the presence of a gynecological cancer in the female. A measurement of the human chorionic gonadotropin beta-subunit core fragment alone was stated to be useful in monitoring progression and regression of such cancers.

[0012] Fung et al, "Biomarker for ovarian and endometrial cancer: hepcidin," U. S. Patent Application 20070054329, published Mar. 8, 2007, describes a method for qualifying ovarian and endometrial cancer status based on measuring hepcidin as a single biomarker, and based on panels of markers including hepcidin plus transthyretin, and those two markers plus at least one biomarker selected from the group consisting of: Apo A1, transferrin, CTAP-III and ITIH4 fragment. An additional panel further includes beta-2 microglobulin. These biomarkers were measured by mass spectrometry, particularly SELDI-MS or by immunoassay. And data was analyzed by ROC curve analysis.

[0013] Fung et al. also described the use of hepcidin levels used in combination with other biomarkers, and concluded that the predictive power of the test was improved. More specifically, increased levels of hepcidin together with decreased levels transthyretin were correlated with ovarian cancer. Increased levels of hepcidin together with decreased levels of transthyretin, together with levels of one or more of Apo A1 (decreased level), transferrin (decreased level), CTAP-III (elevated level) and an internal fragment of ITIH4 (elevated level) were also correlated with ovarian cancer. The foregoing biomarkers were to further be combined with beta-2 microglobulin (elevated level), CA125 (elevated level) and/or other known ovarian cancer biomarkers for use in the disclosed diagnostic test. And hepcidin was said to be hepcidin-25, transthyretin was said to be cysteinylated transthyretin, and/or ITIH4 fragment perhaps being the ITIH4 fragment 1.

[0014] Diamandis, "Multiple marker assay for detection of ovarian cancer," U. S. Patent Application 20060134120 published Jun. 22, 2006, described a method for detecting a plurality of kallikrein markers associated with ovarian cancer and optionally CA125, wherein the kallikrein markers comprise or are selected from the group consisting of kallikrein 5, kallikrein 6, kallikrein 7, kallikrein 8, kallikrein 10, and kallikrein 11. His patent application explained that a significant difference in levels of these kallikreins, which are a subgroup of secreted serine proteases markers, and optionally that also of CA125, relative to the corresponding normal levels, was indicative of ovarian cancer. By repeatedly sampling these markers in the same patient over time, Diamandis also found that a significant difference between the levels of the kallikrein markers, and optionally CA125, in a later sample, relative to an earlier sample, is an indication that a patient's therapy is efficacious for inhibiting ovarian cancer. Samples were evaluated by protein binding techniques, for example, immunoassays, and by nucleotide array, PCR and the like techniques.

[0015] Gorelik et al, Multiplexed Immunobead-Based Cytokine Profiling for Early Detection of Ovarian Cancer" in Cancer Epidemiol Biomarkers Prev. 2005:14(4) 981-7 (April 2005) reported that a panel of multiple cytokines that separately may not show strong correlation with the disease provide diagnostic potential. A related patent application appears to be Lokshin et al., "Multifactorial assay for cancer detection," U. S. Patent Application 20050069963 published Mar. 31, 2005. According to the journal article, a novel multianalyte LabMAP profiling technology was employed that allowed simultaneous measurement of multiple markers. Various concentrations of 24 cytokines (cytokinesi-chemokines, growth, and angiogenic factors) in combination with CA-125 were measured in the blood sera of 44 patients with early-stage ovarian cancer, 45 healthy women, and 37 patients with benign pelvic tumors.

[0016] Of the cytokines discussed by Gorelik et al., six markers, specifically interleukin (IL)-6, IL-8, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP-1), together with CA-125, showed significant differences in serum concentrations between ovarian cancer and control groups. Out of those markers, IL-6, IL-8, VEGF, EGF, and CA-125, were used in a classification tree analysis that reportedly resulted in 84% sensitivity at 95% specificity. The receiver operator characteristic curve (ROC) described using the combination of markers produced sensitivities between 90% and 100% and specificities of 80% to 90%. Interestingly, the receiver operator characteristic curve for CA-125 alone resulted in sensitivities of 70% to 80%. The classification tree analysis described in the paper for discrimination of benign condition from ovarian cancer used CA-125, granulocyte colony-stimulating factor (G-CSF), IL-6, EGF, and VEGF which resulted in 86.5% sensitivity and 93.0% specificity. The authors concluded that simultaneous testing of a panel of serum cytokines and CA-125 using LabMAP technology presented a promising approach for ovarian cancer detection.

[0017] A related patent application by Lokshin, "Enhanced diagnostic multimarker serological profiling," U. S. Patent Application 20070042405 published Feb. 22, 2007 describes various biomarker panels and associated methods for diagnosis of ovarian cancer. One method involves determining the levels of at least four markers in the blood of a patient, where at least two different markers are selected from CA-125, prolactin, HE4 (human epididymis protein 4), sV-CAM and TSH; and where a third marker and a fourth marker are selected from CA-125, prolactin, HE4, sV-CAM. TSH, cytokeratin, sI-CAM, IGFBP-1, eotaxin and FSH, where each of the third marker and fourth marker selected from the above listed markers is different from each other and different from either of the first and second markers, and where dysregulation of at least the four markers indicates high specificity and sensitivity for a diagnosis of ovarian cancer. Another panel includes at least eight markers in the blood of a patient, wherein at least four different markers are selected from the group consisting of CA-125, prolactin, HE4, sV-CAM, and TSH and wherein a fifth marker, a sixth marker, a seventh marker and an eighth marker are selected from the group consisting of CA-125, prolactin, HE4, sV-CAM, TSH, cytokeratin, sI-CAM, IGFBP-1, eotaxin and FSH, and further wherein each of said fifth marker, said sixth marker, said seventh marker and said eighth marker is different from the other and is different from any of said at least four markers, wherein dysregulation of said at least eight markers indicates high specificity and sensitivity for a diagnosis of ovarian cancer.

[0018] The Lokshin (2007) patent application also describes a blood marker panel comprising two or more of EGF (epidermal growth factor), G-CSF (granulocyte colony stimulating factor), IL-6, IL-8, CA-125 (Cancer Antigen 125), VEGF (vascular endothelial growth factor), MCP-I (monocyte chemoattractant protein-1), anti-IL6, anti-TL8, anti-CA-125, anti-c-myc, anti-p53, anti-CEA, anti-CA 15-3, anti-MUC-1, anti-survivin, anti-bHCG, anti-osteopontin, anti-PDGF, anti-Her2/neu, anti-Akt1, anti-cytokeratin 19, cytokeratin 19, EGFR, CEA, kallikrein-8, M-CSF, FasL, ErbB2 and Her2/neu in a sample of the patients blood, where

the presence of two or more of the following conditions indicated the presence of ovarian cancer in the patient: EGF (low), G-CSF (high), IL-6 (high), IL-8 (high), VEGF (high), MCP-1 (low), anti-IL-6 (high), anti-IL-8 (high), anti-CA-125 (high), anti-c-myc (high), anti-p.sup.53 (high), anti-CEA (high), anti-CA 15-3 (high), anti-MUC-1 (high), anti-survivin (high), anti-bHCG (high), anti-osteopontin (high), anti-Her2/neu (high), anti-Akt1 (high), anti-cytokeratin 19 (high), anti-PDGF (high), CA-125 (high), cytokeratin 19 (high), EGFR (low, Her2/neu (low), CEA (high). FasL (high), kallikrein-8 (low), ErbB2 (low) and M-CSF (low). Exemplary panels include, without limitation: CA-125, cytokeratin-19, FasL, M-CSF; cytokeratin-19, CEA, Fas, EGFR, kallikrein-8; CEA, Fas, M-CSF, EGFR, CA-125; cytokeratin 19, kallikrein 8, CEA, CA 125, M-CSF; kallikrein-8, EGFR, CA-125; cytokeratin-19, CEA, CA-125, M-CSF, EGFR; cytokeratin-19, kallikrein-8, CA-125, M-CSF, FasL; cytokeratin-19, kallikrein-8, CEA, M-CSF; cytokeratin-19, kallikrein-8, CEA, CA-125; CA 125, cytokeratin 19, ErbB2; EGF, G-CSF, IL-6, IL-8, VEGF and MCP-1; anti-CA 15-3, anti-IL-8, anti-survivin, anti-p53 and anti c-myc; anti-CA 15-3, anti-IL-8, anti-survivin, anti-p53, anti c-myc, anti-CEA, anti-IL-6, anti-EGF; and anti-bHCG.

[0019] Chan, et al., "Use of biomarkers for detecting ovarian cancer," U.S. Published Patent Application 20050059013, published Mar. 17, 2005 describes a method of qualifying ovarian cancer status in a subject comprising: (a) measuring at least one biomarker in a sample from the subject, wherein the biomarker is selected from the group consisting of ApoA1, transthyretin.DELTA.N10, IAIH4 fragment, and combinations thereof, and (b) correlating the measurement with ovarian cancer status.

[0020] Another embodiment in the Chan application described an additional biomarker selected from CA125, CA125 II, CA15-3, CA19-9, CA72-4, CA 195, tumor associated trypsin inhibitor (TATI), CEA, placental alkaline phosphatase (PLAP), Sialyl TN, galactosyltransferase, macrophage colony stimulating factor (M-CSF, CSF-1), lysophosphatidic acid (LPA), 110 kD component of the extracellular domain of the epidermal growth factor receptor (p110EGFR), tissue kallikreins, for example, kallikrein 6 and kallikrein 10 (NES-1), prostasin, HE4, creatine kinase B (CKB), LASA, HER-2/neu, urinary gonadotropin peptide, Dianon NB 70/K, Tissue peptide antigen (TPA), osteopontin and haptoglobin, and protein variants (e.g., cleavage forms, isoforms) of the markers.

[0021] An ELISA-based blood serum test described the evaluation of four proteins useful in the early diagnosis of epithelial ovarian cancer (leptin, prolactin, osteopontin and insulin-like growth factor). The authors reported that no single protein could completely distinguish the cancer group from the healthy control group. However, the combination of these four proteins provided sensitivity 95%, positive predictive value (PPV) 95%, specificity 95%, and negative predictive value (NPV) 94%, which was said to be a considerable improvement on current methodology. Mor et al., "Serum protein markers for early detection of ovarian cancer," PNAS (102:21) 7677-7682 (2005).

[0022] A related patent application by Mor et al. "Identification of Cancer Protein Biomarkers Using Proteomic Techniques," U.S. Patent Application 2005/0214826. published Sep. 29, 2005 describes biomarkers identified by using a novel screening method. The biomarkers are stated to discriminate between cancer and healthy subjects as well as being useful in the prognosis and monitoring of cancer. Specifically, the abstract of the patent application relates to the use of leptin, prolactin, OPN and IGF-II for these purposes. The disclosed invention is somewhat more generally characterized as involving the comparison of expression of one or more biomarkers in a sample that are selected from the group consisting of: 6Ckine, ACE, BDNF, CA125, E-Selectin, EGF, Eot2, ErbB1, follistatin, HCC4, HVEM, IGF-II, IGFBP-1, IL-17, IL-1sRII, IL-2sRa, leptin, M-CSF R, MIF, MIP-1a, MIP3b, MMP-8, MMP7, MPIF-1, OPN, PARC, PDGF Rb, prolactin, ProteinC, TGF-b RIII, TNF-R1, TNF-a, VAP-1, VEGF R2 and VEGF R3. A significant difference in the expression of these one or more biomarkers in the sample as compared to a predetermined standard of each is said to diagnose or aid in the diagnosis of cancer.

[0023] A patent application by Le Page et al. "Methods of Diagnosing Ovarian Cancer and Kits Therefor," WO2007/030949, published Mar. 22, 2007 describes a method for determining whether a subject is affected by ovarian cancer by detecting the expression levels of FGF-2 and CA125 and, optionally, IL-18.

[0024] Other approaches described in the patent and scientific literature include the analysis of expression of particular gene transcripts in blood cells. See, for example, Liew, "Method for the Detection of Cancer Related Gene Transcripts in Blood," U.S. Published Patent Application 2006/0134637, Jun. 22, 2006. Although gene transcripts specific for ovarian cancer are not identified, transcripts from Tables 3J, 3K and 3X are said to indicate the presence of cancer. See also, Tchagang et al., "Early Detection of Ovarian Cancer Using Group Biomarkers," Mol. Cancer Ther. (1):7 (2008).

[0025] Another diagnostic approach involves detecting circulating antibodies directed against tumor-associated antigens. See, Nelson et al. "Antigen Panels and Methods of Using the Same," U.S. Patent Application 2005/0221305, published Oct. 6, 2005; and Robertson "Cancer Detection Methods and Regents," U.S. Patent Application 2003/0232399, published Dec. 18, 2003.

[0026] What has been urgently needed in the field of gynecologic oncology is a minimally invasive (preferably serum-based) clinical test for assessing and predicting the presence of ovarian cancer that is based on a robust set of biomarkers and sample features identified from a large and diverse set of samples, together with methods and associated computer systems and software tools to predict, diagnose and monitor ovarian cancer with high accuracy at its various stages.

SUMMARY OF THE INVENTION

[0027] The present invention generally relates to cancer biomarkers and particularly to biomarkers associated with ovarian cancer. It provides methods to predict, evaluate diagnose, and monitor cancer, particularly ovarian cancer, by measuring certain biomarkers, and further provides a set or array of reagents to evaluate the expression levels of biomarkers that are associated with ovarian cancer. A preferred set of biomarkers provides a detectable molecular signature of ovarian cancer in a subject. The invention provides a predictive or diagnostic test for ovarian cancer, particularly for epithelial ovarian cancer and more particularly for early-stage ovarian cancer (that is Stage I, Stage II or Stage I and II together).

[0028] More specifically, predictive tests and associated methods and products also provide useful clinical information regarding the stage of ovarian cancer progression, that is: Stage I, Stage II, Stage III and Stage IV and an advanced stage which reflects relatively advanced tumors that cannot readily be classified as either Stage III or Stage IV. Overall, the invention also relates to newly discovered correlations between the relative levels of expression of certain groups of markers in bodily fluids, preferably blood serum and plasma, and a subject's ovarian cancer status.

[0029] In one embodiment, the invention provides a set of reagents to measure the expression levels of a panel or set of biomarkers in a fluid sample drawn from a patient, such as blood, serum, plasma, lymph, cerebrospinal fluid, ascites or urine. The reagents in a further embodiment are a multianalyte panel assay comprising reagents to evaluate the expression levels of these biomarker panels.

[0030] In embodiments of the invention, a subject's sample is prepared from tissue samples such a tissue biopsy or from primary cell cultures or culture fluid. In a further embodiment, the expression of the biomarkers is determined at the polypeptide level. Related embodiments utilize immunoassays, enzyme-linked immunosorbent assays and multiplexed immunoassays for this purpose.

[0031] Preferred panels of biomarkers are selected from the group consisting of the following sets of molecules and their measurable fragments: (a) myoglobin, CRP (C reactive protein), FGF basic protein and CA 19-9; (b) Hepatitis C NS4, Ribosomal P Antibody and CRP; (c) CA 19-9. TGF alpha, EN-RAGE, EGF and HSP 90 alpha antibody, (d) EN-RAGE, EGF, CA 125, Fibrinogen, Apolipoprotein CIII, EGF, Cholera Toxin and CA 19-9; (e) Proteinase 3 (cANCA) antibody, Fibrinogen, CA 125, EGF, CD40, TSH, Leptin, CA 19-9 and lymphotactin; (f) CA125, EGFR, CRP, IL-18, Apolipoprotein CIII, Tenascin C and Apolipoprotein A1; (g) CA125, Beta-2 Microglobulin, CRP, Ferritin, TIMP-1, Creatine Kinase-MB and IL-8; (h) CA125, EGFR, IL-10, Haptoglobin, CRP, Insulin, TIMP-1, Ferritin, Alpha-2 Macroglobulin, Leptin, IL-8, CTGF, EN-RAGE, Lymphotactin, TNF-alpha, IGF-1, TNF RII, von Willebrand Factor and MDC; (i) CA-125, CRP, EGF-R, CA-19-9, Apo-AI, Apo-CIII, IL-6, IL-I8, MIP-1a, Tenascin C and Myoglobin; (j) CA-125, CRP, EGF-R, CA-19-9, Apo-AI, Apo-CIII, IL-6, MIP-1 a, Tenascin C and Myoglobin; and (k) any of the biomarker panels presented in Table II and Table III.

[0032] In another embodiment, the reagents that measure such biomarkers may measure other molecular species that are found upstream or downstream in a biochemical pathway or measure fragments of such biomarkers and molecular species. In some instances, the same reagent may accurately measure a biomarker and its fragments.

[0033] Another embodiment of the present invention relates to binding molecules (or binding reagents) to measure the biomarkers and related molecules and fragments. Contemplated binding molecules includes antibodies, both monoclonal and polyclonal, aptamers and the like.

[0034] Other embodiments include such binding reagents provided in the form of a test kit, optionally together with written instructions for performing an evaluation of biomarkers to predict the likelihood of ovarian cancer in a subject.

[0035] In other of its embodiments, the present invention provides methods of predicting the likelihood of ovarian cancer in a subject based on detecting or measuring the levels in a specimen or biological sample from the subject of the foregoing biomarkers. As described in this specification, a change in the expression levels of these biomarkers, particularly their relative expression levels, as compared with a control group of patients who do not have ovarian cancer, is predictive of ovarian cancer in that subject.

[0036] In other of its aspects, the type of ovarian cancer that is predicted is serous, endometrioid, mucinous, and clear cell tumors. And prediction of ovarian cancer includes the prediction of a specific stage of the disease such as Stage I (IA, IB or IC), II, III and IV tumors.

[0037] In yet another embodiment, the invention relates to creating a report for a physician of the relative levels of the biomarkers and to transmitting such a report by mail, fax, email or otherwise. In an embodiment, a data stream is transmitted via the internet that contains the reports of the biomarker evaluations. In a further embodiment, the report includes the prediction as to the presence or absence of ovarian cancer in the subject or the stratified risk of ovarian cancer for the subject, optionally by subtype or stage of cancer.

[0038] According to another aspect of the invention, the foregoing evaluation of biomarker expression levels is combined for diagnostic purposes with other diagnostic procedures such as gastrointestinal tract evaluation, chest x-ray, HE4 test, CA-125 test, complete blood count, ultrasound or abdominal/pelvic computerized tomography, blood chemistry profile and liver function tests.

[0039] Yet other embodiments of the invention relate to the evaluation of samples drawn from a subject who is symptomatic for ovarian cancer or is at high risk for ovarian cancer. Other embodiments relate to subjects who are asymptomatic of ovarian cancer. Symptomatic subjects have one or more of the following: pelvic mass; ascites; abdominal distention; general abdominal discomfort and/or pain (gas, indigestion, pressure, swelling, bloating, cramps); nausea, diarrhea, constipation, or frequent urination; loss of appetite; feeling of fullness even after a light meal; weight gain or loss with no known reason; and abnormal bleeding from the vagina. The levels of biomarkers may be combined with the findings of such symptoms for a diagnosis of ovarian cancer.

[0040] Embodiments of the invention are highly accurate for determining the presence of ovarian cancer. By "highly accurate" is meant a sensitivity and a specificity each at least about 85 per cent or higher, more preferably at least about 90 per cent or 92 per cent and most preferably at least about 95 per cent or 97 per cent accurate. Embodiments of the invention further include methods having a sensitivity of at least about 85 per cent, 90 per cent or 95 per cent and a specificity of at least about 55 per cent, 65 per cent, 75 per cent, 85 per cent or 90 per cent or higher. Other embodiments include methods having a specificity of at least about 85 per cent, 90 per cent or 95 per cent, and a sensitivity of at least about 55 per cent, 65 per cent, 75 per cent, 85 per cent or 90 per cent or higher.

[0041] Embodiments of the invention relating sensitivity and specificity are determined for a population of subjects who are symptomatic for ovarian cancer and have ovarian cancer as compared with a control group of subjects who are symptomatic for ovarian cancer but who do not have ovarian cancer. In another embodiment, sensitivity and specificity are determined for a population of subjects who are at increased risk for ovarian cancer and have ovarian cancer as

compared with a control group of subjects who are at increased risk for ovarian cancer but who do not have ovarian cancer. And in another embodiment, sensitivity and specificity are determined for a population of subjects who are symptomatic for ovarian cancer and have ovarian cancer as compared with a control group of subjects who are not symptomatic for ovarian cancer but who do not have ovarian cancer.

[0042] In other aspects, the levels of the biomarkers are evaluated by applying a statistical method such as knowledge discovery engine (KDE™), regression analysis, discriminant analysis, classification tree analysis, random forests, ProteomeQuest®, support vector machine, One R, kNN and heuristic naive Bayes analysis, neural nets and variants thereof.

[0043] In another embodiment, a predictive or diagnostic model based on the expression levels of the biomarkers is provided. The model may be in the form of software code, computer readable format or in the form of written instructions for evaluating the relative expression of the biomarkers.

[0044] A patient's physician can utilize a report of the biomarker evaluation, in a broader diagnostic context, in order to develop a relatively more complete assessment of the risk that a given patient has ovarian cancer. In making this assessment, a physician will consider the clinical presentation of a patient, which includes symptoms such as a suspicious pelvic mass and/or ascites, abdominal distention and other symptoms without another obvious source of malignancy. The general lab workup for symptomatic patients currently includes a GI evaluation if clinically indicated, chest x-ray, CA-125 test, CBC, ultrasound or abdominal/pelvic CT if clinically indicated, chemistry profile with LFTs and may include a family history evaluation along with genetic marker tests such as BRCA-1 and BRCA-2. (See, generally, the NCCN Clinical Practice Guidelines in Oncology™ for Ovarian Cancer, V.1.2007.)

[0045] The present invention provides a novel and important additional source of information to assist a physician in stratifying a patient's risk of having ovarian cancer and in planning the next diagnostic steps to take. The present invention is also similarly useful in assessing the risk of ovarian cancer in non-symptomatic, high-risk subjects as well as for the general population as a screening tool. It is contemplated that the methods of the present invention may be used by clinicians as part of an overall assessment of other predictive and diagnostic indicators.

[0046] The present invention also provides methods to assess the therapeutic efficacy of existing and candidate chemotherapeutic agents and other types of cancer treatments. As will be appreciated by persons skilled in the art, the relative expression levels of the biomarker panels—or biomarker profiles—are determined as described above, in specimens taken from a subject prior to and again after treatment or, optionally, at progressive stages during treatment. A change in the relative expression of these biomarkers to a non-cancer profile of expression levels (or to a more nearly non-cancer expression profile) or to a stable, non-changing profile of relative biomarker expression levels is interpreted as therapeutic efficacy. Persons skilled in the art will readily understand that a profile of such expressions levels may become diagnostic for cancer or a pre-cancer, pre-malignant condition or simply move toward such a diagnostic profile as the relative ratios of the biomarkers become more like a cancer-related profile than previously.

[0047] In another embodiment, the invention provides a method for determining whether a subject potentially is developing cancer. The relative levels of expression of the biomarkers are determined in specimens taken from a subject over time, whereby a change in the biomarker expression profile toward a cancer profile is interpreted as a progression toward developing cancer.

[0048] The expression levels of the biomarkers of a specimen may be stored electronically once a subject's analysis is completed and recalled for such comparison purposes at a future time.

[0049] The present invention further provides methods, software products, computer systems and networks, and associated instruments that provide a highly accurate test for ovarian cancer.

[0050] The combinations of markers described in this specification provide sensitive, specific and accurate methods for predicting the presence of or detecting ovarian cancer at various stages of its progression. The evaluation of samples as described may also correlate with the presence of a pre-malignant or a pre-clinical condition in a patient. Thus, it is contemplated that the disclosed methods are useful for predicting or detecting the presence of ovarian cancer in a sample, the absence of ovarian cancer in a sample drawn from a subject, the stage of an ovarian cancer, the grade of an ovarian cancer, the benign or malignant nature of an ovarian cancer, the metastatic potential of an ovarian cancer, the histological type of neoplasm associated with the ovarian cancer, the indolence or aggressiveness of the cancer, and other characteristics of ovarian cancer that are relevant to prevention, diagnosis, characterization, and therapy of ovarian cancer in a patient.

[0051] It is further contemplated that the methods disclosed are also useful for assessing the efficacy of one or more test agents for inhibiting ovarian cancer, assessing the efficacy of a therapy for ovarian cancer, monitoring the progression of ovarian cancer, selecting an agent or therapy for inhibiting ovarian cancer, monitoring the treatment of a patient afflicted with ovarian cancer, monitoring the inhibition of ovarian cancer in a patient, and assessing the carcinogenic potential of a test compound by evaluating biomarkers of test animals following exposure.

DETAILED DESCRIPTION

[0052] The biomarker panels and associated methods and products were identified through the analysis of analyte levels of various molecular species in human blood serum drawn from subjects having ovarian cancer of various stages and subtypes, subjects having non-cancer gynecological disorders and normal subjects. The immunoassays described below were courteously performed by our colleagues at Rules-Based Medicine of Austin, Tex. using their Multi-Analyte Profile (MAP) Luminex® platform (www.rules-basedmedicine.com).

[0053] While a preferred sample is blood scrum, it is contemplated that an appropriate sample can be derived from any biological source or sample, such as tissues, extracts, cell cultures, including cells (for example, tumor cells), cell lysates, and physiological fluids, such as, for example, whole blood, plasma, serum, saliva, ductal lavage, ocular lens fluid, cerebral spinal fluid, sweat, urine, milk, ascites fluid, synovial fluid, peritoneal fluid and the like. The

sample can be obtained from animals, preferably mammals, more preferably primates, and most preferably humans using species specific binding agents that are equivalent to those discussed below in the context of human sample analysis. It is further contemplated that these techniques and marker panels may be used to evaluate drug therapy in rodents and other animals, including transgenic animals, relevant to the development of human and veterinary therapeutics.

[0054] The sample can be treated prior to use by conventional techniques, such as preparing plasma from blood, diluting viscous fluids, and the like. Methods of sample treatment can involve filtration, distillation, extraction, concentration, inactivation of interfering components, addition of chaotropes, the addition of reagents, and the like. Nucleic acids (including silencer, regulatory and interfering RNA) may be isolated and their levels of expression for the analytes described below also used in the methods of the invention.

Samples and Analytical Platform

[0055] The set of blood serum samples that was analyzed to generate most of the data discussed below contained 150 ovarian cancer samples and 150 non-ovarian cancer samples. Subsets of these samples were used as described. The ovarian cancer sample samples further comprised the following epithelial ovarian cancer subtypes: serous (64), clear cell (22). endometrioid (35), mucinous (15), mixed, that is, consisting of more than one subtype (14). The stage distribution of the ovarian cancer samples was: Stage I (41), Stage II (23), Stage III (68), Stage IV (12) and unknown stage (6).

[0056] The non-ovarian cancer sample set includes the following ovarian conditions: benign (104), normal ovary (29) and "low malignant potential/borderline (3). The sample set also includes serum from patients with other cancers: cervical cancer (7), endometrial cancer (6) and uterine cancer (1).

[0057] Analyte levels in the samples discussed in this specification were measured using a high-throughput, multi-analyte immunoassay platform. A preferred platform is the Luminex® MAP system as developed by Rules-Based Medicine, Inc. in Austin, Tex. It is described on the company's website and also, for example, in publications such as Chandler et al., "Methods and kits for the diagnosis of acute coronary syndrome, U. S. Patent Application 2007/0003981, published Jan. 4, 2007, and a related application of Spain et al., "Universal Shotgun Assay," U. S. Patent Application 2005/0221363, published Oct. 6, 2005. This platform has previously been described in Lokshin (2007) and generated data used in other analyses of ovarian cancer biomarkers. However, any immunoassay platform or system may be used.

[0058] In brief, to describe a preferred analyte measurement system, the MAP platform incorporates polystyrene microspheres that are dyed internally with two spectrally distinct fluorochromes. By using accurate ratios of the fluorochromes, an array is created consisting of 100 different microsphere sets with specific spectral addresses. Each microsphere set can display a different surface reactant. Because microsphere sets can be distinguished by their spectral addresses, they can be combined, allowing up to 100 different analytes to be measured simultaneously in a single reaction vessel. A third fluorochrome coupled to a reporter molecule quantifies the biomolecular interaction that has occurred at the microsphere surface. Microspheres are interrogated individually in a rapidly flowing fluid stream as they pass by two separate lasers in the Luminex® analyzer. High-speed digital signal processing classifies the microsphere based on its spectral address and quantifies the reaction nil the surface in a few seconds per sample.

[0059] Skilled artisans will recognize that a wide variety of analytical techniques may be used to determine the levels of biomarkers in a sample as is described and claimed in this specification. Other types of binding reagents available to persons skilled in the art may be utilized to measure the levels of the indicated analytes in a sample. For example, a variety of binding agents or binding reagents appropriate to evaluate the levels of a given analyte may readily be identified in the scientific literature. Generally, an appropriate binding agent will bind specifically to an analyte, in other words, it reacts at a detectable level with the analyte but does not react detectably (or reacts with limited cross-reactivity) with other or unrelated analytes. It is contemplated that appropriate binding agents include polyclonal and monoclonal antibodies, aptamers, RNA molecules and the like. Spectrometric methods also may be used to measure the levels of analytes, including immunofluorescence, mass spectrometry, nuclear magnetic resonance and optical spectrometric methods. Depending on the binding agent to be utilized, the samples may be processed, for example, by dilution, purification, denaturation, digestion, fragmentation and the like before analysis as would be known to persons skilled in the art. Also, gene expression, for example, in a tumor cell or lymphocyte also may be determined.

[0060] It is also contemplated that the identified biomarkers may have multiple epitopes for immunassays and/or binding sites for other types of binding agents. Thus, it is contemplated that peptide fragments or other epitopes of the identified biomarkers, isoforms of specific proteins and even compounds upstream or downstream in a biological pathway or that have been post-translationaily modified may be substituted for the identified analytes or biomarkers so long as the relevant and relative stoichiometries are taken into account appropriately. Skilled artisans will recognize that alternative antibodies and binding agents can be used to determine the levels of any particular analyte, so long as their various specificities and binding affinities are factored into the analysis.

[0061] A variety of algorithms may be used to measure or determine the levels of expression of the analytes or biomarkers used in the methods and test kits of the present invention. It is generally contemplated that such algorithms will be capable of measuring analyte levels beyond the measurement of simple cut-off values. Thus, it is contemplated that the results of such algorithms will generically be classified as multivariate index analyses by the U.S. Food and Drug Administration. Specific types of algorithms include: knowledge discovery engine (KDE™), regression analysis, discriminant analysis, classification tree analysis, random forests, ProteomeQuest®, support vector machine, One R, kNN and heuristic naive Bayes analysis, neural nets and variants thereof.

ANALYLSIS AND EXAMPLES

[0062] The following discussion and examples are provided to describe and illustrate the present invention. As such, they should not be construed to limit the scope of the

invention. Those skilled in the art will well appreciate that many other embodiments also fall within the scope of the invention, as it is described in this specification and the claims.

Analysis of Data To Find Informative Biomarker Panels Using the KDE™

[0063] Correlogic has described the use of evolutionary and pattern recognition algorithms in evaluating complex data sets, including the Knowledge Discovery Engine (KDE™) and ProteomeQuest®. See, for example, Hitt et al., U.S. Pat. No. 6,925,389, "Process for Discriminating Between Biological States Based on Hidden Patterns From Biological Data" (issued Aug. 2, 2005); Hitt, U.S. Pat. No. 7,096,206, "Heuristic Method of Classification," (issued Aug. 22, 2006) and Hitt, U.S. Pat. No. 7,240,038, "Heuristic Method of Classification," (to be issued Jul. 3, 2007). The use of this technology to evaluate mass spectral data derived from ovarian cancer samples is further elucidated in Hitt et al., "Multiple high-resolution serum proteomic features for ovarian cancer detection." U. S. Published Patent Application 2006/0064253. published Mar. 23, 2006.

[0064] When analyzing the data set by Correlogic's Knowledge Discovery Engine, the following five-biomarker panels were found to provide sensitivities and specificities for various stages of ovarian cancer as set forth in Table 1. Specifically, KDE Model 1 [2_0008_20] returned a relatively high accuracy for Stage I ovarian cancer and included these markers: Cancer Antigen 19-9 (CA19-9, Swiss-Prot Accession Number: Q9BXJ9), C Reactive Protein (CRP, Swiss-Prot Accession Number: P02741), Fibroblast Growth Factor-basic Protein (FGF-basic, Swiss-Prot Accession Number: P09038) and Myoglobin (Swiss-Prot Accession Number: P02144). KDE Model 2 [4_0002-10] returned a relatively high accuracy for Stage III, IV and "advanced" ovarian cancer and included these markers: Hepatitis C NS4 Antibody (Hep C NS4 Ab), Ribosomal P Antibody and CRP. KDE Model 3 [4_0009_140] returned a relatively high accuracy for Stage I and included these markers: CA 19-9, TGF alpha, EN-RAGE (Swiss-Prot Accession Number: P80511), Epidermal Growth Factor (EGF, Swiss-Prot Accession Number: P01133) and HSP 90 alpha antibody. KDE Model 4 [4_0026_100] returned a relatively high accuracy for Stage II and Stages III, IV and "advanced" ovarian cancers and included these markers: EN-RAGE, EGF, Cancer Antigen 125 (CA125, Swiss-Prot Accession Number: Q14596), Fibrinogen (Swiss-Prot Accession Number: Alpha chain P02671; Beta chain P02675; Gamma chain P02679), Apolipoprotein CIII (ApoCIII, Swiss-Prot Accession Number: P02656), Cholera Toxin and CA 19-9. KDE Model 5 [4_0027_20] also returned a relatively high accuracy for Stage II and Stages III, IV and "advanced" ovarian cancers and included these markers: Proteinase 3 (cANCA) antibody. Fibrinogen, CA 125, EGF, CD40 (Swiss-Prot Accession Number: Q6P2H9), Thyroid Stimulating Hormone (TSH, Swiss-Prot Accession Number: Alpha P01215; Beta P01222 P02679, Leptin (Swiss-Prot Accession Number: P41159), CA 19-9 and Lymphotactin (Swiss-Prot Accession Number: P47992). It is contemplated that skilled artisans could use the KDE analytical tools to identify other, potentially useful sets of biomarkers for predictive or diagnostic value based on the levels of selected analytes. Note that the KDE algorithm may select and utilize various markers based on their relative abundances; and that a given marker, for example the level of cholera toxin in Model IV may be zero but is relevant in combination with the other markers selected in a particular grouping.

[0065] Skilled artisans will recognize that a limited size data set as was used in this specification may lead to different results, for example, different panels of markers and varying accuracies when comparing the relative performance of KDE with other analytical techniques to identify informative panels of biomarkers. These particular KDE models were built on a relatively small data set using 40 stage I ovarian cancers and 40 normal/benigns and were tested blindly on the balance of the stage II, III/IV described above. Thus, the specificity is of the stage I samples reflects sample set size and potential overfitting. The drop in specificity for the balance of the non-ovarian cancer samples also is expected given the relatively larger size of the testing set relative to the training set. Overall, the biomarker panel developed for the stage I samples also provides potentially useful predictive and diagnostic assays for later stages of ovarian cancer given the high sensitivity values.

[0066] However, these examples of biomarker panels illustrate that there are a number of parameters that can be adjusted to impact model performance. For instance in these cases a variety of different numbers of features are combined together, a variety of match values are used, a variety of different lengths of evolution of the genetic algorithm are used and models differing in the number of nodes are generated. By routine experimentation apparent to one skilled in the art, combinations of these parameters can be used to generate other predictive models based on biomarker panels having clinically relevant performance.

TABLE I

Results of Analysis Using Knowledge Discovery Engine to develop a stage I specific classification model.

Model Name	Feature	Match	Generation	Node	Sensitivity Stage I	Specificity Stage I	Accuracy Stage I	Sensitivity Stage II	Sensitivity Stage III-IV	Specificity
2_0008_20	4	0.9	20	12	75	100	87.5	60.9	46.5	82.0
4_0002_10	3	0.7	10	4	75	100	87.5	69.6	82.6	56
4_0009_140	5	0.6	140	5	75	100	87.5	43.5	39.5	71.6
4_0026_100	9	0.7	100	5	87.5	100	93.8	78.3	84.9	67
4_0027_20	9	0.8	20	5	87.5	100	93.8	78.3	84.9	60.6

Methods and Analysis To Find Informative Biomarker Panels Using Random Forests

[0067] A preferred analytical technique, known to skilled artisans, is that of Breiman, Random Forests. Machine Learning, 2001. 45:5-32; as further described by Segel,

Machine Learning Benchmarks and Random Forest Regression, 2004; and Robnik-Sikonja, Improving Random Forests, in Machine Learning, ECML, 2004 Proceedings, J. F. B. e. al., Editor, 2004, Springer: Berlin. Other variants of Random Forests are also useful and contemplated for the methods of the present invention, for example, Regression Forests, Survival Forests, and weighted population Random Forests.

[0068] A modeling set of samples was used as described above for diagnostic models built with the KDE algorithm. Since each of the analyte assays is an independent measurement of a variable, under some circumstances, known to those skilled in the art, it is appropriate to scale the data to adjust for the differing variances of each assay. In such cases, biweight, MAD or equivalent scaling would be appropriate, although in some cases, scaling would not be expected to have a significant impact. A bootstrap layer on top of the Random Forests was used in obtaining the results discussed below.

[0069] In preferred embodiments of the present invention, contemplated panels of biomarkers are:

[0070] a. Cancer Antigen 125 (CA125, Swiss-Prot Accession Number: Q14596) and Epidermal Growth Factor Receptor (EGF-R, Swiss-Prot Accession Number: P00533).

[0071] b. CA125 and C Reactive Protein (CRP, Swiss-Prot Accession Number: P02741).

[0072] c. CA125, CRP and EGF-R.

[0073] d. Any one or more of CA125, CRP and EGF-R, plus any one or more of Ferritin (Swiss-Prot Accession Number: Heavy chain P02794; Light chain P02792), Interleukin-8 (IL-8, Swiss-Prot Accession Number: P10145), and Tissue Inhibitor of Metalloproteinases 1 (TIMP-1, Swiss-Prot Accession Number: P01033),

[0074] e. Any one of the biomarker panels presented in Table II and Table III.

[0075] f. Any of the foregoing panels of biomarkers (a-e) plus any one or more of the other biomarkers in the following list if not previously included in the foregoing panels (a-e). These additional biomarkers were identified empirically or by a literature review: Alpha-2 Macroglobulin (A2M, Swiss-Prot Accession Number: P01023), Apolipoprotein A1-1 (ApoA1, Swiss-Prot Accession Number: P02647), Apolipoprotein C-III (ApoCIII, Swiss-Prot Accession Number: P02656), Apolipoprotein H (ApoH, Swiss-Prot Accession Number: P02749), Beta-2 Microglobulin (B2M, Swiss-Prot Accession Number: P23560), Betacellulin (Swiss-Prot Accession Number: P35070), C Reactive Protein (CRP, Swiss-Prot Accession Number: P02741). Cancer Antigen 19-9 (CA19-9, Swiss-Prot Accession Number: Q9BXJ9), Cancer Antigen 125 (CA125, Swiss-Prot Accession Number: Q14596), Collagen Type 2 Antibody, Creatine Kinase-MB (CK-MB, Swiss-Prot Accession Number: Brain P12277; Muscle P06732), C Reactive Protein (CRP, Swiss-Prot Accession Number: P02741), Connective Tissue Growth Factor (CTGF, Swiss-Prot Accession Number: P29279), Double Stranded DNA Antibody (dsDNA Ab), EN-RAGE (Swiss-Prot Accession Number: P80511), Eotaxin (C—C motif chemokine 11, small-inducible cytokine A11 and Eosinophil chemotactic protein, Swiss-Prot Accession Number: P51671), Epidermal Growth Factor Receptor (EGF-R, Swiss-Prot Accession Number: P00533), Ferritin (Swiss-Prot Accession Number: Heavy chain P02794; Light chain P02792), Follicle-stimulating hormone (FSH, Follicle-stimulating hormone beta subunit, FSH-beta, FSH-B, Follitropin beta chain, Follitropin subunit beta, Swiss-Prot Accession Number: P01225), Haptoglobin (Swiss-Prot Accession Number: P00738), HE4 (Major epididymis-specific protein E4, Epididymal secretory protein E4, Putative protease inhibitor WAP5 and WAP four-disulfide core domain protein 2, Swiss-Prot Accession Number: Q14508), Insulin (Swiss-Prot Accession Number: P01308), Insulin-like Growth Factor 1 (IGF-1, Swiss-Prot Accession Number: P01343), Insulin like growth factor II (IGF-II, Somatomedin-A, Swiss-Prot Accession Number: P01344), Insulin Factor VII (Swiss-Prot Accession Number: P08709), Interleukin-6 (IL-6, Swiss-Prot Accession Number: P05231), Interleukin-8 (IL-8, Swiss-Prot Accession Number: P10145), Interleukin-10 (IL-10, Swiss-Prot Accession Number: P22301), Interleukin-18 (IL-18, Swiss-Prot Accession Number: Q14116), Leptin (Swiss-Prot Accession Number: P41159), Lymphotactin (Swiss-Prot Accession Number: P47992), Macrophage-derived Chemokine (MDC, Swiss-Prot Accession Number: O00626), Macrophage Inhibitory Factor (SWISS PROT), Macrophage Inflammatory Protein 1 alpha (MIP-1alpha, Swiss-Prot Accession Number: P10147), Macrophage migration inhibitory factor (MIF, Phenylpyruvate tautomerase, Glycosylation-inhibiting factor, GIF, Swiss-Prot Accession Number: P14174), Myoglobin (Swiss-Prot Accession Number: P02144), Ostopontin (Bone sialoprotein 1, Secreted phosphoprotein 1, SPP-1, Urinary stone protein, Nephropontin, Uropontin, Swiss-Prot Accession Number: P10451), Pancreatic Islet Cells (GAD) Antibody, Prolactin (Swiss-Prot Accession Number: P01236), Stem Cell Factor (SCF, Swiss-Prot Accession Number: P21583), Tenascin C (Swiss-Prot Accession Number: P24821), Tissue Inhibitor of Metalloproteinases 1 (TIMP-1, Swiss-Prot Accession Number: P01033), Tumor Necrosis Factor-alpha (TNF-alpha, Swiss-Prot Accession Number: P01375), Tumor Necrosis Factor RII (TNF-RII, Swiss-Prot Accession Number: Q92956), von Willebrand Factor (vWF, Swiss-Prot Accession Number: P04275) and the other biomarkers identified as being informative for cancer in the references cited in this specification.

[0076] Using the Random Forests analytical approach, a preferred seven biomarker panel was identified that has a high predictive value for Stage I ovarian cancer. It includes: ApoA1, ApoCIII, CA125, CRP, EGF-R, IL-18 and Tenascin. In the course of building and selecting the relatively more accurate models for Stage I cancers generated by Random Forests using these biomarkers, the sensitivity for Stage I ovarian cancers ranged from about 80% to about 85%. Sensitivity was also about 95 for Stage II and about 94% sensitive for Stage III/IV. The overall specificity was about 70%.

[0077] Similarly, a preferred seven biomarker panel was identified that has a high predictive value for Stage II. It includes: B2M, CA125, CK-MB, CRP, Ferritin, IL-8 and TIMP1. A preferred model for Stage II had a sensitivity of about 82% and a specificity of about 88%.

[0078] For Stage III, Stage IV and advanced ovarian cancer, the following 19 biomarker panel was identified: A2M, CA125, CRP, CTGF, EGF-R, EN-RAGE, Ferritin, Haptoglobin, IGF-1, IL-8, IL-10, Insulin, Leptin, Lymphotactin, MDC, TIMP-1, TNF-alpha, TNF-RII, vWF. A preferred model for Stage III/IV had a sensitivity of about 86% and a specificity of about 89%.

[0079] Other preferred biomarker or analyte panels for detecting, diagnosing and monitoring ovarian cancer are

shown in Table II and in Table III. These panels include CA-125, CRP and EGF-R and, in most cases, CA19-9. In Table II, 20 such panels of seven analytes each selected from 20 preferred analytes are displayed in columns numbered 1 through 20. In Table III, another 20 such panels of seven analytes each selected from 23 preferred analytes are displayed in columns numbered 1 through 20.

TABLE II

	Additional Biomarker Panels																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
CA125	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
CRP	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
EGF-R	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
CA19-9	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Haptoglobin																				
Serum Amyloid P			x			x			x											
Apo AI							x		x											
IL-6			x	x				x		x								x		x
Myoglobin					x					x	x	x		x	x	x	x	x	x	x
MIP-1□	x	x	x	x			x	x	x	x	x	x	x			x				
EN-RAGE																				
CK-MB																				
vWF		x		x			x													
Leptin																x	x			
Apo CIII					x	x													x	
Growth Hormone											x		x	x	x		x	x		
IL-10																				
IL-18	x				x	x		x				x		x					x	x
Myeloperoxidase										x			x							
VCAM-1	x	x													x					

TABLE III

	Additional Biomarker Panels																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
CA125	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
CRP	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
EGF-R	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
CA19-9	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
Haptoglobin																				
Serum Amyloid P							x					x					x			
Apo AI			x							x										
IL-6											x	x		x	x			x	x	
Myoglobin	x	x	x	x	x	x	x	x	x	x										
MIP-1□	x		x	x	x	x	x	x	x			x		x	x	x		x		x
EN-RAGE																				
CK-MB																			x	
vWF											x		x			x			x	
Leptin	x	x								x										
Apo CIII								x			x		x			x	x	x		
Growth Hormone																				
IL-10															x					x
IL-18																				
Myeloperoxidase		x				x						x								
VCAM-1																				
Insulin				x																
Ferritin					x								x	x			x			x
Haptoglobin									x											

[0080] Other preferred biomarker panels (or models) for all stages of ovarian cancer include: (a) CA-125, CRP, EGF-R, CA-19-9, Apo-AI, Apo-CIII, IL-6, IL-18, MIP-1a, Tenascin C and Myoglobin; (b) CA125, CRP, CA19-9, EGF-R, Myoglobin, IL-18, Apo CIII; and (e) CA125, CRP, EGF-R, CA19-9, Apo CIII, MTP-1a, Myoglobin, IL-18, IL-6, Apo AI, Tenascin C, vWF, Haptoglobin, IL-10. Optionally, any one or more of the following biomarkers may be added to these or to any of the other biomarker panels disclosed above in text or tables (to the extent that any such panels are not already specifically identified therein): vWF, Haptoglobin, IL-10, IGF-I, IGF-II, Prolactin, HE4, ACE, ASP and Resistin.

[0081] Any two or more of the preferred biomarkers described above will have predictive value, however, adding one or more of the other preferred markers to any of the analytical panels described herein may increase the panel's predictive value for clinical purposes. For example, adding one or more of the different biomarkers listed above or otherwise identified in the references cited in this specification may also increase the biomarker panel's predictive value and are therefore expressly contemplated. Skilled artisans can readily assess the utility of such additional

biomarkers. It is contemplated that additional biomarker appropriate for addition to the sets (or panels) of biomarkers disclosed or claimed in this specification will not result in a decrease in either sensitivity or specificity without a corresponding increase in either sensitivity or specificity or without a corresponding increase in robustness of the biomarker panel overall. A sensitivity and/or specificity of at least about 80% or higher are preferred, more preferably at least about 85% or higher, and most preferably at least about 90% or 95% or higher.

[0082] To practice the methods of the present invention, appropriate cut-off levels for each of the biomarker analytes must be determined for cancer samples in comparison with control samples. As discussed above, it is preferred that at least about 40 cancer samples and 40 benign samples (including benign, non-malignant disease and normal subjects) be used for this purpose. preferably case matched by age, sex and gender. Larger sample sets are preferred. A person skilled in the art would measure the level of each biomarker in the selected biomarker panel and then use an algorithm, preferably such as Random Forest, to compare the level of analytes in the cancer samples with the level of analytes in the control samples. In this way, a predictive profile can be prepared based on informative cutoffs for the relevant disease type. The use of a separate validation set of samples is preferred to confirm the cut-off values so determined. Case and control samples can be obtained by obtaining consented (or anonymized) samples in a clinical trial or from repositories like the Screening Study for Prostate, Lung, Colorectal, and Ovarian Cancer—PLCO Trial sponsored by the National Cancer Institute (http://www.cancer.gov/clinicaltrials/PLCO-1) or The Gynecologic Oncology Group (http://www.gog.org/). Samples obtained in multiple sites are also preferred.

[0083] The results of analysis of patients' specimens using the disclosed predictive biomarker panels may be output for the benefit of the user or diagnostician, or may otherwise be displayed on a medium such as, but not limited to, a computer screen, a computer readable medium, a piece of paper, or any other visible medium.

[0084] The foregoing embodiments and advantages of this invention are set forth, in part, in the preceding description and examples and, in part, will be apparent to persons skilled in the art from this description and examples and may be further realized from practicing the invention as disclosed herein. For example, the techniques of the present invention are readily applicable to monitoring the progression of ovarian cancer in an individual, by evaluating a specimen or biological sample as described above and then repeating the evaluation at one or more later points in time, such that a difference in the expression or disregulation of the relevant biomarkers over time is indicative of the progression of the ovarian cancer in that individual or the responsiveness to therapy. All references, patents, journal articles, web pages and other documents identified in this patent application are hereby incorporated by reference in their entireties.

OVARIAN CANCER BIOMARKERS—REFERENCES

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1. A set of reagents to measure the levels of biomarkers in a specimen, wherein the biomarkers comprise a panel of biomarkers and their measurable fragments selected from one of the following panels:

(a) CA125, Apo A1, HE4, and FSH, and one or more of the following: CD 40 Antigen (CD40), Eotaxin 1 (CCL11), EN-RAGE (S100A12), Ferritin (FTL), Growth Hormone (GH, somatotropin, human growth hormone, HGH), Haptoglobin (Hp), Insulin like Growth Factor I (IGF-I), Interleukin 8 (IL-8, CXCL8), Leptin (LEP), Macrophage Derived Chemokine (MDC, CCL22), Macrophage Inflammatory Protein 1 alpha (MIP-1α, CCL3), Myoglobin (Mb), Stem Cell Factor (SCF), Tumor Necrosis Factor alpha (TNFα, TNF), and von Willebrand Factor (VWF);

(b) CA125, Apo A1, HE4, FSH, and transferrin; and one or more of the following: CD 40 Antigen (CD40), Eotaxin 1 (CCL11), EN-RAGE (S100A12), Ferritin (FTL), Growth Hormone (GH, somatotropin, human growth hormone, HGH), Haptoglobin (Hp), Insulin like Growth Factor I (IGF-I), Interleukin 8 (IL-8, CXCL8), Leptin (LEP), Macrophage Derived Chemokine (MDC, CCL22), Macrophage Inflammatory Protein 1 alpha (MIP-1α, CCL3), Myoglobin (Mb), Stem Cell Factor (SCF), Tumor Necrosis Factor alpha (TNFα, TNF), and von Willebrand Factor (VWF);

(c) CA125, Apo A1, HE4, FSH, and transthyretin; and one or more of the following: CD 40 Antigen (CD40), Eotaxin 1 (CCL11), EN-RAGE (S100A12), Ferritin (FTL), Growth Hormone (GH, somatotropin, human growth hormone, HGH), Haptoglobin (Hp), Insulin like Growth Factor I (IGF-I), Interleukin 8 (IL-8, CXCL8), Leptin (LEP), Macrophage Derived Chemokine (MDC, CCL22), Macrophage Inflammatory Protein 1 alpha (MIP-1α, CCL3), Myoglobin (Mb), Stem Cell Factor (SCF), Tumor Necrosis Factor alpha (TNFα, TNF), and von Willebrand Factor (VWF);

(d) CA125, Apo A1, Beta-2 Microglobulin, and FSH; and one or more of the following: CD 40 Antigen (CD40), Eotaxin 1 (CCL11), EN-RAGE (S100A12), Ferritin (FTL), Growth Hormone (GH, somatotropin, human growth hormone, HGH), Haptoglobin (Hp), Insulin like Growth Factor I (IGF-I), Interleukin 8 (IL-8, CXCL8), Leptin (LEP), Macrophage Derived Chemokine (MDC, CCL22), Macrophage Inflammatory Protein 1 alpha (MIP-1α, CCL3), Myoglobin (Mb), Stem Cell Factor (SCF), Tumor Necrosis Factor alpha (TNFα, TNF), and von Willebrand Factor (VWF);

(e) CA125, Apo A1, transferrin, and FSH; and one or more of the following: CD 40 Antigen (CD40), Eotaxin 1 (CCL11), EN-RAGE (S100A12), Ferritin (FTL), Growth Hormone (GH, somatotropin, human growth hormone, HGH), Haptoglobin (Hp), Insulin like Growth Factor I (IGF-I), Interleukin 8 (IL-8, CXCL8), Leptin (LEP), Macrophage Derived Chemokine (MDC, CCL22), Macrophage Inflammatory Protein 1 alpha (MIP-1α, CCL3), Myoglobin (Mb), Stem Cell Factor (SCF), Tumor Necrosis Factor alpha (TNFα, TNF), and von Willebrand Factor (VWF);

(f) CA125, Apo A1, transthyretin, and FSH; and one or more of the following: CD 40 Antigen (CD40), Eotaxin 1 (CCL11), EN-RAGE (S100A12), Ferritin (FTL), Growth Hormone (GH, somatotropin, human growth hormone, HGH), Haptoglobin (Hp), Insulin like Growth Factor I (IGF-I), Interleukin 8 (IL-8, CXCL8), Leptin (LEP), Macrophage Derived Chemokine (MDC, CCL22), Macrophage Inflammatory Protein 1 alpha (MIP-1α, CCL3), Myoglobin (Mb), Stem Cell Factor (SCF), Tumor Necrosis Factor alpha (TNFα, TNF), and von Willebrand Factor (VWF); and

(g) CA125, Apo A1, Beta-2 Microglobulin, transferrin, transthyretin, and FSH; and one or more of the following: CD 40 Antigen (CD40), Eotaxin 1 (CCL11), EN-RAGE (S100A12), Ferritin (FTL), Growth Hormone (GH, somatotropin, human growth hormone, HGH), Haptoglobin (Hp), Insulin like Growth Factor I (IGF-I), Interleukin 8 (IL-8, CXCL8), Leptin (LEP), Macrophage Derived Chemokine (MDC, CCL22), Macrophage Inflammatory Protein 1 alpha (MIP-1α, CCL3), Myoglobin (Mb), Stem Cell Factor (SCF), Tumor Necrosis Factor alpha (TNFα, TNF), and von Willebrand Factor (VWF).

2. (canceled)

3. The set of reagents of claim **1**, wherein the reagents are binding molecules.

4. The set of reagents of claim **3**, wherein the binding molecules are antibodies.

5. A test kit comprising the set of reagents of claim **1**.

6. A method of predicting the likelihood of cancer in a subject, comprising: detecting the levels of biomarkers in a specimen using the set of reagents of claim **1**, wherein a change in the levels of the biomarkers, as compared with a control group of patients who do not have cancer, is predictive of cancer in that subject.

7. The method of claim **6**, wherein the cancer is ovarian cancer.

8. The method of claim **7**, wherein a change in the relative levels of the biomarkers is determined.

9. The method of claim **7**, wherein the specimen is selected from the group consisting of blood, serum, plasma, lymph, cerebrospinal fluid, ascites, urine and tissue biopsy.

10. The method of claim **7**, wherein the ovarian cancer is selected from the group consisting of serous, endometrioid, mucinous, and clear cell cancer.

11. The method of claim **7**, wherein the prediction of ovarian cancer includes a stage selected from the group consisting of Stage IA, IB, IC, II, III and IV tumors.

12. The method of claim **7**, further comprising creating a report of the relative levels of the biomarkers.

13. The method of claim **12**, wherein the report includes the prediction as to the presence or absence of ovarian cancer in the subject or the stratified risk of ovarian cancer for the subject, optionally by stage of cancer.

14. The method of claim **7**, wherein the sample is taken from a subject selected from the group consisting of subjects who are symptomatic for ovarian cancer and subjects who are at high risk for ovarian cancer.

15. The method of claim **7**, wherein the method has a sensitivity of at least about 85 per cent and a specificity of at least about 85 per cent.

16. The method of claim **15**, wherein the sensitivity and specificity are determined for a population of women who are symptomatic for ovarian cancer and have ovarian cancer as compared with a control group of women who are symptomatic for ovarian cancer but who do not have ovarian cancer.

17. A predictive or diagnostic model based on levels of the panels of biomarkers of claim **1**.

18. A multianalyte panel assay comprising the set of reagents of claim **1**.

19. A method to assess the therapeutic efficacy of a cancer treatment, comprising: comparing the biomarker profiles in specimens taken from a subject before and after the treatment or during the course of treatment with a set of reagents according to claim **1**, wherein a change in the biomarker profile over time toward a non-cancer profile or to a stable profile is interpreted as efficacy.

20. A method for determining whether a subject potentially is developing cancer, comprising: comparing the biomarker profiles in specimens taken from a subject at two or more points in time with a set of reagents according to claim **1**, wherein a change in the biomarker profile toward a cancer profile, is interpreted as a progression toward developing cancer.

* * * * *