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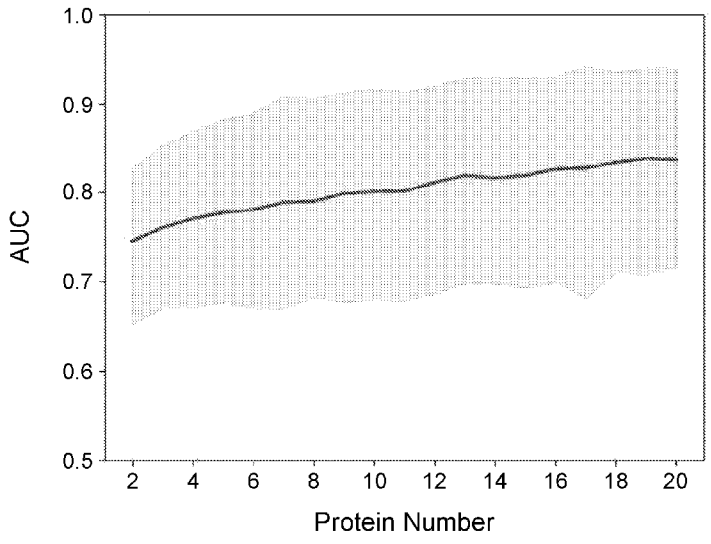

FIG. 1

(57) Abstract: Methods of evaluating a subject for ovarian cancer or detecting ovarian cancer in a subject, the methods comprising determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1. The methods may further comprise applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative of the subject having ovarian cancer. In addition, methods of treatment comprising administering a treatment to the subject when the subject is evaluated or detected to have ovarian cancer.

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TITLE

OVARIAN CANCER DETECTION PROTEINS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 63/451,622, filed on March 12, 2023, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Ovarian cancer is a major health concern among women. In the U.S. alone for 2023, the number of estimated new ovarian cancer cases is 19,710, accounting for about 2% of all estimated new cancer cases in women (Siegel *et al.*, 2022). However, the number of estimated deaths due to ovarian cancer for 2023 is 13,320, which represents almost 5% of all cancer-related deaths in women (*id.*). Thus, while ovarian cancer is not among the most prevalent cancers in women, it is disproportionately more deadly, attributable at least in part to the fact that four out of five patients who are found to have ovarian cancer are diagnosed with an advanced form of the disease (Howlader *et al.*, 2017).

[0003] Early detection of ovarian cancer can have a significant impact on survival. For instance, for epithelial tumors, which represent about 90% of ovarian cancers, the five-year survival rate is 47%, but it is 89% if the cancer is diagnosed when the cancer is at stage I, 71% when diagnosed at stage II, 41% when diagnosed at stage III, and only 20% when diagnosed at stage IV (Torre *et al.*, 2018). Similarly, stromal tumors, which account for about 5% of ovarian tumors, are associated with a five-year survival rate of 98% if the cancer is diagnosed at stage I, but 41% if diagnosed at stage IV (*id.*).

[0004] Unfortunately, there are no obvious symptoms associated with early stages of ovarian cancer. The most common sign of advanced stages of the disease is swelling of the abdomen caused by ascites (Goff *et al.*, 2004), and studies have indicated that some women experience persistent nonspecific symptoms in the months prior to diagnosis, including back pain, abdominal distension, pelvic or abdominal pain, difficulty eating or feeling full quickly, vomiting, indigestion, altered bowel habits, or urinary urgency or frequency (Bankhead *et al.*, 2008; Hamilton *et al.*, 2009). Moreover, there is currently no test recommended for screening for ovarian cancer. Studies have assessed the use of transvaginal ultrasound in

combination with levels of the tumor marker CA125 for early detection, but such use did not result in a reduction in ovarian cancer mortality (Pinsky *et al.*, 2016).

[0005] Therefore, there is an urgent unmet clinical need to improve the detection and diagnosis of ovarian cancer.

SUMMARY OF THE INVENTION

[0006] Some of the main aspects of the present invention are summarized below. Additional aspects are described in the Detailed Description of the Invention, Examples, Drawings, and Claims sections of this disclosure. The description in each section of this disclosure is intended to be read in conjunction with the other sections. Furthermore, the various embodiments described in each section of this disclosure can be combined in various different ways, and all such combinations are intended to fall within the scope of the present invention.

[0007] One aspect of the invention relates to a method of evaluating a subject for ovarian cancer, the method comprising determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**. In some embodiments, the method further comprises applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative of the subject having ovarian cancer. In some embodiments, the method further comprises administering a treatment to the subject.

[0008] Another aspect of the invention relates to a method of treating ovarian cancer in a subject, comprising acquiring results from a method of evaluating a subject for ovarian cancer as described herein, and administering a treatment to the subject.

[0009] Another aspect of the invention relates to a method of detecting ovarian cancer in a subject, the method comprising determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**; and applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected.

[0010] Yet another aspect of the invention relates to a method of treating ovarian cancer in a subject, comprising acquiring results from a method of detecting ovarian cancer in a subject as described herein, and administering a treatment to the subject.

[0011] Another aspect of the invention relates to a method of treating ovarian cancer in a subject in whom ovarian cancer was detected, the method comprising administering a treatment for ovarian cancer to the subject, in which ovarian cancer was detected in the subject by a method comprising determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**; and applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected.

[0012] In some embodiments, the ovarian cancer is early-stage.

[0013] In some embodiments, the subject is asymptomatic of ovarian cancer.

[0014] Another aspect of the invention relates to a method of evaluating a treatment for ovarian cancer in a subject, the method comprising administering a treatment for ovarian cancer, and determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**.

[0015] Another aspect of the invention relates to a method of evaluating the efficacy of a treatment for ovarian cancer in a subject, the method comprising administering a treatment for ovarian cancer to the subject, and determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**.

[0016] Another aspect of the invention relates to a method of treating ovarian cancer in a subject, the method comprising administering a treatment for ovarian cancer to the subject, and determining in a biological sample from the subject a concentration of one or more proteins to evaluate the efficacy of the treatment, wherein the one or more proteins are selected from **Table 1**.

[0017] Another aspect of the invention relates to a method of adjusting a treatment for ovarian cancer in a subject, the method comprising administering a treatment for ovarian cancer to the subject, and determining in a biological sample from the subject a concentration of one or more proteins, wherein the one or more proteins are selected **Table 1**.

[0018] Yet another aspect of the invention relates to a method of treating ovarian cancer in a subject, the method comprising administering a treatment for ovarian cancer to the subject, and determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether the treatment requires adjustment, wherein the one or more proteins are selected from **Table 1**.

[0019] Another aspect of the invention relates to a method of monitoring for ovarian cancer recurrence in a subject, the method comprising administering a treatment for ovarian cancer to the subject, and determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether the treatment requires adjustment, wherein the one or more proteins are selected from **Table 1**. In some embodiments, the method further comprises administering an adjusted treatment when it is determined that the treatment requires adjustment.

[0020] Another aspect of the invention relates to a method of treating ovarian cancer in a subject, the method comprising administering a treatment for ovarian cancer to the subject, and determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether cancer is recurring, wherein the one or more proteins are selected from **Table 1**. In some embodiments, the method further comprises administering a second treatment when it is determined that the cancer is recurring.

[0021] In some embodiments, the biological sample is selected from a plasma sample, serum sample, saliva sample, cerebrospinal fluid (CSF)sample, sweat sample, urine sample, or tear sample. In preferred embodiments, the biological sample is a urine sample.

[0022] In some embodiments, the one or more proteins are selected from **Table 2**.

[0023] In some embodiments, the one or more proteins are selected from **Table 3**.

[0024] In some embodiments, the one or more proteins are selected from **Table 4**. In certain embodiments, the one or more proteins are each protein from **Table 4**.

[0025] Another aspect of the invention relates to a method of measuring amounts of proteins in a subject, the method comprising determining individual amounts of one or more proteins selected from **Table 1**.

[0026] Yet another aspect of the invention relates to a method of measuring amounts of proteins in a subject, the method comprising determining individual amounts of one or more proteins selected from **Table 2**.

[0027] Another aspect of the invention relates to a method of measuring amounts of proteins in a subject, the method comprising determining individual amounts of one or more proteins selected from **Table 3**.

[0028] Another aspect of the invention relates to a method of measuring amounts of proteins in a subject, the method comprising determining individual amounts of one or more proteins selected from **Table 4**. In certain embodiments, the method comprises determining individual amounts of each protein from **Table 4**.

[0029] Another aspect of the invention relates to a kit comprising one or more components that can be used to perform assays for detecting one or more proteins of **Table 1**, or one or more proteins of **Table 2**, or one or more proteins of **Table 3**, or one or more proteins of **Table 4**. In some embodiments, the one or more proteins are selected from **Table 2**. In certain embodiments, the one or more proteins are selected from **Table 3**. In some embodiments, the one or more proteins are selected from **Table 4**. In certain embodiments, the one or more proteins are each protein from **Table 4**.

BRIEF DESCRIPTION OF THE FIGURES

[0030] **FIG. 1** shows accuracy, measured as area-under-the-curve (AUC) of a receiver operating characteristic (ROC) curve, of detecting ovarian cancer in a subject using random combinations of two to 20 proteins selected from **Table 1**, as described in the Example. The process of selecting the random combinations of each number of proteins (two proteins, three proteins, etc.) was performed for 1000 iterations.

[0031] **FIG. 2** shows an ROC curve generated by application of a classifier, which depicts the high diagnostic utility of detecting ovarian cancer in a subject using the panel of 22 proteins listed in **Table 2**, as described in the Example.

[0032] **FIG. 3** shows an ROC curve generated by application of a classifier, which depicts the high diagnostic utility of detecting ovarian cancer in a subject using the panel of 42 proteins listed in **Table 3**, as described in the Example.

[0033] **FIG. 4** shows ROC curves generated by application of a classifier, which depict the high diagnostic utiltiy of detecting ovarian cancer in a subject using each of the 15 proteins listed in **Table 4**, both individualy (solid lines) and in combination (starred line), as described in the Example.

DETAILED DESCRIPTION OF THE INVENTION

[0034] The practice of the present invention can employ, unless otherwise indicated, conventional techniques of proteomics, bioinformatics, oncology, and pharmacology, which are within the skill of the art.

[0035] In order that the present invention can be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the disclosure. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention is related.

[0036] Any headings provided herein are not limitations of the various aspects or embodiments of the invention, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0037] All references cited in this disclosure are hereby incorporated by reference in their entireties. In addition, any manufacturers' instructions or catalogues for any products cited or mentioned herein are incorporated by reference. Documents incorporated by reference into this text, or any teachings therein, can be used in the practice of the present invention. Documents incorporated by reference into this text are not admitted to be prior art.

Definitions

[0038] The phraseology or terminology in this disclosure is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0039] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents, unless the context clearly dictates otherwise. The terms "a" (or "an") as well as the terms "one or more" and "at least one" can be used interchangeably.

[0040] Furthermore, "and/or" is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" is intended to include A and B, A or B, A (alone), and B (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended

to include A, B, and C; A, B, or C; A or B; A or C; B or C; A and B; A and C; B and C; A (alone); B (alone); and C (alone).

[0041] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range, and any individual value provided herein can serve as an endpoint for a range that includes other individual values provided herein. For example, a set of values such as 1, 2, 3, 8, 9, and 10 is also a disclosure of a range of numbers from 1-10. Where a numeric term is preceded by "about," the term includes the stated number and values ±10% of the stated number. The headings provided herein are not limitations of the various aspects or embodiments of the invention, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0042] Wherever embodiments are described with the language "comprising," otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are included.

[0043] An "effective amount" of a composition as disclosed herein is an amount sufficient to carry out a specifically stated purpose. An "effective amount" can be determined empirically and in a routine manner, in relation to the stated purpose, route of administration, and dosage form.

[0044] The term "subject" or "individual" or "patient" means any subject, preferably a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, sports animals, and zoo animals including, *e.g.*, humans, non-human primates, dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, and so on.

[0045] The term "early-stage" in the context of cancer (*e.g.*, "early-stage cancer" or cancer that "is early-stage") refers generally to a level of advancement of the cancer prior to the cancer spreading to lymph nodes or tissues that are distant from the tissue of origin. In some embodiments, an early-stage cancer can refer to a cancer that is a Stage 0, Stage I, or Stage II cancer, based on the stage classification known in the art that grades cancer from Stage 0 (*e.g.*, carcinoma *in situ*, where the cancer is still only in the layer of cells where it started and has not advanced farther), through Stages I-III (*e.g.*, cancer is present—the higher

the number, the larger the tumor and the more it has spread into nearby tissues), and to Stage IV (*e.g.*, the cancer has spread to distant parts of the body). In some embodiments, this stage classification incorporates the TNM System, which evaluates the cancer based on the size and extent of the main tumor ("T"), the number of nearby lymph nodes that have cancer ("N"), and the extent to which the cancer has metastasized ("M").

[0046] The term "symptomatic" means to exhibit one or more signs or features that are regarded as indicative, or are known to be associated with, a disease or condition. A subject may be considered as "symptomatic" of cancer based on symptoms that are known in the art to be associated with cancer in general or for specific types of cancer. Examples include, but are not limited to, fatigue; lump or area of thickening that can be felt under the skin; weight changes, including unintended loss or gain; skin changes, such as yellowing, darkening, or redness of the skin, sores that will not heal, or changes to existing moles; changes in bowel or bladder habits; persistent cough or trouble breathing; difficulty swallowing; hoarseness; persistent indigestion or discomfort after eating; persistent, unexplained muscle or joint pain; persistent, unexplained fevers or night sweats; and unexplained bleeding or bruising. Symptoms that can occur with ovarian cancer in particular include, but are not limited to, bloating, pelvic or abdominal pain; difficulty eating or feeling full quickly; urinary symptoms such as urgency or frequency; fatigue; upset stomach; back pain; pain during sex; constipation; change in menstruation such as heavier-than-normal or irregular bleeding; and abdominal swelling with weight loss.

[0047] A subject may be considered as "suspected of having a cancer" due to the presence of symptoms, *i.e.*, the subject is symptomatic; genetic markers (*e.g.*, mutations in BRCA1, BRCA2, RAS, BRAF, *etc.*); patient's habits or medical history; patient's family medical history; examination or tests known in the art for which the outcome is associated with cancer or risk of cancer, *etc.*

[0048] The term "asymptomatic" means to not exhibit any signs or features that are regarded as indicative, or are known to be associated with, a disease or condition.

[0049] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder. Thus, those in need of treatment include those already with the disorder. In certain embodiments, a subject is successfully

"treated" for a disease or disorder if the patient shows, *e.g.*, total, partial, or transient alleviation or elimination of symptoms associated with the disease or disorder.

[0050] The term "ROC" or "ROC curve" is used to refer to a receiver operator characteristic curve. A ROC curve can be a graphical representation of the performance of a classifier system. For any given method, a ROC can be generated by plotting the sensitivity against the specificity. The sensitivity and specificity of a method for detecting the presence of a cancer or a specific type of cancer can be determined at various concentrations of proteins in a sample from the subject. The AUC of a ROC curve is a metric that can provide a measure of diagnostic utility of a method, taking into account both the sensitivity and specificity of the method. The AUC can range from 0.5 to 1.0, where a value closer to 0.5 can indicate that the method has limited diagnostic utility (*e.g.*, lower sensitivity and/or specificity) and a value closer to 1.0 indicates the method has greater diagnostic utility (*e.g.*, higher sensitivity and/or specificity).

[0051] The term "third party" means a person or group different from the two persons or groups primarily involved. For example, in a multi-step method involving a subject, a third party can be a person/group other than the subject and the person/group primarily responsible for the performance of the steps. In such an example, a third party may perform one of the steps in the method. As another example, in a treatment method involving administration of a treatment to a subject, a third party may be a person/group other than the subject and the person/group administering the treatment.

[0052] The term "cancer recurrence" refers to a return of cancer after a period of remission. The cancer can reappear in the same, or close to, the place that it was previously found (local recurrence); in the lymph nodes and tissue located in the vicinity of the original cancer (regional recurrence); or in areas farther away from the original cancer (distant recurrence).

Methods of the Invention

[0053] The present invention involves the use of proteins in the detection of evaluation of ovarian cancer in subjects (also referred to herein as "ovarian cancer detection

proteins"). Such use can be applied in methods of evaluating a subject for ovarian cancer, methods of treating subjects for ovarian cancer, among others.

[0054] The proteins can be used to detect or evaluate ovarian cancer based on a biological sample from the subject. The biological sample may be any biological sample capable of being obtained from the subject, and encompass fluids, solids, tissues, and gases. In some embodiments, the sample may be a blood product, such as plasma, serum and the like. In some embodiments, the sample may be a urine sample, saliva sample, CSF sample, sweat sample, or tear sample.

[0055] In preferred embodiments, the biological sample is advantageously a urine sample. Compared to blood or plasma samples, there is no homeostasis mechanism in urine that can regulate the presence of proteins in the course of maintaining relatively constant physical/chemical properties within the body (Jing, 2018). It is possible that potential biomarkers may be cleared from plasma or blood by the inherent homeostasis mechanism in order to avoid possible damage or interference to the body (*id.*). On the other hand, the waste materials in the urine are the cleared objects of the blood homeostasis mechanism and therefore may better reflect changes that are produced *in vivo* by the presence of a disease such as ovarian cancer and that would not be cleared by any homeostasis mechanism (*id.*). In addition, urine collection is less traumatic to the body and involves no infliction of pain, is safer and less costly, and is easier and simpler to store (*id.*).

[0056] An aspect of the present invention relates to a method of evaluating a subject for a cancer that is associated with the ovary; or a method of evaluating a subject for ovarian cancer. The method comprises determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**.

[0057] In preferred embodiments, the sample is already separated/obtained/collected from the subject at the time of the evaluation. In some embodiments, the sample is separated from the subject at home and/or by the subject prior to the evaluation.

[0058] In embodiments of the invention, the method identifies whether the subject has ovarian cancer. The method may further comprise applying a classifier to the concentration of the one or more ovarian cancer detection proteins. The classifier identifies whether the concentration of the one or more ovarian cancer detection proteins is indicative that the subject has ovarian cancer.

[0059] In embodiments of the invention, the methods of evaluating a subject further comprise administering a treatment. In some embodiments, the treatment is administered when it is determined that the subject has ovarian cancer.

[0060] To this end, an aspect of the present invention relates to a method of treating ovarian cancer in a subject, comprising (a) acquiring results from methods of evaluating a subject for ovarian cancer as described herein, and (b) administering a treatment to the subject. In some embodiments, the results from methods of evaluating a subject for ovarian cancer are provided by a third party. In some embodiments, the treatment is responsive to the results, *e.g.*, responsive to having ovarian cancer.

[0061] Another aspect of the present invention relates to a method of treating ovarian cancer in a subject, in which the method comprises (a) acquiring results from an evaluation of the subject that determined the subject has ovarian cancer; (b) administering a treatment to the subject, *e.g.*, a treatment for ovarian cancer, in which the evaluation comprises: (I) determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**, and (II) applying a classifier to the concentration of the one or more proteins to identify whether the subject has ovarian cancer. In some embodiments, the results in (a) are acquired from a third party.

[0062] An aspect of the present invention relates to a method of detecting ovarian cancer in a subject, the method comprising determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**, and applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected.

[0063] In embodiments of the invention, the method of detecting ovarian cancer in a subject further comprises administering a treatment. In some embodiments, the treatment is administered when ovarian cancer is detected.

[0064] To this end, an aspect of the present invention relates to a method of treating ovarian cancer in a subject, comprising (a) acquiring results from a method of detecting ovarian cancer in a subject as described herein, and (b) administering a treatment to the subject. In some embodiments, the results from the method of detecting ovarian cancer in a subject are provided by a third party. In some embodiments, the treatment is responsive to the results, *e.g.*, responsive to ovarian cancer being detected.

[0065] An aspect of the invention relates to a method of treating ovarian cancer in a subject in whom ovarian cancer was detected, the method comprising administering a treatment for the ovarian cancer; in which the ovarian cancer had been detected in the subject by a method comprising determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**, and applying a classifier to the concentration of the one or more proteins to identify whether the subject has ovarian cancer. In some embodiments, the method of detecting the ovarian cancer was performed by a third party.

[0066] Yet another aspect of the present invention relates to a method of treating ovarian cancer in a subject, in which the method comprises (a) determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**; (b) applying a classifier to the concentration of the one or more proteins to identify that the subject has ovarian cancer; and (c) administering a treatment to the subject, *e.g.*, a treatment for ovarian cancer.

[0067] Another aspect of the present invention relates to a method of treating cancer in a patient who has been or was determined to have ovarian cancer, comprising administering a treatment for ovarian cancer to the patient, in which the patient was determined to have ovarian cancer by a method comprising (a) determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**, and (b) applying a classifier to the concentration of the one or more proteins. The classifier identifies whether the concentration of the one or more proteins is indicative that the subject has ovarian cancer.

[0068] In some embodiments, the subject is asymptomatic for ovarian cancer. In some embodiments, the methods may be performed as part of, or may be included within, or may overlap with, a screening for ovarian cancer in the subject. In some embodiments, the subject is undergoing a screen for ovarian cancer.

[0069] In some embodiments, the subject is suspected of having ovarian cancer, such as symptomatic of having ovarian cancer.

[0070] In addition, an aspect of the present invention relates to a method of evaluating a treatment for ovarian cancer in a subject. The method comprises (a) administering a treatment for ovarian cancer, and (b) determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**. In preferred embodiments, the sample is already separated/obtained from the subject at the time of performing (b). In some

embodiments, administration of the treatment in (a) may be performed by a third party. In other embodiments, determining the concentration of the one or more proteins in (b) may be performed by a third party.

[0071] In embodiments of the invention, the one or more proteins identifies whether the subject has ovarian cancer after treatment. Thus, the method may further comprise applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative of the subject having ovarian cancer.

[0072] The treatment may be any known treatment for cancer as known in the art and as described herein. The administration of the treatment in (a) may comprise a single administration or occurrence of a therapy, or may comprise multiple administrations or occurrences of a therapy.

[0073] The determination in a biological sample from the subject a concentration of one or more proteins in (b) may be performed more than once. The determination may overlap with the administration of the treatment in (a) or may occur after the administration of the treatment in (a).

[0074] In embodiments in which determination in a biological sample from the subject a concentration of one or more proteins in (b) is occurring after the administration of the treatment in (a), the determination may occur immediately after the administration of the treatment or a period of time after the administration of the treatment. The period of time may be one day or more, or one week or more, or one month or more, or one year or more; including one day, or two days, or three days, or four days, or five days, or six days, or about one week, or about two weeks, or about three weeks, or about four weeks, or about five weeks, or about six weeks, or about seven weeks, or about eight weeks, or about nine weeks, or about ten weeks, or about 11 weeks, or about 12 weeks, or about one month, or about two months, or about three months, or about four months, or about five months, or about six months, or about seven months, or about eight months, or about nine months, or about ten months, or about 11 months, or about 12 months, or about one year, or about two years, or about three years, or about four years, or about five years, or about six years, or about seven years, or about eight years, or about nine years, or about ten years, or about 11 years, or about 12 years, or about 13 years, or about 14 years, or about 15 years, or about 16 years, or about 17 years, or about 18 years, or about 19 years, or about 20 years, or about 21 years, or about

22 years, or about 23 years, or about 24 years, or about 25 years, or about 26 years, or about 27 years, or about 28 years, or about 29 years, or about 30 years, or more; including any ranges formed with these time periods as endpoints, for examples about 4 weeks to about 13 years, about 7 months to about 3 years, *etc.*

[0075] In some embodiments, the presence of ovarian cancer after treatment may be indicative that the treatment was not effective. Thus, another aspect of the invention is a method of evaluating the efficacy of an ovarian cancer treatment, comprising (a) administering a treatment for ovarian cancer, and (b) determining in a biological sample from the subject a concentration of one or more proteins, as described herein. Yet another aspect is a method of treatment, comprising (a) administering a treatment for ovarian cancer, and (b) determining in a biological sample from the subject a concentration of one or more proteins, as described herein, to evaluate whether the treatment was effective.

[0076] In some embodiments, the presence of ovarian cancer after treatment may be indicative that the treatment requires adjustment. Thus, another aspect of the invention is a method of adjusting a treatment for ovarian cancer, comprising (a) administering a treatment for ovarian cancer, and (b) determining in a biological sample from the subject a concentration of one or more proteins, as described herein, to evaluate whether the treatment requires adjustment; such method may further comprise administering a second treatment. The second treatment may be different from the original treatment, for example, a different therapy or different dosage of the same therapy.

[0077] In some embodiments, the presence of ovarian cancer after treatment may be indicative of cancer recurrence. Thus, another aspect of the invention is a method of monitoring for ovarian cancer recurrence, comprising (a) administering a treatment for ovarian cancer, and (b) determining in a biological sample from the subject a concentration of one or more proteins, as described herein. Yet another aspect is a method of treatment, comprising (a) administering a treatment for ovarian cancer, and (b) determining in a biological sample from the subject a concentration of one or more proteins, as described herein, to evaluate cancer recurrence. In some embodiments, the method may further comprise administering a second treatment when it is determined that the ovarian cancer is recurring. The second treatment may be different from the original treatment, for example, a different therapy or different dosage of the same therapy.

[0078] An aspect of the present invention relates to a method of measuring amounts of proteins in a subject, the method comprising determining individual amounts of one or more proteins selected from **Table 1**. In some embodiments, the individual amounts of the one or more proteins is determined in a biological sample from the subject.

[0079] In some embodiments, the biological sample is a plasma sample, serum sample, saliva sample, CSF sample, sweat sample, urine sample, or tear sample. In preferred embodiments, the biological sample is a urine sample.

[0080] In embodiments of the invention, the methods may further comprise obtaining or collecting a biological sample from the subject before determining the concentration of one or more proteins in the biological sample. The collection of the biological sample may be performed in a home (*e.g.*, the home of the subject) or at a medical facility (*e.g.*, doctor's office, hospital, urgent care center, *etc.*).

[0081] In some embodiments, the determination of the concentration of one or more proteins in the biological sample may be performed in a home (*e.g.*, the home of the subject) or at a medical facility (*e.g.*, doctor's office, hospital, urgent care center, *etc.*).

[0082] In some embodiments of the invention, the one or more proteins may be selected from **Table 2**. In some embodiments of the invention, the one or more proteins may be each protein of **Table 2**.

[0083] In some embodiments of the invention, the one or more proteins may be selected from **Table 3**. In some embodiments of the invention, the one or more proteins may be each protein of **Table 3**.

[0084] In some embodiments of the invention, the one or more proteins may be selected from **Table 4**. In some embodiments of the invention, the one or more proteins may be each protein of **Table 4**.

[0085] In some embodiments of the present invention, for any of the ovarian cancer detection proteins, the methods may comprise determining the concentration of two or more, or three or more, or four or more, or five or more, or six or more, or seven or more, or eight or more, or nine or more, or ten or more, or about 15 or more, or about 20 or more, or about 25 or more, or about 30 or more, or about 35 or more, or about 40 proteins or more, or about 40 or more, or about 45 proteins or more, or about 50 proteins or more, or about 55 proteins or more, or about 60 proteins or more, proteins; including any number of proteins chosen

from two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44,45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, or 65; and including any ranges thereof, for example, about two to 65 proteins, or about two to 60 proteins, or about two to 55 proteins, or about two to 50 proteins, or about two to 45 proteins, or about two to 40 proteins, or about two to 35 proteins, or about two to 30 proteins, or about two to 25 proteins, or about two to 20 proteins, or about two to 15 proteins, or about two to ten proteins, or about two to nine proteins, or about two to eight proteins, or about two to seven proteins, or about two to six proteins, or about two to five proteins, or about two to four proteins, or about two or three proteins, or about three to 65 proteins, or about three to 60 proteins, or about three to 55 proteins, or about three to 50 proteins, or about three to 45 proteins, or about three to 40 proteins, or about three to 35 proteins, or about three to 30 proteins, or about three to 25 proteins, or about three to 20 proteins, or about three to 15 proteins, or about three to ten proteins, or about three to nine proteins, or about three to eight proteins, or about three to seven proteins, or about three to six proteins, or about three to five proteins, or about three or four proteins, or about five to 65 proteins, or about five to 60 proteins, or about five to 55 proteins, or about five to 50 proteins, or about five to 45 proteins, or about five to 40 proteins, or about five to 35 proteins, or about five to 30 proteins, or about five to 25 proteins, or about five to 20 proteins, or about five to 15 proteins, or about five to ten proteins, or about five to nine proteins, or about five to eight proteins, or about five to seven proteins, or about five or six proteins, or about ten to 65 proteins, or about ten to 60 proteins, or about ten to 55 proteins, or about ten to 50 proteins, or about ten to 45 proteins, or about ten to 40 proteins, or about ten to 35 proteins, or about ten to 30 proteins, or about ten to 25 proteins, or about ten to 20 proteins, or about ten to 15 proteins, or about 15 to 65 proteins, or about 15 to 60 proteins, or about 15 to 55 proteins, or about 15 to 50 proteins, or about 15 to 45 proteins, or about 15 to 40 proteins, or about 15 to 35 proteins, or about 15 to 30 proteins, or about 15 to 25 proteins, or about 15 to 20 proteins, or about 20 to 65 proteins, or about 20 to 60 proteins, or about 20 to 55 proteins, or about 20 to 50 proteins, or about 20 to 45 proteins, or about 20 to 40 proteins, or about 20 to 35 proteins, or about 20 to 30 proteins, or about 20 to 25 proteins, or about 25 to 65 proteins, or about 25 to 60 proteins, or about 25 to 55 proteins, or about 25 to 50 proteins, or about 25 to 45 proteins, or about 25 to 40 proteins, or about 25 to 30 proteins, or about 30 to 65 proteins, or about 30 to 60 proteins, or about 30 to 55 proteins, or about 30 to 50 proteins, or about 30 to 45 proteins, or about 30

to 40 proteins, or about 30 to 35 proteins, or about 35 to 65 proteins, or about 35 to 60 proteins, or about 35 to 55 proteins, or about 35 to 50 proteins, or about 35 to 45 proteins, or about 35 to 40 proteins, or about 40 to 65 proteins, or about 40 to 60 proteins, or about 40 to 55 proteins, or about 40 to 50 proteins, or about 40 to 45 proteins, or about 45 to 65 proteins, or about 45 to 60 proteins, or about 45 to 55 proteins, or about 45 to 50 proteins, or about 50 to 65 proteins, or about 50 to 60 proteins, or about 50 to 55 proteins, or about 55 to 65 proteins, or about 55 to 60 proteins, or about 60 to 65 proteins.

[0086] In some embodiments, the methods may comprise determining the concentration of each protein of **Table 1**. In certain embodiments, the methods may comprise determining the concentration of each protein of **Table 2**. In certain embodiments, the methods may comprise determining the concentration of each protein of **Table 3**. In certain embodiments, the methods may comprise determining the concentration of each protein of **Table 4**.

[0087] In some embodiments, the number of proteins for which the concentration is determined may be sufficient to achieve an AUC of a ROC curve of at least about 0.6. In certain embodiments, the number of proteins for which the concentration is determined may be sufficient to achieve an AUC of a ROC curve of at least about 0.7, or at least about 0.8, or at least about 0.9.

[0088] In embodiments of the invention, the ovarian cancer is early-stage. In some embodiments, the ovarian cancer is stage I. In some embodiments, the ovarian cancer is stage II.

[0089] In some embodiments, the ovarian cancer is stage III. In some embodiments, the ovarian cancer is stage IV. In some embodiments, the ovarian cancer is stage V.

[0090] The treatment administered to the subjects according to the methods described herein may be treatments known in the art. Examples of such treatments include, but are not limited to, surgery, radiation therapy, chemotherapy, hormone therapy, targeted therapy, and any combination thereof. Examples of surgery may include, but are not limited, to bilateral salpingo-oophorectomy (removal of the ovaries and fallopian tubes); hysterectomy, such as a simple hysterectomy (removal of the uterus and cervix) or radical hysterectomy (removal of the entire uterus, tissues next to the uterus, and the upper part of the vagina); omentectomy (removal of omentum); lymph node dissection (removal of lymph nodes, such as those in the

pelvis or around the aorta); and any combination thereof. Examples of radiation therapy include, but are not limited to, brachytherapy, external beam radiation therapy, and a combination thereof. Examples of chemotherapy include, but are not limited to, paclitaxel, albumin-bound paclitaxel, altretamine, capecitabine, cisplatin, cyclophosphamide, dactinomycin, etoposide, gemcitabine, ifosfamide, irinotecan, doxorubicin, melphalan, pemetrexed, topotecan, vinblastine, vinorelbine, and any combination thereof. Examples of hormone therapy include, but are not limited to, luteinizing-hormone-releasing hormone agonists such as goserelin and/or leuprolide; tamoxifen; aromatase inhibitors such as letrozole, anastrozole, and/or exemestane; and any combination thereof. Examples of targeted therapy include, but are not limited to, angiogenesis inhibitors such as bevacizumab; poly(ADP)-ribose polymerase (PARP) inhibitors such as olaparib, rucaparib, and/or niraparib; drugs that target folate receptor-alpha such as mirvetuximab soravtansine; drugs that target cells with *NTRK* gene changes such as larotrectinib and/or entrectinib; and a combination thereof.

[0091] In certain embodiments, a cancer patient subjected to a method of the invention is successfully treated if the patient's survival is longer than the median survival of patients having ovarian cancer. Survival can be overall survival, *i.e.*, length of time a patient lives, or progression-free survival, *i.e.*, length of time a patient is treated without progression of the disease. Survival can be measured from the date of diagnosis or from the date that treatment commences. Overall survival, median overall survival, progression-free survival, and median progression-free survival can be determined by methods known in the art and/or by those described herein.

[0092] In certain embodiments a patient with ovarian cancer subjected to a method of the invention is successfully treated if the patient has an improved response to the anti-cancer therapy compared with a patient having ovarian cancer who has not been subjected to a method of the invention. For example, treatment of ovarian cancer would be successful in a subject treated by the methods of the invention if the subject has an improved response compared to the median response of patients who have not been treated by the methods of the invention. Response to anti-cancer treatment can be measured by known methods appropriate to the cancer type, for instance, using Response Evaluation Criteria in Solid Tumors (RECIST). Patients evaluated using RECIST can have a complete response (CR), a partial response (PR), stable disease (SD), or progressive disease (PD). An improved

response can also be assessed by other criteria, for example, duration of response, reduction in tumor volume, minimum residual disease (MRD), and the like.

Protein Concentration Measurement and Application of Classifiers

[0093] The concentration of proteins in the sample may be measured using protein quantitation techniques known in the art. Such techniques include, but are not limited to, enzyme-linked immunosorbent assays, chemiluminescence immunoassays, immunohistochemistry, liquid-bead immunoassays, mass spectrometry, aptamer-based assays, reverse phase protein arrays, proximity extension assay (PEA), and a combination thereof.

[0094] In the methods described herein, the concentration of the two or more proteins are used and combined with mathematical, statistical, and machine-learning methods to create secondary features. One or more proteins with and without secondary features and baseline features, including age, sex, race and ethnicity, past medical history, family history, patient's lab values, comorbidities, and concomitant medications, are used in one or more predictive models to calculate a score.

[0095] Machine learning and statistical analyses techniques used to generate features and the final score for the cancer are included but not limited to the following concepts and methods: Supervised learning concepts may include AODE; Artificial neural network, such as Backpropagation, Auto encoders, Hopfield networks, Boltzmann machines, Restricted Boltzmann Machines, and Spiking neural networks; Bayesian statistics, such as Bayesian network and Bayesian knowledge base; Case-based reasoning; Gaussian process regression; Gene expression programming; Group method of data handling (GMDH); Inductive logic programming; Instance-based learning; Lazy learning; Learning Automata; Learning Vector Quantization; Logistic Model Tree; Minimum message length (decision trees, decision graphs, etc.), such as Nearest Neighbor Algorithm and Analogical modeling; Probably approximately correct learning (PAC) learning; Ripple down rules, a knowledge acquisition methodology; Symbolic machine learning algorithms; Support vector machines; Random Forests; Ensembles of classifiers, such as Bootstrap aggregating (bagging) and Boosting (meta -algorithm); Ordinal classification; Information fuzzy networks (IFN); Conditional Random Field; ANOVA; Linear classifiers, such as Fisher's linear discriminant, Linear regression, Logistic regression, Multinomial logistic regression, I Bayes classifier,

Perceptron, Support vector machines; Quadratic classifiers; k -nearest neighbor; Boosting; Decision trees, such as C4.5, Random forests, ID3, CART, SLIQ SPRINT; Bayesian networks, sucINaive Bayes; and Hidden Markov models . Unsupervised learning concepts may include; Expectation –maximization algorithm; Vector Quantization; Generative topographic map; Information bottleneck method; Artificial neural network, such as Self - organizing map; Association rule learning, such as Apriori algorithm, Eclat algorithm, and FP growth algorithm; Hierarchical clusterings such as Single linkage clustering and Conceptual clustering; Cluster analysis, such as K -means algorithm, Fuzzy clustering, DBSCAN, and OPTICS algorithm; and Outlier Detection, such as Local Outlier Factor. Semi-supervised learning concepts may include; Generative models; Low –density separation; Graph-based methods, and Co -training. Reinforcement learning concepts may include Temporal difference learning; Q -learning, Learning Automata, and SARSA. Deep learning concepts may include Deep belief networks; Deep Boltzmann machines; Deep Convolutional neural networks; Deep Recurrent neural networks; and Hierarchical temporal memory.

[0096] For concentrations obtained from detection proteins, one or more features are fed into one or more computation models. The classifiers are used to calculate a score for the patient. The scores of different classifiers are combined to identify the patient as having the specific cancer or not. The computational model may use one or more proteins or secondary features with and without baseline features that could generate a (ROC curve greater than or equal to 0.6. This step determines if the sample indicates the presence of the cancer.

[0097] Protein concentrations and/or secondary features are fed into one or more predictive models. The features could be similar or different from what was used in determining cancer status. The classifiers are used to calculate a score for the patient for ovarian cancer. The predictive models use the proteins or derived secondary features that could generate a ROC curve greater than or equal to 0.6.

[0098] Generally, machine learning algorithms are used to construct models that accurately assign class labels to examples based on the input features that describe the example.

[0099] Embodiments of the present disclosure can be further defined by reference to the following non-limiting examples. It will be apparent to those skilled in the art that many modifications, both to materials and methods, can be practiced without departing from the scope of the present disclosure.

Kit

[00100] An aspect of the present invention relates to a kit for use in detecting one or more ovarian cancer detection proteins, *i.e.*, one or more proteins of **Table 1**, or one or more proteins of **Table 2**, or one or more proteins of **Table 3**, or one or more proteins of **Table 4**, or each protein of **Table 4**, which can be used to perform the methods described herein. The kit may comprise one or more components that can be used to perform assays such as enzyme-linked immunosorbent assays, chemiluminescence immunoassays, immunohistochemistry, liquid-bead immunoassays, mass spectrometry, aptamer-based assays, reverse phase protein arrays, PEA, or a combination thereof. Such components include, but are not limited to, antibodies or antigen binding fragments thereof that bind one or more proteins of **Table 1**, or one or more proteins of **Table 2**, or one or more proteins of **Table 3**, or one or more proteins of **Table 4**, or each protein of **Table 4**.

[00101] In some embodiments, the kit comprises antibodies or antigen binding fragments thereof that bind two or more, or three or more, or four or more, or five or more, or six or more, or seven or more, or eight or more, or nine or more, or ten or more, or about 15 or more, or about 20 or more, or about 25 or more, or about 30 or more, or about 35 or more, or about 40 proteins or more, or about 40 or more, or about 45 proteins or more, or about 50 proteins or more, or about 55 proteins or more, or about 60 proteins or more proteins; including any number of proteins chosen from two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44,45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, or 65; and including any ranges thereof, for example, about two to 65 proteins, or about two to 60 proteins, or about two to 55 proteins, or about two to 50 proteins, or about two to 45 proteins, or about two to 40 proteins, or about two to 35 proteins, or about two to 30 proteins, or about two to 25 proteins, or about two to 20 proteins, or about two to 15 proteins, or about two to ten proteins, or about two to nine proteins, or about two to eight proteins, or about two to seven proteins, or about two to six proteins, or about two to five proteins, or about two to four proteins, or about two or three proteins, or about three to 65 proteins, or about three to 60 proteins, or about three to 55 proteins, or about three to 50 proteins, or about three to 45 proteins, or about three to 40 proteins, or about three to 35 proteins, or about three to 30 proteins, or about three to 25 proteins, or about three to 20 proteins, or about three to 15 proteins, or about three to ten proteins, or about three to nine

proteins, or about three to eight proteins, or about three to seven proteins, or about three to six proteins, or about three to five proteins, or about three or four proteins, or about five to 65 proteins, or about five to 60 proteins, or about five to 55 proteins, or about five to 50 proteins, or about five to 45 proteins, or about five to 40 proteins, or about five to 35 proteins, or about five to 30 proteins, or about five to 25 proteins, or about five to 20 proteins, or about five to 15 proteins, or about five to ten proteins, or about five to nine proteins, or about five to eight proteins, or about five to seven proteins, or about five or six proteins, or about ten to 65 proteins, or about ten to 60 proteins, or about ten to 55 proteins, or about ten to 50 proteins, or about ten to 45 proteins, or about ten to 40 proteins, or about ten to 35 proteins, or about ten to 30 proteins, or about ten to 25 proteins, or about ten to 20 proteins, or about ten to 15 proteins, or about 15 to 65 proteins, or about 15 to 60 proteins, or about 15 to 55 proteins, or about 15 to 50 proteins, or about 15 to 45 proteins, or about 15 to 40 proteins, or about 15 to 35 proteins, or about 15 to 30 proteins, or about 15 to 25 proteins, or about 15 to 20 proteins, or about 20 to 65 proteins, or about 20 to 60 proteins, or about 20 to 55 proteins, or about 20 to 50 proteins, or about 20 to 45 proteins, or about 20 to 40 proteins, or about 20 to 35 proteins, or about 20 to 30 proteins, or about 20 to 25 proteins, or about 25 to 65 proteins, or about 25 to 60 proteins, or about 25 to 55 proteins, or about 25 to 50 proteins, or about 25 to 45 proteins, or about 25 to 40 proteins, or about 25 to 30 proteins, or about 30 to 65 proteins, or about 30 to 60 proteins, or about 30 to 55 proteins, or about 30 to 50 proteins, or about 30 to 45 proteins, or about 30 to 40 proteins, or about 30 to 35 proteins, or about 35 to 65 proteins, or about 35 to 60 proteins, or about 35 to 55 proteins, or about 35 to 50 proteins, or about 35 to 45 proteins, or about 35 to 40 proteins, or about 40 to 65 proteins, or about 40 to 60 proteins, or about 40 to 55 proteins, or about 40 to 50 proteins, or about 40 to 45 proteins, or about 45 to 65 proteins, or about 45 to 60 proteins, or about 45 to 55 proteins, or about 45 to 50 proteins, or about 50 to 65 proteins, or about 50 to 60 proteins, or about 50 to 55 proteins, or about 55 to 65 proteins, or about 55 to 60 proteins, or about 60 to 65 proteins.

[00102] In some embodiments, the kit may also comprise one or more enzymes, substrates, labels, or other components useful for performing the assays.

[00103] In some embodiments, the kit further comprises one or more of the following: one or more containers for collecting or holding the sample (*e.g.*, urine sample), controls, directions for performing the methods, any necessary software for analysis and presentation of results.

[00104] One skilled in the art will readily recognize that the disclosed one or more components can be readily incorporated into any of the established kit formats that are well known in the art.

EXAMPLE

[00105] Analyses were performed to identify the ovarian cancer detection proteins of the present invention.

Sample Collection

Urine samples were collected from a patient population diagnosed with ovarian cancer, and from healthy individuals without ovarian cancer.

Protein Measurement

[00106] While any protein measurement technique could have been used, including enzyme-linked immunosorbent assays (ELISA), chemiluminescence immunoassays (CLIA), immunohistochemistry (IHC), liquid-bead immunoassays, mass spectrometry, aptamer-based assays, reverse phase protein arrays (RPPA), etc., a proximity extension assay (PEA) was employed to evaluate proteins in urine. In PEA, each protein was recognized by two antibodies for proper detection. In proximity assays, each of the two antibodies was conjugated to one of two different DNA oligonucleotides, and the reagents were incubated with the samples in solution. The proximity reactions underwent a dilution step. Oligonucleotides on pairs of antibodies that remain in proximity by virtue of having bound the same protein molecule then underwent DNA ligation (proximity ligation assay) or DNA polymerization (proximity extension assay). The effect of the ligation or polymerization reactions was to create amplifiable reporter DNA strands for sensitive readout via, for example, real-time PCR or next-generation sequencing, and the assays could be performed in high multiplex. By constructing the assays so that only proper pairs of antibodies can yield detection signals, but no other combination of antibodies, the detection of many different proteins in parallel was possible without eroding detection specificity by reactions of noncognate pairs.

[00107] The analytical performance of the panels was validated for sensitivity, dynamic range, specificity, precision, and scalability. The analytical measuring range was defined by the lower limit of quantification (LLOQ) and upper limit of quantification

(ULOQ) and reported in pg/mL. The high dose hook effect (a state of antigen excess relative to the reagent antibodies resulting in falsely lower values) was also determined for each analyte.

[00108] All assays were thoroughly validated for precision (repeatability and reproducibility). Intra-assay variation (within-run) was calculated as the mean CV for individual samples, within each separate run during the validation studies. Inter-assay variation (between-runs) was calculated as the mean CV, for the same individual samples, among separate runs during the validation studies.

[00109] Each protein analyte was addressed by a matched pair of antibodies, coupled to unique, partially complementary oligonucleotides and measured by quantitative real-time PCR. Validation of the readout specificity for all of the panels was carried out using a simple, sequential approach in which pools of protein analytes were tested.

Feature Selection

[00110] Proteins were used to create features that could be used for the classification of samples. The proteins were categorized based on their concentration or their patterns of change detected by different statistical or machine-learning techniques to create new features.

[00111] Machine learning and statistical analyses techniques used to generate features and the final score for the cancer were included but not limited to the following concepts and methods: supervised learning concepts that may include AODE; artificial neural network, such as Backpropagation, Auto encoders, Hopfield networks, Boltzmann machines, Restricted Boltzmann Machines, and Spiking neural networks; Bayesian statistics, such as Bayesian network and Bayesian knowledge base; case-based reasoning; Gaussian process regression; gene expression programming; group method of data handling (GMDH); inductive logic programming; instance-based learning; lazy learning; learning Automata; learning vector quantization; logistic model tree; minimum message length (decision trees, decision graphs, etc.), such as nearest neighbor algorithm and analogical modeling; probability approximately correct learning (PAC) learning; ripple down rules, a knowledge acquisition methodology; symbolic machine learning algorithms; support vector machines; random forests; ensembles of classifiers, such as bootstrap aggregating (bagging) and boosting (meta -algorithm); ordinal classification; information fuzzy networks (IFN); conditional random field; ANOVA; linear classifiers, such'as Fisher's linear discriminant,

linear regression, logistic regression, multinomial logistic relion, naive Bayes classifier, Perceptron, support vector machines; quadratic classifiers; k -nearest neighbor; boosting; decision trees, such as C4.5, random forests, ID3, CART, SLIQ SPRINT; Bayesian netI, such as Naive Bayes; and Hidden Markov models. cUnsupervised learning concepts may include; expectation –maximization algorithm; vector quantization; generative topographic map; information bottleneck method; artificial neural network, such as self -organizing map; association rule learning, such as Apriori algorithm, Eclat algorithm, and FP growth algorithm; hierarchical clusterings such as single linkage clustering and conceptual clustering; cluster analysis, such as K -means algorithm, fuzzy clustering, DBSCAN, and OPTICS algorithm; and outlier detection, such as local outlier factor. Semi-supervised learning concepts may include: generative models; low–density separation; graph-based methods, and co -training. Reinforcement learning concepts may include temporal difference learning; Q -learning, learning automata, and SARSA. Deep learning concepts may include deep belief networks; deep Boltzmann machines; deep convolutional neural networks; deep recurrent neural networks; and hierarchical temporal memory.

Ovarian Cancer Detection Proteins

[00112] One or more features were fed into one or more computation models. The classifiers were used to calculate a score for the patient. The scores of different classifiers were combined to identify the patient as having ovarian cancer or not. The computational model only selected protein or protein combinations that could generate a receiver operating characteristic (ROC) curve of greater than or equal to 0.6. The resulting ovarian cancer detection proteins are shown in **Table 1**. **FIG. 1** shows that the accuracy is over 0.7 when any two proteins through any 20 proteins are randomly selected.

[00113] The model also identified particular substes of the proteins of **Table 1** from which one or more proteins can be selected from to detect ovarian cancer. Such subsets are presented in **Table 2**, **Table 3**, and **Table 4**. In addition, it was determined that panels of the proteins of **Table 2**, **Table 3**, and **Table 4** each exhibits high diagnostic utiltiy: the ROC curve generated from the panel of all of the proteins listed in **Table 2** has an AUC of about 0.894 (*see* **FIG. 2**), the ROC curve generated from the panel of all of the proteins listed in **Table 3** has an AUC of about 0.942 (*see* **FIG. 3**), and the ROC curve generated from the panel of all of the proteins listed in **Table 4** has an AUC of about 0.974 (*see* **FIG. 4**).

Table 1. Ovarian cancer detection proteins.

Gene Name	Uniprot ID	AUC	Gene Name	Uniprot ID	AUC
NRGN	Q92686	0.741	LEPR	P48357	0.749
FGFR4	P22455	0.785	INHBC	P55103	0.762
KLRD1	Q13241	0.745	CEP170	Q5SW79	0.742
REG1B	P48304	0.762	OGN	P20774	0.718
FSTL1	Q12841	0.785	A1BG	P04217	0.759
IMPACT	Q9P2X3	0.705	PDIA2	Q13087	0.767
SCGB1A1	P11684	0.657	IL18RAP	O95256	0.740
PRSS2	P07478	0.709	YTHDF3	Q7Z739	0.740
RARRES1	P49788	0.474	ALPI	P09923	0.697
LIF	P15018	0.738	RRAS	P10301	0.645
MNDA	P41218	0.733	PF4	P02776	0.775
CGB3; CGB5; CGB8	P0DN86	0.770	ITIH5	Q86UX2	0.736
CFHR4	Q92496	0.705	FCRLB	Q6BAA4	0.731
PBLD	P30039	0.742	CELA2A	P08217	0.725
C9orf40	Q8IXQ3	0.784	RTBDN	Q9BSG5	0.539
KIRREL2	Q6UWL6	0.711	MYCBP2	O75592	0.705
AHSG	P02765	0.751	ADGRD1	Q6QNK2	0.649
PRDX2	P32119	0.729	ANKRD54	Q6NXT1	0.581
ALPP	P05187	0.661	WNT9A	O14904	0.719
CDH1	P12830	0.766	SLIT2	O94813	0.675
FSHB	P01225	0.748	SLA2	Q9H6Q3	0.679
PPBP	P02775	0.733	PZP	P20742	0.720
MYDGF	Q969H8	0.689	AFP	P02771	0.632
PGD	P52209	0.725	AMY2A	P04746	0.704
AGRN	O00468	0.712	ANGPT2	O15123	0.678
ANGPTL4	Q9BY76	0.720	FZD8	Q9H461	0.618
ROR1	Q01973	0.730	GlPC3	Q8TF64	0.713
DCN	P07585	0.775	THTPA	Q9BU02	0.713
TREM2	Q9NZC2	0.795	SUSD1	Q6UWL2	0.660
CA1	P00915	0.725	DNM3	Q9UQ16	0.711
RNASE3	P12724	0.643	LGALS9	O00182	0.727
IFI30	P13284	0.770	TG	P01266	0.620
ULBP2	Q9BZM5	0.753			

Table 2. Subset of ovarian cancer detection proteins from Table 1, which together can achieve an AUC of 0.894.

Gene Name	Uniprot ID	Gene Name	Uniprot ID
TREM2	Q9NZC2	INHBC	P55103
FSTL1	Q12841	A1BG	P04217
FGFR4	P22455	ULBP2	Q9BZM5
C9orf40	Q8IXQ3	AHSG	P02765
DCN	P07585	LEPR	P48357
PF4	P02776	FSHB	P01225
CGB3; CGB5; CGB8	P0DN86	KLRD1	Q13241
IFI30	P13284	PBLD	P30039
PDIA2	Q13087	CEP170	Q5SW79
CDH1	P12830	NRGN	Q92686
REG1B	P48304	YTHDF3	Q7Z739

Table 3. Subset of ovarian cancer detection proteins from Table 1, which together can achieve an AUC of 0.942.

Gene Name	Uniprot ID	Gene Name	Uniprot ID
FGFR4	P22455	PF4	P02776
FSTL1	Q12841	ITIH5	Q86UX2
RARRES1	P49788	FCRLB	Q6BAA4
CGB3; CGB5; CGB8	P0DN86	RTBDN	Q9BSG5
PBLD	P30039	MYCBP2	O75592
C9orf40	Q8IXQ3	ADGRD1	Q6QNK2
KIRREL2	Q6UWL6	ANKRD54	Q6NXT1
ALPP	P05187	WNT9A	O14904
CDH1	P12830	SLIT2	O94813
FSHB	P01225	SLA2	Q9H6Q3
PGD	P52209	PZP	P20742
ROR1	Q01973	AFP	P02771
TREM2	Q9NZC2	AMY2A	P04746
IFI30	P13284	ANGPT2	O15123
ULBP2	Q9BZM5	FZD8	Q9H461
LEPR	P48357	GIPC3	Q8TF64
A1BG	P04217	THTPA	Q9BU02
PDIA2	Q13087	SUSD1	Q6UWL2
IL18RAP	O95256	DNM3	Q9UQ16
YTHDF3	Q7Z739	LGALS9	O00182
ALPI	P09923	TG	P01266

Table 4. Subset of ovarian cancer detection proteins from Table 1, which together can achieve an AUC of 0.974.

Gene Name	Uniprot ID	Gene Name	Uniprot ID
FSTL1	Q12841	PGD	P52209
RARRES1	P49788	ULBP2	Q9BZM5
PBLD	P30039	IL18RAP	O95256
C9orf40	Q8IXQ3	PF4	P02776
KIRREL2	Q6UWL6	ITIH5	Q86UX2
ALPP	P05187	AFP	P02771
CDH1	P12830	RTBDN	Q9BSG5
FSHB	P01225		

EMBODIMENTS

[00114] Select embodiments of the present invention are as follows:

Embodiment 1. A method of evaluating a subject for ovarian cancer, the method comprising:

determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**;

thereby evaluating the subject for cancer.

Embodiment 2. The method of Embodiment 1, further comprising applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative of the subject having ovarian cancer.

Embodiment 3. The method of Embodiment 1 or 2, further comprising administering a treatment to the subject.

Embodiment 4. A method of treating ovarian cancer in a subject, comprising

(a) acquiring results from the method of Embodiments 1 or 2; and

(b) administering a treatment to the subject.

Embodiment 5. The method of Embodiment 4, wherein the treatment is responsive to the results acquired in (a).

Embodiment 6. The method of Embodiment 4 or 5, wherein (a) comprises:

(i) determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**; and

(ii) applying a classifier to the concentration of the one or more proteins to identify whether the subject has ovarian cancer.

Embodiment 7. A method of treating ovarian cancer in a subject, the method comprising:

(a) acquiring results from an evaluation of the subject that determined the subject has ovarian cancer;

(b) administering a treatment to the subject,

wherein the evaluation comprises:

(i) determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**; and

(ii) applying a classifier to the concentration of the one or more proteins to identify whether the subject has ovarian cancer.

Embodiment 8. The method of any one of Embodiments 4-8, wherein the results in (a) are acquired from a third party.

Embodiment 9. A method of detecting ovarian cancer in a subject, the method comprising:

determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**; and

applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected.

Embodiment 10. The method of Embodiment 9, further comprising administering a treatment to the subject.

Embodiment 11. A method of treating ovarian cancer in a subject, comprising

(a) acquiring results from the method of Embodiment 9; and

(b) administering a treatment to the subject.

Embodiment 12. The method of Embodiment 11, wherein the treatment is responsive to the results acquired in (a).

Embodiment 13. A method of treating ovarian cancer in a subject, the method comprising

determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**;

applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected; and

administering a treatment to the subject when ovarian cancer is detected.

Embodiment 14. A method of treating ovarian cancer in a subject in whom ovarian cancer was detected, the method comprising administering a treatment for ovarian cancer to the subject, wherein ovarian cancer was detected in the subject by a method comprising:

determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**; and

applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected.

Embodiment 15. The method of Embodiment 14, wherein the method of detecting ovarian cancer was performed by a third party.

Embodiment 16. The method of any one of Embodiments 1-15, wherein the ovarian cancer is early-stage.

Embodiment 17. The method of any one of Embodiments 1-16, wherein the subject is asymptomatic of ovarian cancer.

Embodiment 18. The method of Embodiment 17, wherein the subject is undergoing a screen for ovarian cancer.

Embodiment 19. The method of any one of Embodiments 1-18, wherein the subject is symptomatic of ovarian cancer.

Embodiment 20. A method of evaluating a treatment for ovarian cancer in a subject, the method comprising:

administering a treatment for ovarian cancer, and

determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**;

thereby evaluating the treatment.

Embodiment 21. A method of evaluating the efficacy of a treatment for ovarian cancer in a subject, the method comprising

(a) administering a treatment for ovarian cancer to the subject, and

(b) determining in a biological sample from the subject a concentration of one or more proteins selected from **Table 1**;

thereby evaluating the efficacy of the treatment.

Embodiment 22. A method of treating ovarian cancer in a subject, the method comprising

(a) administering a treatment for ovarian cancer to the subject, and

(b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate the efficacy of the treatment, wherein the one or more proteins are selected from **Table 1**.

Embodiment 23. A method of adjusting a treatment for ovarian cancer in a subject, the method comprising

(a) administering a treatment for ovarian cancer to the subject,

(b) determining in a biological sample from the subject a concentration of one or more proteins, wherein the one or more proteins are selected **Table 1**, and

(c) administering an adjusted treatment to the subject when it is determined that the adjusted treatment is necessary.

Embodiment 24. A method of treating ovarian cancer in a subject, the method comprising

(a) administering a treatment for ovarian cancer to the subject, and

(b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether the treatment requires adjustment, wherein the one or more proteins are selected from **Table 1**.

Embodiment 25. The method of Embodiment 24, further comprising administering an adjusted treatment when it is determined that the adjusted treatment is necessary.

Embodiment 26. A method of monitoring for ovarian cancer recurrence in a subject, comprising

(a) administering a treatment for ovarian cancer to the subject, and

(b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether the ovarian cancer is recurring, wherein the one or more proteins are selected from **Table 1**.

Embodiment 27. A method of treating ovarian cancer in a subject, the method comprising

(a) administering a treatment for ovarian cancer to the subject, and

(b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether cancer is recurring, wherein the one or more proteins are selected from **Table 1**.

Embodiment 28. The method of Embodiment 26 or 27, further comprising administering a second treatment when it is determined that the cancer is recurring.

Embodiment 29. The method of any one of Embodiments 20-28, further comprising applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative of the subject having ovarian cancer.

Embodiment 30. The method of any one of Embodiments 1-29, wherein the biological sample is selected from a plasma sample, serum sample, saliva sample, CSF sample, sweat sample, urine sample, or tear sample.

Embodiment 31. The method of Embodiment 30, wherein the biological sample is a urine sample.

Embodiment 32. The method of any one of Embodiments 1-31, further comprising collecting the biological sample from the subject.

Embodiment 33. The method of Embodiment 32, wherein the collection of the biological sample is performed in the home of the subject.

Embodiment 34. The method of Embodiment 33, wherein the collection of the biological sample is performed in a medical facility.

Embodiment 35. The method of any one of Embodiments 1-34, wherein the determination of the concentration of the one or more proteins is performed in the home of the subject.

Embodiment 36. The method of any one of Embodiments 1-34, wherein the determination of the concentration of the one or more proteins is performed in a medical facility.

Embodiment 37. The method of any one of Embodiments 1-36, wherein the number of proteins for which the concentration is determined is sufficient to achieve an area-under-the-curve (AUC) of a ROC curve of at least about 0.6.

Embodiment 38. The method of Embodiment 37, wherein the number of proteins for which the concentration is determined is sufficient to achieve an AUC of a ROC curve of at least about 0.7.

Embodiment 39. The method of Embodiment 38, wherein the number of proteins for which the concentration is determined is sufficient to achieve an AUC of a ROC curve of at least about 0.8.

Embodiment 40. The method of any one of Embodiments 1-39, wherein the concentration of the two or more proteins is determined by one or more assays.

Embodiment 41. The method of any one of Embodiments 20-40, wherein the administration of the treatment in (a) is performed by a third party.

Embodiment 42. The method of any one of Embodiments 20-40, wherein the determination in a urine sample from the subject a concentration of one or more proteins in (b) is performed by a third party.

Embodiment 43. A method of measuring amounts of proteins in a subject, the method comprising determining individual amounts of one or more proteins selected from **Table 1**.

Embodiment 44. The method of any one of Embodiments 1-43, wherein the one or more proteins are selected from **Table 2**.

Embodiment 45. The method of any one of Embodiments 1-43, wherein the one or more proteins are selected from **Table 3**.

Embodiment 46. The method of any one of Embodiments 1-43, wherein the one or more proteins are selected from **Table 4**.

Embodiment 47. The method of any one of Embodiments 1-46, wherein two or more proteins are selected.

Embodiment 48. The method of any one of Embodiments 1-46, wherein three or more proteins are selected.

Embodiment 49. The method of any one of Embodiments 1-46, wherein five or more proteins are selected.

Embodiment 50. The method of any one of Embodiments 1-46, wherein ten or more proteins are selected.

Embodiment 51. The method of any one of Embodiments 1-45, wherein 20 or more proteins are selected.

Embodiment 52. The method of any one of Embodiments 1-43 or 45, wherein 30 or more proteins are selected.

Embodiment 53. The method of any one of Embodiments 1-43 or 45, wherein 40 or more proteins are selected.

Embodiment 54. The method of any one of Embodiments 1-43, wherein 50 or more proteins are selected.

Embodiment 55. The method of any one of Embodiments 1-43, wherein 60 or more proteins are selected.

Embodiment 56. The method of any one of Embodiments 1-46, wherein all proteins are selected.

Embodiment 57. The method of any one of Embodiments 1-43, wherein no more than about 60 proteins are selected.

Embodiment 58. The method of any one of Embodiments 1-43, wherein no more than about 50 proteins are selected.

Embodiment 59. The method of any one of Embodiments 1-43 or 45, wherein no more than about 40 proteins are selected.

Embodiment 60. The method of any one of Embodiments 1-43 or 45, wherein no more than about 30 proteins are selected.

Embodiment 61. The method of any one of Embodiments 1-45, wherein no more than about 20 proteins are selected.

Embodiment 62. The method of any one of Embodiments 1-46, wherein no more than about ten proteins are selected.

Embodiment 63. The method of any one of Embodiments 1-46, wherein no more than about five proteins are selected.

REFERENCES

Bankhead CR, *et al.*, Identifying symptoms of ovarian cancer: a qualitative and quantitative study, *BJOG*, 2008, 115: 1008–1014.

Goff BA, *et al.*, Frequency of symptoms of ovarian cancer in women presenting to primary care clinics, *JAMA*, 2004, 291:2705–2712.

Hamilton W, *et al.*, Risk of ovarian cancer in women with symptoms in primary care: population based case-control study. *BMJ*, 2009, 339: b2998.

Howlader N, et al. (eds). *SEER Cancer Statistics Review, 1975–2014*, National Cancer Institute; Bethesda, MD, https://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission, posted to the SEER web site, April 2017.

Jing, J., Urine biomarkers in the early stages of diseases: current status and perspective, *Discov. Med.*, 2018, 25: 57-65.

Pinsky PF, *et al.*, Extended mortality results for ovarian cancer screening in the PLCO trial with median 15years follow-up, *Gynecol. Oncol.*, 2016, 143: 270–275.

Siegel RL, *et al.*, Cancer statistics, 2023, *CA Cancer J. Clin.*, 2022, 72: 7-33.

Torre LA, *et al.*, Ovarian cancer statistics, 2018, *CA Cancer J. Clin.*, 2018, 68: 284-296.

CLAIMS

1. A method of evaluating a subject for ovarian cancer, the method comprising:

determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1;

thereby evaluating the subject for cancer.

2. The method of claim 1, further comprising applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative of the subject having ovarian cancer.

3. The method of claim 1 or 2, further comprising administering a treatment to the subject.

4. A method of treating ovarian cancer in a subject, comprising

(a) acquiring results from the method of claim 1 or 2; and

(b) administering a treatment to the subject.

5. The method of claim 4, wherein the treatment is responsive to the results acquired in (a).

6. The method of claim 4 or 5, wherein (a) comprises:

(i) determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1; and

(ii) applying a classifier to the concentration of the one or more proteins to identify whether the subject has ovarian cancer.

7. A method of treating ovarian cancer in a subject, the method comprising:

(a) acquiring results from an evaluation of the subject that determined the subject has ovarian cancer;

(b) administering a treatment to the subject,

wherein the evaluation comprises:

(i) determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1; and

(ii) applying a classifier to the concentration of the one or more proteins to identify whether the subject has ovarian cancer.

8. The method of any one of claims 4-8, wherein the results in (a) are acquired from a third party.

9. A method of detecting ovarian cancer in a subject, the method comprising:

determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1; and

applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected.

10. The method of claim 9, further comprising administering a treatment to the subject.

11. A method of treating ovarian cancer in a subject, comprising

(a) acquiring results from the method of claim 9; and

(b) administering a treatment to the subject.

12. The method of claim 11, wherein the treatment is responsive to the results acquired in (a).

13. A method of treating ovarian cancer in a subject, the method comprising

determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1;

applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected; and

administering a treatment to the subject when ovarian cancer is detected.

14. A method of treating ovarian cancer in a subject in whom ovarian cancer was detected, the method comprising administering a treatment for ovarian cancer to the subject, wherein ovarian cancer was detected in the subject by a method comprising:

determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1; and

applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected.

15. The method of claim 14, wherein the method of detecting ovarian cancer was performed by a third party.

16. The method of any one of claims 1-15, wherein the ovarian cancer is early-stage.

17. The method of any one of claims 1-16, wherein the subject is asymptomatic of ovarian cancer.

18. The method of claim 17, wherein the subject is undergoing a screen for ovarian cancer.

19. The method of any one of claims 1-18, wherein the subject is symptomatic of ovarian cancer.

20. A method of evaluating a treatment for ovarian cancer in a subject, the method comprising:

(a) administering a treatment for ovarian cancer, and

(b) determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1;

thereby evaluating the treatment.

21. A method of evaluating the efficacy of a treatment for ovarian cancer in a subject, the method comprising

(a) administering a treatment for ovarian cancer to the subject, and

(b) determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1;

thereby evaluating the efficacy of the treatment.

22. A method of treating ovarian cancer in a subject, the method comprising

(a) administering a treatment for ovarian cancer to the subject, and

(b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate the efficacy of the treatment, wherein the one or more proteins are selected from Table 1.

23. A method of adjusting a treatment for ovarian cancer in a subject, the method comprising

(a) administering a treatment for ovarian cancer to the subject,

(b) determining in a biological sample from the subject a concentration of one or more proteins, wherein the one or more proteins are selected Table 1, and

(c) administering an adjusted treatment to the subject when it is determined that the adjusted treatment is necessary.

24. A method of treating ovarian cancer in a subject, the method comprising

(a) administering a treatment for ovarian cancer to the subject, and

(b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether the treatment requires adjustment, wherein the one or more proteins are selected from Table 1.

25. The method of claim 24, further comprising administering an adjusted treatment when it is determined that the adjusted treatment is necessary.

26. A method of monitoring for ovarian cancer recurrence in a subject, the method comprising

(a) administering a treatment for ovarian cancer to the subject, and

(b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether the ovarian cancer is recurring, wherein the one or more proteins are selected from Table 1.

27. A method of treating ovarian cancer in a subject, the method comprising

(a) administering a treatment for ovarian cancer to the subject, and

(b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether cancer is recurring, wherein the one or more proteins are selected from Table 1.

28. The method of claim 26 or 27, further comprising administering a second treatment when it is determined that the cancer is recurring.

29. The method of any one of claims 20-28, further comprising applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative of the subject having ovarian cancer.

30. The method of any one of claims 1-29, wherein the biological sample is selected from a plasma sample, serum sample, saliva sample, CSF sample, sweat sample, urine sample, or tear sample.

31. The method of claim 30, wherein the biological sample is a urine sample.

32. The method of any one of claims 1-31, further comprising collecting the biological sample from the subject.

33. The method of claim 32, wherein the collection of the biological sample is performed in the home of the subject.

34. The method of claim 33, wherein the collection of the biological sample is performed in a medical facility.

35. The method of any one of claims 1-34, wherein the determination of the concentration of the one or more proteins is performed in the home of the subject.

36. The method of any one of claims 1-34, wherein the determination of the concentration of the one or more proteins is performed in a medical facility.

37. The method of any one of claims 1-36, wherein the number of proteins for which the concentration is determined is sufficient to achieve an area-under-the-curve (AUC) of a ROC curve of at least about 0.6.

38. The method of claim 37, wherein the number of proteins for which the concentration is determined is sufficient to achieve an AUC of a ROC curve of at least about 0.7.

39. The method of claim 38, wherein the number of proteins for which the concentration is determined is sufficient to achieve an AUC of a ROC curve of at least about 0.8.

40. The method of any one of claims 1-39, wherein the concentration of the two or more proteins is determined by one or more assays.

41. The method of any one of claims 20-40, wherein the administration of the treatment in (a) is performed by a third party.

42. The method of any one of claims 20-40, wherein the determination in a urine sample from the subject a concentration of one or more proteins in (b) is performed by a third party.

43. A method of measuring amounts of proteins in a subject, the method comprising determining individual amounts of one or more proteins selected from Table 1.

44. The method of any one of claims 1-43, wherein the one or more proteins are selected from Table 2.

45. The method of any one of claims 1-43, wherein the one or more proteins are selected from Table 3.

46. The method of any one of claims 1-43, wherein the one or more proteins are selected from Table 4.

47. The method of any one of claims 1-46, wherein two or more proteins are selected.

48. The method of any one of claims 1-46, wherein three or more proteins are selected.

49. The method of any one of claims 1-46, wherein five or more proteins are selected.

50. The method of any one of claims 1-46, wherein ten or more proteins are selected.

51. The method of any one of claims 1-45, wherein 20 or more proteins are selected.

52. The method of any one of claims 1-43 or 45, wherein 30 or more proteins are selected.

53. The method of any one of claims 1-43 or 45, wherein 40 or more proteins are selected.

54. The method of any one of claims 1-43, wherein 50 or more proteins are selected.

55. The method of any one of claims 1-43, wherein 60 or more proteins are selected.

56. The method of any one of claims 1-46, wherein all proteins are selected.

57. The method of any one of claims 1-43, wherein no more than about 60 proteins are selected.

58. The method of any one of claims 1-43, wherein no more than about 50 proteins are selected.

59. The method of any one of claims 1-43 or 45, wherein no more than about 40 proteins are selected.

60. The method of any one of claims 1-43 or 45, wherein no more than about 30 proteins are selected.

61. The method of any one of claims 1-45, wherein no more than about 20 proteins are selected.

62. The method of any one of claims 1-46, wherein no more than about ten proteins are selected.

63. The method of any one of claims 1-46, wherein no more than about five proteins are selected.

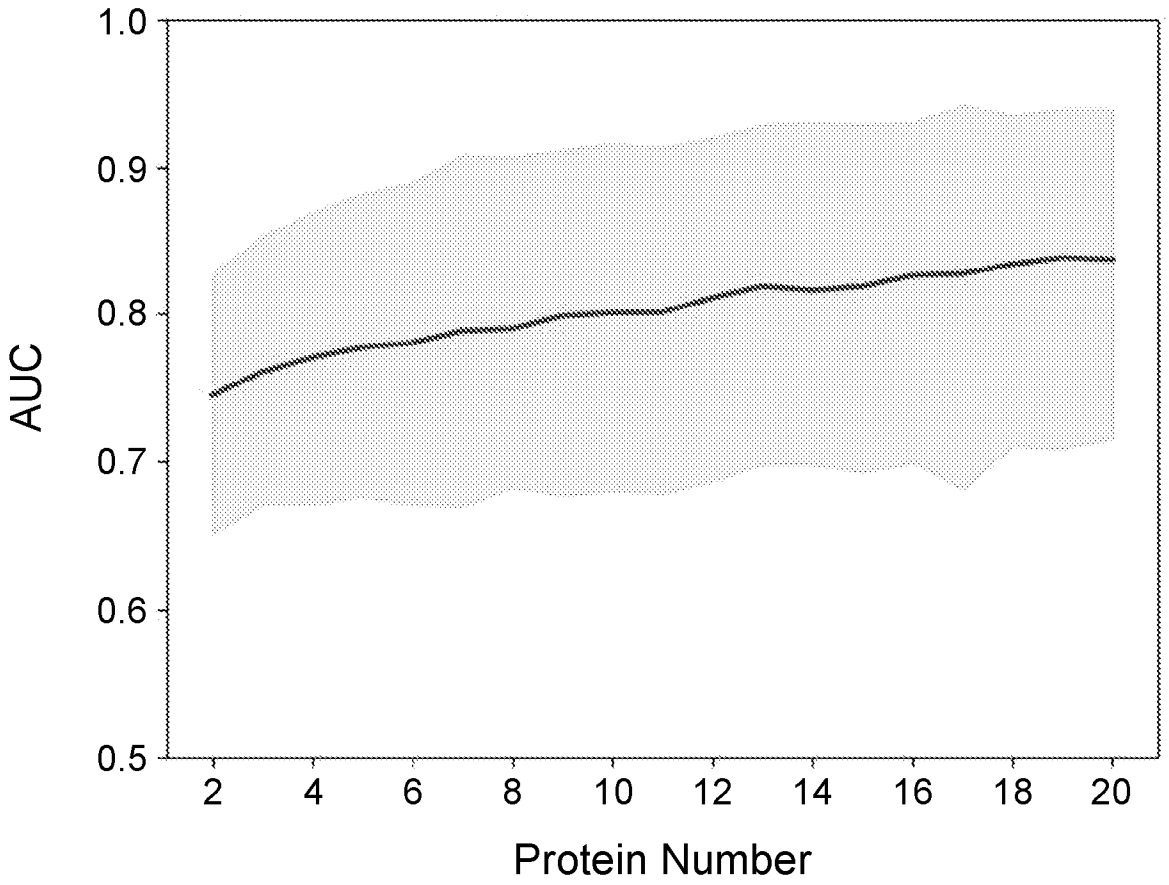
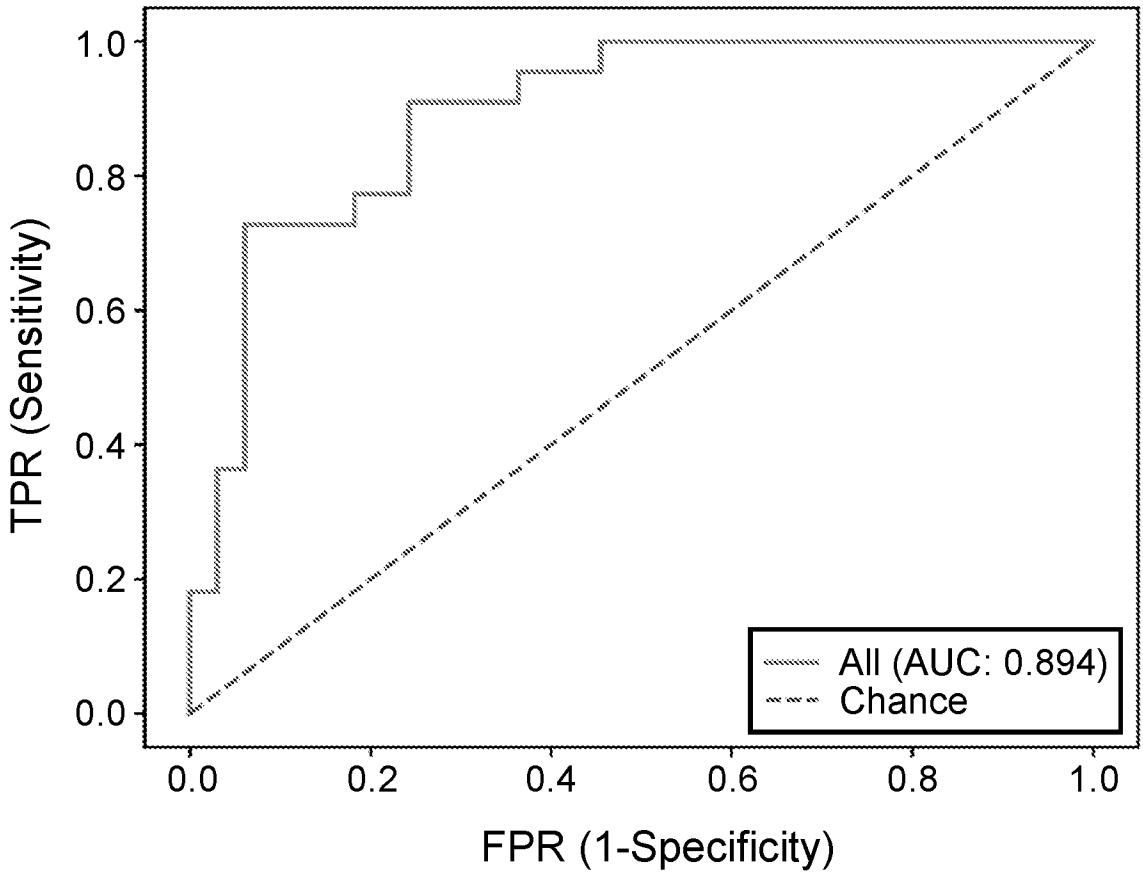


FIG. 1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0.6
0.4
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TPR (Sensitivity)
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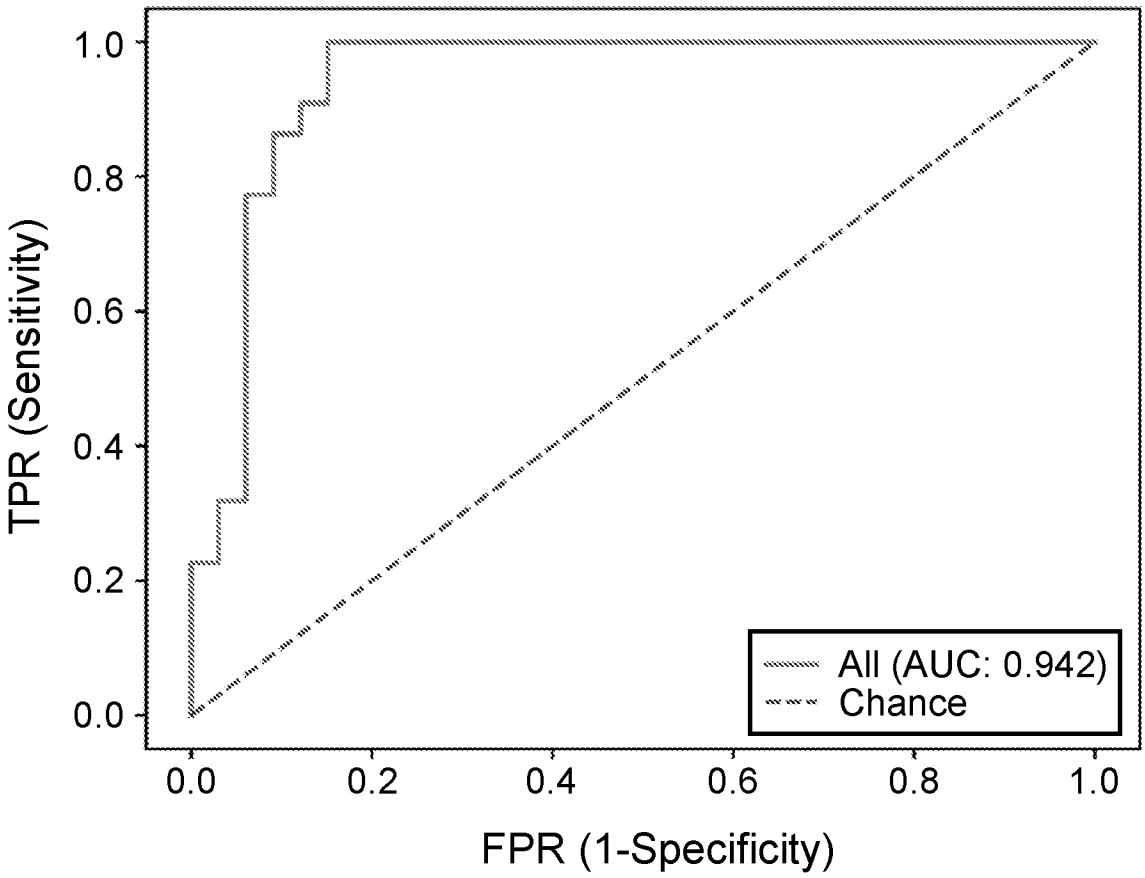
0.4
0.6
0.8
1.0
FPR (1-Specificity)
All (AUC: 0.894)
Chance

FIG. 2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0.8
0.6
0.4
0.2
0.0
TPR (Sensitivity)
0.0
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0.4
0.6
0.8
1.0
FPR (1-Specificity)
All (AUC: 0.942)
Chance

FIG. 3

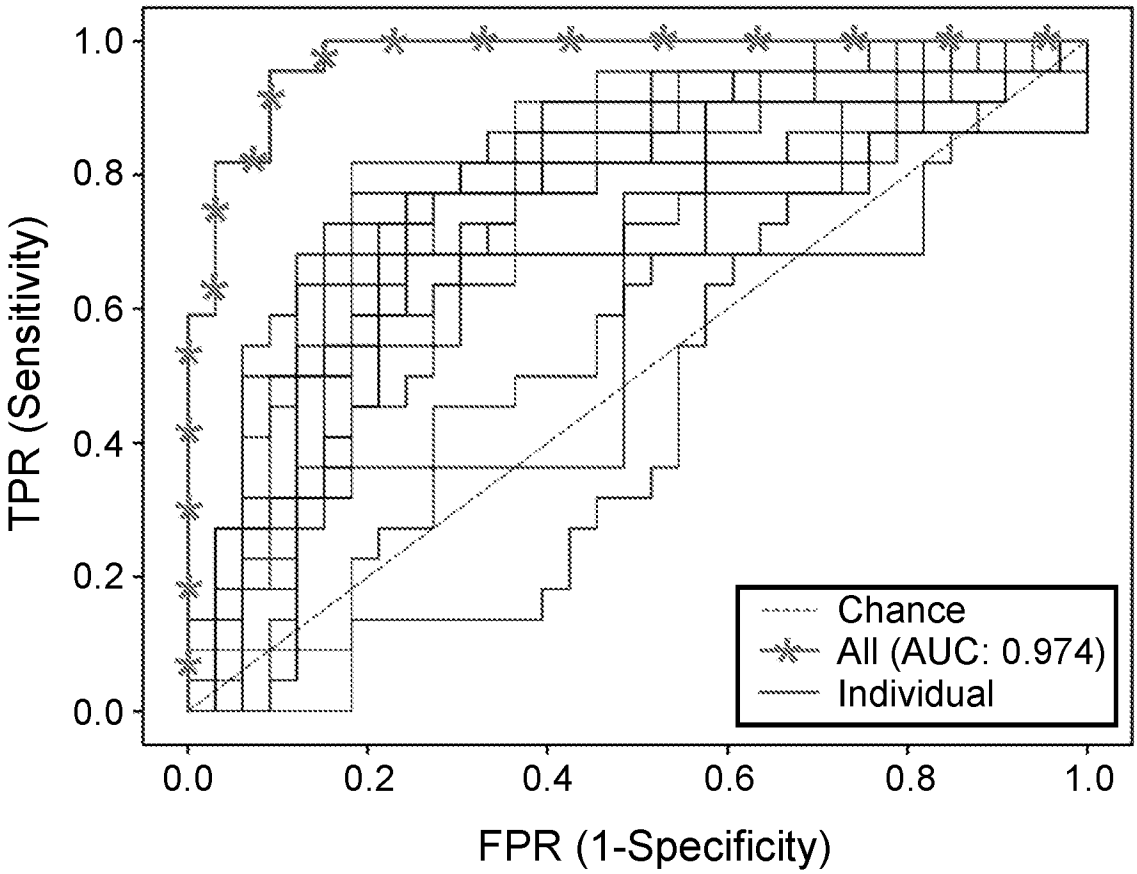
1.0
0.8
0.6
0.4
0.2
0.0
TPR (Sensitivity)
0.0
0.2
0.4
0.6
0.8
1.0
FPR (1-Specificity)
Chance
All (AUC: 0.974)
Individual

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/019543

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61P 35/00*** (2024.01); ***C12Q 1/6886*** (2024.01); ***G01N 33/574*** (2024.01)

CPC: ***G01N 33/57449; A61P 35/00; C12Q 1/6886;*** *C12Q 2600/106; C12Q 2600/112; C12Q 2600/118; C12Q 2600/158; G01N 2800/52; G01N 2800/7028*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/0220580 A1 (BROWN et al.) 07 August 2014 (07.08.2014) entire document	1-3
A	US 2020/0377956 A1 (THE JOHNS HOPKINS UNIVERSITY et al.) 03 December 2020 (03.12.2020) entire document	1-3
A	US 2018/0340945 A1 (NANOSOMIX INC.) 29 November 2018 (29.11.2018) entire document	1-3
A	US 2014/0228233 A1 (PAWLOWSKI et al.) 14 August 2014 (14.08.2014) entire document	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 May 2024 (23.05.2024)	**05 July 2024 (05.07.2024)**

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US **Commissioner for Patents** **P.O. Box 1450, Alexandria, VA 22313-1450**	**MATOS** **TAINA**
Facsimile No. **571-273-8300**	Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/019543

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☑ Claims Nos.: **6, 8, 16-19, 29-42, 44-63**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-3 are drawn to methods of evaluating a subject for ovarian cancer.

Group II+: claims 4, 5, 7, 11-15, 22, 24, 25, 27, and 28 are drawn to methods of treating ovarian cancer in a subject.

Group III+: claims 9 and 10 are drawn to methods of detecting ovarian cancer in a subject.

Group IV+: claims 20, 21, 23, and 26 are drawn to methods of evaluating a treatment for ovarian cancer in a subject, methods of evaluating the efficacy of a treatment for ovarian cancer in a subject, methods of adjusting a treatment for ovarian cancer in a subject, and methods of monitoring for ovarian cancer recurrence in a subject.

Group V+: claim 43 is drawn to a method of measuring amounts of proteins in a subject.

The first invention of Group I+ is restricted to a protein selected to be NRGN, and methods of evaluating a subject for ovarian cancer comprising the same. The first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Specifically, the first named invention was selected based on the first listed compound species presented in the claims (see claim 1). It is believed that claims 1-3 read on this first named invention and thus these claims will be searched without fee to the extent that they read on a protein selected to be NRGN, and methods comprising the same.

The first invention of Group II+ is restricted to a protein selected to be NRGN, and methods of treating ovarian cancer in a subject comprising the same.

The first invention of Group III+ is restricted to a protein selected to be NRGN, and methods of detecting ovarian cancer in a subject comprising the same.

The first invention of Group IV+ is restricted to a protein selected to be NRGN, and methods of evaluating a treatment for ovarian cancer in a subject comprising the same, methods of evaluating the efficacy of a treatment for ovarian cancer in a subject comprising the same, methods of adjusting a treatment for ovarian cancer in a

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

subject comprising the same, and methods of monitoring for ovarian cancer recurrence in a subject comprising the same.

The first invention of Group V+ is restricted to a protein selected to be NRGN, and a method of measuring amounts of proteins in a subject comprising the same.

Applicant is invited to elect additional proteins to be searched by paying an additional fee for each election. An exemplary election would be a protein selected to be FGFR4, and methods of evaluating a subject for ovarian cancer comprising the same. Additional proteins will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+-V+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+, II+, III+, IV+, and V+ formulas do not share a significant structural element responsible for methods requiring the selection of alternative proteins where "one or more proteins selected from Table 1."

The special technical features of Groups I+, methods of evaluating a subject for ovarian cancer, are not present in Groups II+-V+; the special technical features of Groups II+, methods of treating ovarian cancer in a subject, are not present in Groups I+, or III+-V+; the special technical features of Group III+, methods of detecting ovarian cancer in a subject, are not present in Groups I+, II+, IV+ or V+; the special technical features of Groups IV+, methods of evaluating a treatment for ovarian cancer in a subject, methods of evaluating the efficacy of a treatment for ovarian cancer in a subject, methods of adjusting a treatment for ovarian cancer in a subject, and methods of monitoring for ovarian cancer recurrence in a subject, are not present in Groups I+-III+, or V+; and the special technical features of Groups V+, a method of measuring amounts of proteins in a subject, are not present in Groups I+-IV+.

Additionally, even if Groups I+-V+ were considered to share the technical features of a method of evaluating a subject for ovarian cancer, the method comprising: determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1; thereby evaluating the subject for cancer; A method of treating ovarian cancer in a subject, comprising (a) acquiring results from the method; and (b) administering a treatment to the subject; A method of treating ovarian cancer in a subject, the method comprising: (a) acquiring results from an evaluation of the subject that determined the subject has ovarian cancer; (b) administering a treatment to the subject, wherein the evaluation comprises: (i) determining in a biological sample from the subject a concentration of one or more proteins; and (ii) applying a classifier to the concentration of the one or more proteins to identify whether the subject has ovarian cancer; A method of detecting ovarian cancer in a subject, the method comprising: determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1; and applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected; A method of treating ovarian cancer in a subject, comprising (a) acquiring results from the method; and (b) administering a treatment to the subject; A method of treating ovarian cancer in a subject, the method comprising determining in a biological sample from the subject a concentration of one or more proteins; applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected; and administering a treatment to the subject when ovarian cancer is detected; A method of treating ovarian cancer in a subject in whom ovarian cancer was detected, the method comprising administering a treatment for ovarian cancer to the subject, wherein ovarian cancer was detected in the subject by a method comprising: determining in a biological sample from the subject a concentration of one or more proteins; and applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected; A method of evaluating a treatment for ovarian cancer in a subject, the method comprising: (a) administering a treatment for ovarian cancer, and (b) determining in a biological sample from the subject a concentration of one or more proteins; thereby evaluating the treatment; A method of evaluating the efficacy of a treatment for ovarian cancer in a subject, the method comprising (a) administering a treatment for ovarian cancer to the subject, and (b) determining in a biological sample from the subject a

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

concentration of one or more proteins; thereby evaluating the efficacy of the treatment; A method of treating ovarian cancer in a subject, the method comprising (a) administering a treatment for ovarian cancer to the subject, and (b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate the efficacy of the treatment; A method of adjusting a treatment for ovarian cancer in a subject, the method comprising (a) administering a treatment for ovarian cancer to the subject, (b) determining in a biological sample from the subject a concentration of one or more proteins, and (c) administering an adjusted treatment to the subject when it is determined that the adjusted treatment is necessary; A method of treating ovarian cancer in a subject, the method comprising (a) administering a treatment for ovarian cancer to the subject, and (b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether the treatment requires adjustment; A method of monitoring for ovarian cancer recurrence in a subject, the method comprising (a) administering a treatment for ovarian cancer to the subject, and (b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether the ovarian cancer is recurring; A method of treating ovarian cancer in a subject, the method comprising (a) administering a treatment for ovarian cancer to the subject, and (b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether cancer is recurring; and A method of measuring amounts of proteins in a subject, the method comprising determining individual amounts of one or more proteins, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2020/0377956 A1 to The Johns Hopkins University et al. teaches a method of evaluating a subject for ovarian cancer, the method comprising: determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1; thereby evaluating the subject for cancer (Methods and materials for assessing and treating cancer, Title; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]; Examples of genetic biomarkers that can be detected using any of the variety of techniques described herein include, without limitation,… CDH1, Para. [0705]; Instant Table 1 discloses CDH1); A method of treating ovarian cancer in a subject, comprising (a) acquiring results from the method; and (b) administering a treatment to the subject (methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]; the subject is also administered a treatment, Para. [0135]); A method of treating ovarian cancer in a subject, the method comprising: (a) acquiring results from an evaluation of the subject that determined the subject has ovarian cancer; (b) administering a treatment to the subject, wherein the evaluation comprises: (i) determining in a biological sample from the subject a concentration of one or more proteins; and (ii) applying a classifier to the concentration of the one or more proteins to identify whether the subject has ovarian cancer (Methods and materials for assessing and treating cancer, Title; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]; the subject is also administered a treatment, Para. [0135]; Applying a mathematical model to the data can generate one or more classifiers, Para. [0393]); A method of detecting ovarian cancer in a subject, the method comprising: determining in a biological sample from the subject a concentration of one or more proteins selected from Table 1; and applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected (Methods and materials for assessing and treating cancer, Title; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]; Examples of genetic biomarkers that can be detected using any of the variety of techniques described herein include, without limitation,… CDH1, Para. [0705]; Instant Table 1 discloses CDH1); A method of treating ovarian cancer in a subject, comprising (a) acquiring results from the method; and (b) administering a treatment to the subject (Methods and materials for assessing and treating

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

cancer, Title; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]; the subject is also administered a treatment, Para. [0135]; Applying a mathematical model to the data can generate one or more classifiers, Para. [0393]); A method of treating ovarian cancer in a subject, the method comprising determining in a biological sample from the subject a concentration of one or more proteins; applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected; and administering a treatment to the subject when ovarian cancer is detected (Methods and materials for assessing and treating cancer, Title; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]; the subject is also administered a treatment, Para. [0135]; Applying a mathematical model to the data can generate one or more classifiers, Para. [0393]); A method of treating ovarian cancer in a subject in whom ovarian cancer was detected, the method comprising administering a treatment for ovarian cancer to the subject, wherein ovarian cancer was detected in the subject by a method comprising: determining in a biological sample from the subject a concentration of one or more proteins; and applying a classifier to the concentration of the one or more proteins that identifies whether the concentration of the one or more proteins is indicative that ovarian cancer is detected (Methods and materials for assessing and treating cancer, Title; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]; the subject is also administered a treatment, Para. [0135]; Applying a mathematical model to the data can generate one or more classifiers, Para. [0393]); A method of evaluating a treatment for ovarian cancer in a subject, the method comprising: (a) administering a treatment for ovarian cancer, and (b) determining in a biological sample from the subject a concentration of one or more proteins; thereby evaluating the treatment (methods provided herein can be used to determine the efficacy of the treatment, Para. [0212]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]); A method of evaluating the efficacy of a treatment for ovarian cancer in a subject, the method comprising (a) administering a treatment for ovarian cancer to the subject, and (b) determining in a biological sample from the subject a concentration of one or more proteins; thereby evaluating the efficacy of the treatment (methods provided herein can be used to determine the efficacy of the treatment, Para. [0212]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g., … ovarian cancer…), Para. [0121]); A method of treating ovarian cancer in a subject, the method comprising (a) administering a treatment for ovarian cancer to the subject, and (b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate the efficacy of the treatment (Methods and materials for assessing and treating cancer, Title; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]; the subject is also administered a treatment, Para. [0135]); A method of adjusting a treatment for ovarian cancer in a subject, the method comprising (a) administering a treatment for ovarian cancer to the subject, (b) determining in a biological sample from the subject a concentration of one or more proteins, and (c) administering an adjusted treatment to the subject when it is determined that the adjusted treatment is necessary (Methods and materials for assessing and treating cancer, Title; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]; the subject is also administered a treatment, Para. [0135]; After receiving this higher amount, the subject can be monitored for both responsiveness to the treatment and toxicity symptoms, and adjustments made accordingly, Para. [0867]); A method of treating ovarian cancer in a subject, the method comprising (a) administering a treatment for ovarian cancer to the subject, and (b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether the treatment requires adjustment (Methods and materials for assessing and treating cancer, Title; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]; the subject is also administered a treatment, Para. [0135]; After receiving this higher amount, the subject can be monitored for both responsiveness to the treatment and toxicity symptoms, and adjustments made accordingly, Para. [0867]); A method of monitoring for ovarian cancer recurrence in a subject, the method comprising (a) administering a treatment for ovarian cancer to the subject, and (b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether the ovarian cancer is recurring (Assays as described herein may improve management and prognosis though the earlier detection of recurrence, Para. [0217]; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]); A method of treating ovarian cancer in a subject, the method comprising (a) administering a treatment for ovarian cancer to the subject, and (b) determining in a biological sample from the subject a concentration of one or more proteins to evaluate whether cancer is recurring (Methods and materials for assessing and treating cancer, Title; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g.,… ovarian cancer…), Para. [0121]; Assays as described herein may improve management and prognosis though the earlier detection of recurrence, Para. [0217]; methods and materials provided herein provide high sensitivity in the detection or diagnosis of cancer… cancers that can be detected using methods and materials provided herein include cancers of the female reproductive tract (e.g., ovarian cancer…), Para. [0121]; The present disclosure provides methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]); and A method of measuring amounts of proteins in a subject, the method comprising determining individual amounts of one or more proteins (methods of identifying the presence of cancer in a subject based on one or more protein biomarkers (e.g., protein concentrations in whole blood or plasma), Para. [0367]).

The inventions listed in Groups I+-V+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/019543

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-3**

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.