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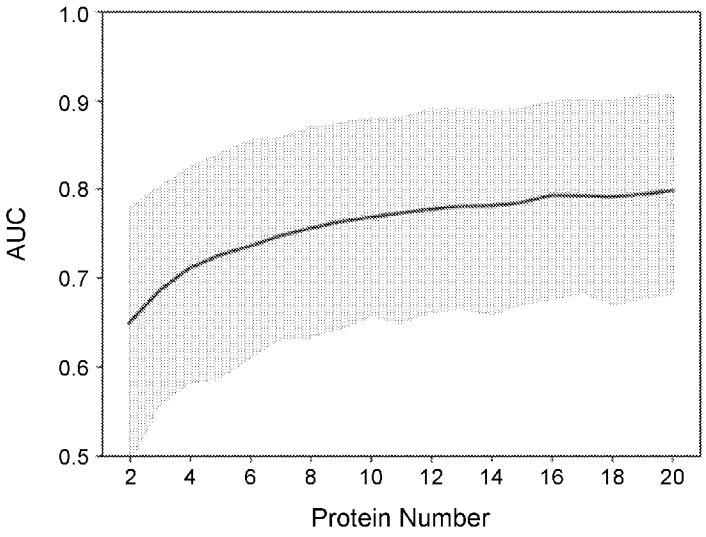

FIG. 1

(57) Abstract: Methods of evaluating a subject for endometrial cancer or detecting endometrial 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 endometrial cancer. In addition, methods of treatment comprising administering a treatment to the subject when the subject is evaluated or detected to have endometrial cancer.

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TITLE

ENDOMETRIAL CANCER DETECTION PROTEINS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 63/451,628, filed on March 12, 2023, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Endometrial cancer is a major health concern among women. In 2018, the total number of new cases reported worldwide was 382,069, and the total number of deaths was 89,929 (Bray *et al.*, 2018). Endometrial cancer is the most common cancer of the female reproductive organs; for 2023, the estimated number of new cases in the U.S. is 66,200, which is over 57% of all predicted new cancer cases of the female genital system (Siegel *et al.*, 2022).

[0003] Early diagnosis for endometrial cancer has a significant impact on survival rate. According to the American Cancer Society, five-year survival of patients diagnosed with endometrial cancer at the localized stage (*i.e.*, no sign that the cancer has spread outside the uterus, approximately Stage I) is 96% (American Cancer Society, 2023) But the five-year survival rate drops to 72% for patients with regional stage endometrial cancer (*i.e.*, cancer has spread from the uterus to nearby structures or lymph nodes, approximately Stage II/III), and to 20% for patients with distant stage endometrial cancer (*i.e.*, cancer has spread to distant parts of the body such as the lungs, liver, or bones, approximately Stage IV) (*id.*).

[0004] Diagnosis of endometrial cancer currently relies on the presentation of symptoms. Abnormal uterine bleeding (AUB) is the most common symptom, occurring in 90% of endometrial cancer patients (Coll-de la Rubia *et al.*, 2020). However, AUB is common to other diseases as well, and only 10-15% of women with AUB will develop endometrial cancer (Dueholm *et al.*, 2019; Clarke *et al.*, 2018). To distinguish endometrial cancer from other diseases, patients have to undergo a multi-step diagnosis process that includes a gynecological examination, transvaginal ultrasonography, and a pathological

examination of an endometrial biopsy (Coll-de la Rubia *et al.*, 2020), all of which can be uncomfortable/painful, time-consuming, and expensive.

[0005] Therefore, there is an urgent unmet clinical need to improve the detection and diagnosis of endometrial 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 endometrial 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 endometrial 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 endometrial cancer in a subject, comprising acquiring results from a method of evaluating a subject for endometrial cancer as described herein, and administering a treatment to the subject.

[0009] Another aspect of the invention relates to a method of detecting endometrial 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 endometrial cancer is detected.

[0010] Yet another aspect of the invention relates to a method of treating endometrial cancer in a subject, comprising acquiring results from a method of detecting endometrial 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 endometrial cancer in a subject in whom endometrial cancer was detected, the method comprising administering a treatment for endometrial cancer to the subject, in which endometrial 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 endometrial cancer is detected.

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

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

[0014] Another aspect of the invention relates to a method of evaluating a treatment for endometrial cancer in a subject, the method comprising administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising administering a treatment for endometrial 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 endometrial cancer recurrence in a subject, the method comprising administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising administering a treatment for endometrial 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 endometrial 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 endometrial cancer in a subject using the panel of 21 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 endometrial cancer in a subject using the panel of 40 proteins listed in **Table 3**, as described in the Example.

[0033] **FIG. 4** shows ROC curves generated by application of a classifier, which depicts the high diagnostic utility of detecting endometrial cancer in a subject using each of the seven proteins listed in **Table 4**, both individually (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 endometrial cancer in particular include, but are not limited to, usual vaginal bleeding, spotting, or other discharge; pelvic pain; a mass; and 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 endometrial cancer in subjects (also referred to herein as "endometrial cancer detection proteins"). Such use can be applied in methods of evaluating a subject for endometrial cancer, methods of treating subjects for endometrial cancer, among others.

[0054] The proteins can be used to detect or evaluate endometrial 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 endometrial 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 endometrium; or a method of evaluating a subject for endometrial 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 endometrial cancer. The method may further comprise applying a classifier to the concentration of the one or more endometrial cancer detection proteins. The classifier identifies whether the concentration of the one or more endometrial cancer detection proteins is indicative that the subject has endometrial 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 endometrial cancer.

[0060] To this end, an aspect of the present invention relates to a method of treating endometrial cancer in a subject, comprising (a) acquiring results from methods of evaluating

a subject for endometrial cancer as described herein, and (b) administering a treatment to the subject. In some embodiments, the results from methods of evaluating a subject for endometrial cancer are provided by a third party. In some embodiments, the treatment is responsive to the results, *e.g.*, responsive to having endometrial cancer.

[0061] Another aspect of the present invention relates to a method of treating endometrial cancer in a subject, in which the method comprises (a) acquiring results from an evaluation of the subject that determined the subject has endometrial cancer; (b) administering a treatment to the subject, *e.g.*, a treatment for endometrial 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 endometrial 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 endometrial 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 endometrial cancer is detected.

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

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

[0065] An aspect of the invention relates to a method of treating endometrial cancer in a subject in whom endometrial cancer was detected, the method comprising administering a treatment for the endometrial cancer; in which the endometrial 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 endometrial cancer. In some embodiments, the method of detecting the endometrial cancer was performed by a third party.

[0066] Yet another aspect of the present invention relates to a method of treating endometrial 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 endometrial cancer; and (c) administering a treatment to the subject, *e.g.*, a treatment for endometrial 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 endometrial cancer, comprising administering a treatment for endometrial cancer to the patient, in which the patient was determined to have endometrial 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 endometrial cancer.

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

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

[0070] In addition, an aspect of the present invention relates to a method of evaluating a treatment for endometrial cancer in a subject. The method comprises (a) administering a treatment for endometrial 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 endometrial 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 endometrial 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 endometrial 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 endometrial cancer treatment, comprising (a) administering a treatment for endometrial 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 endometrial 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 endometrial 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 endometrial cancer, comprising (a) administering a treatment for endometrial 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 endometrial cancer after treatment may be indicative of cancer recurrence. Thus, another aspect of the invention is a method of monitoring for endometrial cancer recurrence, comprising (a) administering a treatment for endometrial 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 endometrial 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 endometrial 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 endometrial 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, or 63; and including any ranges thereof, for example, about two to 63 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 63 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 63 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 63 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 63 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 63 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 63 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 63 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 63 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 63 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 63 proteins, or about 45 to 60 proteins, or about 45 to 55 proteins, or about 45 to 50 proteins, or about 50 to 63 proteins, or about 50 to 60 proteins, or about 50 to 55 proteins, or about 55 to 63 proteins, or about 55 to 60 proteins, or about 60 to 63 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 endometrial cancer is early-stage. In some embodiments, the endometrial cancer is stage I. In some embodiments, the endometrial cancer is stage II.

[0089] In some embodiments, the endometrial cancer is stage III. In some embodiments, the endometrial cancer is stage IV. In some embodiments, the endometrial 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, immunotherapy; targeted therapy; and any combination thereof. Examples of surgery may include, but are not limited, to 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); bilateral salpingo-oophorectomy (removal of the ovaries and fallopian tubes); 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, carboplatin, doxorubicin, cisplatin, docetaxel, and any combination thereof. Examples of hormone therapy include, but are not limited to, progestins such as medroxyprogesterone acetate and/or megestrol acetate; tamoxifen; luteinizing hormone-releasing hormone agonists such as goserelin and/or leuprolide; aromatase inhibitors such as letrozole, anastrozole, and/or exemestane; and any combination thereof. Examples of targeted therapy include, but are not limited to, lenvatinib; bevacizumab; mTOR inhibitors such as everolimus and/or temsirolimus; and any combination thereof. Examples of immunotherapy include, but are not limited to, immune checkpoint inhibitors such as pembrolizumab, dostarlimab, and/or dostarlimab.

[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 endometrial 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 endometrial 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 endometrial cancer who has not been subjected to a method of the invention. For example, treatment of endometrial 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 endometrial 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 endometrial 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, or 63; and including any ranges thereof, for example, about two to 63 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 63 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 63 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 63 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 63 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 63 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 63 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 63 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 63 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 63 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 63 proteins, or about 45 to 60 proteins, or about 45 to 55 proteins, or about 45 to 50 proteins, or about 50 to 63 proteins, or about 50 to 60 proteins, or about 50 to 55 proteins, or about 55 to 63 proteins, or about 55 to 60 proteins, or about 60 to 63 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 endometrial cancer detection proteins of the present invention.

Sample Collection

Urine samples were collected from a patient population diagnosed with endometrial cancer, and from healthy individuals without endometrial 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. Across the assays, the mean intra-assay and inter-assay variations observed were 8% and 11%, respectively.

[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.

Endometrial 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 endometrial 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 endometrial cancer detection proteins are shown in **Table 1**. **FIG. 1** shows that the accuracy is over 0.6 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 endometrial 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 0.891 (*see* **FIG. 2**), the ROC curve generated from the panel of all of the proteins listed in **Table 3** has an AUC of about 0.921 (*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 0.942 (*see* **FIG. 4**).

Table 1. Endometrial cancer detection proteins.

Gene Name	Uniprot ID	AUC	Gene Name	Uniprot ID	AUC
GUCA2A	Q02747	0.652	GM2A	P17900	0.607
SPINK1	P00995	0.719	AMBP	P02760	0.665
IGFBP2	P18065	0.671	MSMB	P08118	0.624
LONP1	P36776	0.664	PTX3	P26022	0.668
FSTL1	Q12841	0.719	SEMA3F	Q13275	0.610
TFF1	P04155	0.654	PPM1A	P35813	0.680
SCGB1A1	P11684	0.581	HJV	Q6ZVN8	0.450
TOM1L2	Q6ZVM7	0.616	BLVRB	P30043	0.679
PRSS2	P07478	0.629	KLRD1	Q13241	0.663
CGB3; CGB5; CGB8	P0DN86	0.693	NEO1	Q92859	0.605
C9orf40	Q8IXQ3	0.687	LMOD2	Q6P5Q4	0.623
TRIM21	P19474	0.708	FLT3	P36888	0.682
KLK3	P07288	0.574	SLC27A4	Q6P1M0	0.645
NADK	O95544	0.665	CALB2	P22676	0.674
AHSG	P02765	0.614	IGFBP3	P17936	0.606
TSLP	Q969D9	0.653	IGF2R	P11717	0.599
ABO	P16442	0.663	LEFTY2	O00292	0.653
CCN5	O76076	0.676	CES2	O00748	0.657
HMGCL	P35914	0.672	GYS1	P13807	0.664
TNFSF8	P32971	0.680	STEAP4	Q687X5	0.618
APPL2	Q8NEU8	0.687	CDH17	Q12864	0.667
SCRN1	Q12765	0.734	GSTP1	P09211	0.654
VEGFB	P49765	0.525	CNTF	P26441	0.596
RNASE3	P12724	0.584	CRYM	Q14894	0.678
TYMP	P19971	0.711	LEG1	Q6P5S2	0.656
CD14	P08571	0.565	VNN1	O95497	0.685
ENO1	P06733	0.709	CSNK2A1	P68400	0.643
ECHS1	P30084	0.646	APOF	Q13790	0.627
ANXA10	Q9UJ72	0.685	TBCC	Q15814	0.674
NMI	Q13287	0.667	ZP4	Q12836	0.702
SLAMF6	Q96DU3	0.657	CD276	Q5ZPR3	0.664
NCLN	Q969V3	0.646			

Table 2. Subset of endometrial cancer detection proteins from Table 1, which together can achieve an AUC of 0.891.

Gene Name	Uniprot ID	Gene Name	Uniprot ID
SCRN1	Q12765	ANXA10	Q9UJ72
SPINK1	P00995	FLT3	P36888
FSTL1	Q12841	TNFSF8	P32971
TYMP	P19971	PPM1A	P35813

ENO1	P06733	BLVRB	P30043
TRIM21	P19474	CRYM	Q14894
ZP4	Q12836	CCN5	O76076
CGB3; CGB5; CGB8	P0DN86	TBCC	Q15814
C9orf40	Q8IXQ3	CALB2	P22676
APPL2	Q8NEU8	HMGCL	P35914
VNN1	O95497		

Table 3. Subset of endometrial cancer detection proteins from Table 1, which together can achieve an AUC of 0.921.

Gene Name	Uniprot ID	Gene Name	Uniprot ID
SPINK1	P00995	FLT3	P36888
IGFBP2	P18065	SLC27A4	Q6P1M0
FSTL1	Q12841	CALB2	P22676
C9orf40	Q8IXQ3	IGFBP3	P17936
TRIM21	P19474	IGF2R	P11717
CCN5	O76076	LEFTY2	O00292
APPL2	Q8NEU8	CES2	O00748
SCRN1	Q12765	GYS1	P13807
VEGFB	P49765	STEAP4	Q687X5
TYMP	P19971	CDH17	Q12864
ENO1	P06733	GSTP1	P09211
NMI	Q13287	CNTF	P26441
PTX3	P26022	CRYM	Q14894
SEMA3F	Q13275	LEG1	Q6P5S2
PPM1A	P35813	VNN1	O95497
HJV	Q6ZVN8	CSNK2A1	P68400
BLVRB	P30043	APOF	Q13790
KLRD1	Q13241	TBCC	Q15814
NEO1	Q92859	ZP4	Q12836
LMOD2	Q6P5Q4	CD276	Q5ZPR3

Table 4. Subset of endometrial cancer detection proteins from Table 1, which together can achieve an AUC of 0.942.

Gene Name	Uniprot ID	Gene Name	Uniprot ID
C9orf40	Q8IXQ3	FLT3	P36888
ENO1	P06733	SPINK1	P00995
APPL2	Q8NEU8	APOF	Q13790
GYS1	P13807		

EMBODIMENTS

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

Embodiment 1. A method of evaluating a subject for endometrial 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 endometrial cancer.

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

Embodiment 4. A method of treating endometrial 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 endometrial cancer.

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

(a) acquiring results from an evaluation of the subject that determined the subject has endometrial 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 endometrial 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 endometrial 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 endometrial cancer is detected.

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

Embodiment 11. A method of treating endometrial 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 endometrial 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 endometrial cancer is detected; and

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

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

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

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

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

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

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

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

administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising

(a) administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising

(a) administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising

(a) administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising

(a) administering a treatment for endometrial 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 endometrial cancer recurrence in a subject, comprising

(a) administering a treatment for endometrial 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 endometrial cancer is recurring, wherein the one or more proteins are selected from **Table 1**.

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

(a) administering a treatment for endometrial 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 endometrial 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 1-45, 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, 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, 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 1-45, 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

American Cancer Society (https://www.cancer.org/cancer/endometrial-cancer/detection-diagnosis-staging/survival-rates.html), accessed on March 9, 2023.

Bray F., *et al.*, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA Cancer J. Clin.*, 2018, 68: 394-424.

Clarke MA., et al., Association of endometrial cancer risk with postmenopausal bleeding in women: a systematic review and meta-analysis, *JAMA Intern. Med.*, 2018, 178: 1210-1222.

Coll-de la Rubia E., *et al.*, Prognostic biomarkers in endometrial cancer: a systematic review and meta-analysis, *J. Clin. Med.*, 2020, 9:1900.

Dueholm M., *et al.*, Ultrasound scoring of endometrial pattern for fast-track identification or exclusion of endometrial cancer in women with postmenopausal bleeding, *J. Minim. Invasive Gynecol.*, 2019, 26: 516-525.

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

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

CLAIMS

1. A method of evaluating a subject for endometrial 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 endometrial cancer.

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

4. A method of treating endometrial 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 endometrial cancer.

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

(a) acquiring results from an evaluation of the subject that determined the subject has endometrial 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 endometrial 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 endometrial 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 endometrial cancer is detected.

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

11. A method of treating endometrial 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 endometrial 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 endometrial cancer is detected; and

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

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

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

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

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

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

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

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

(a) administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising

(a) administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising

(a) administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising

(a) administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising

(a) administering a treatment for endometrial 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 endometrial cancer recurrence in a subject, the method comprising

(a) administering a treatment for endometrial 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 endometrial cancer is recurring, wherein the one or more proteins are selected from Table 1.

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

(a) administering a treatment for endometrial 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 claim 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 endometrial 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 1-45, 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, 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, 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 1-45, 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.

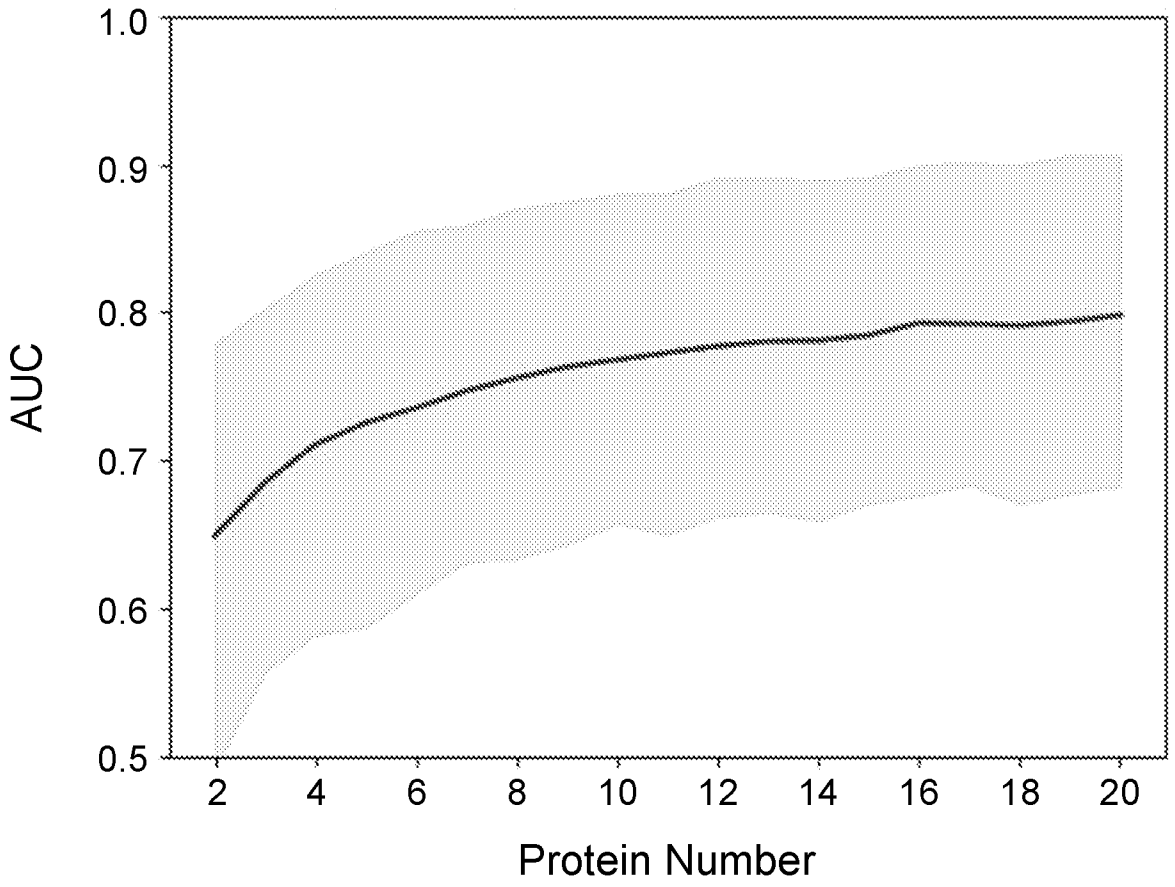
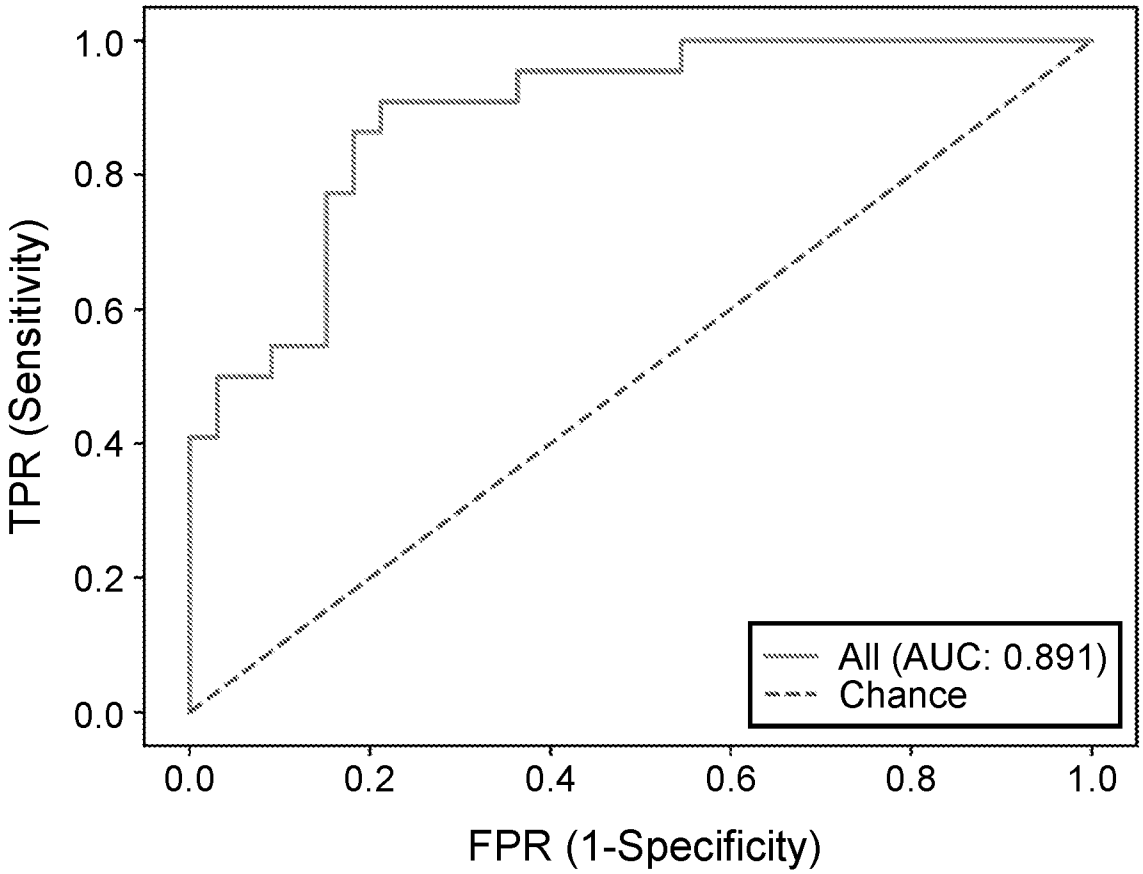


FIG. 1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0.4
0.2
0.0
TPR (Sensitivity)
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0.2
0.4
0.6
0.8
1.0
FPR (1-Specificity)
All (AUC: 0.891)
Chance

FIG. 2

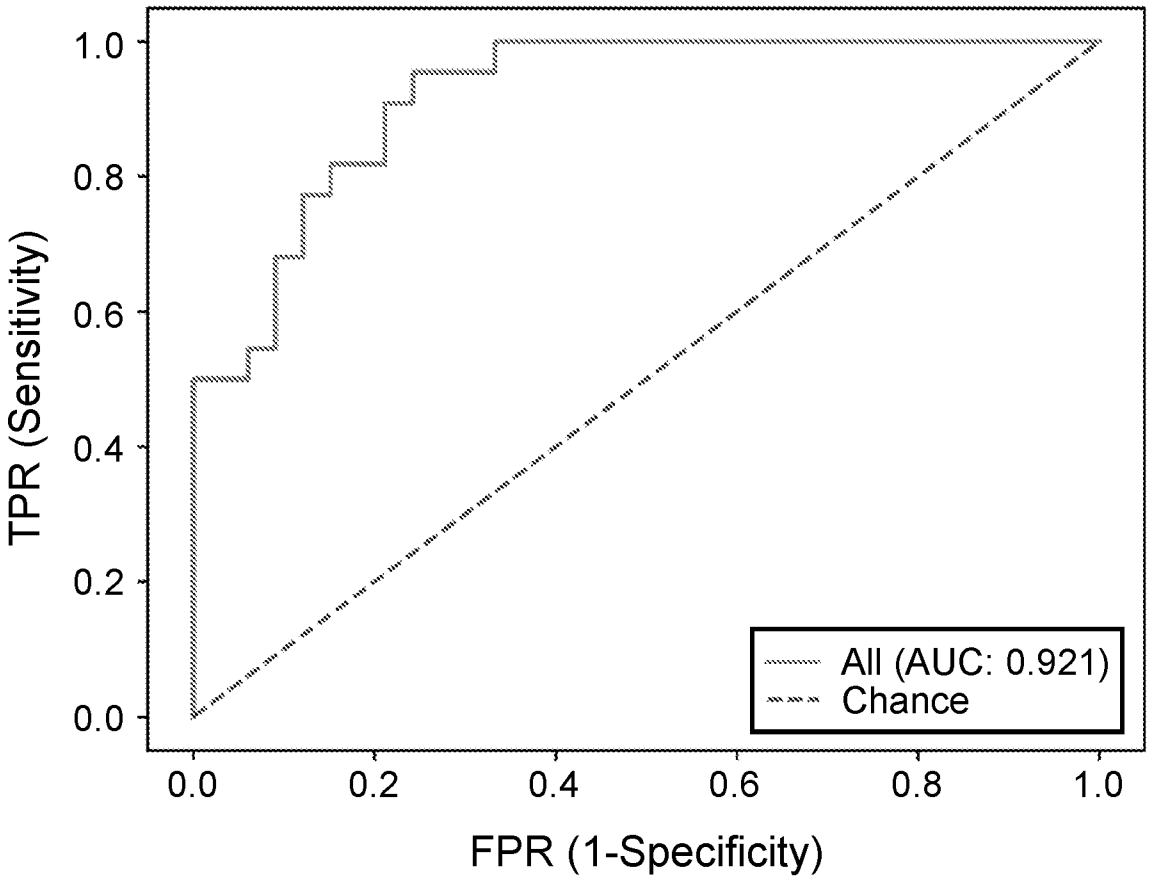
1.0
0.8
0.6
0.4
0.2
0.0
TPR (Sensitivity)
0.0
0.2
0.4
0.6
0.8
1.0
FPR (1-Specificity)
All (AUC: 0.921)
Chance

FIG. 3

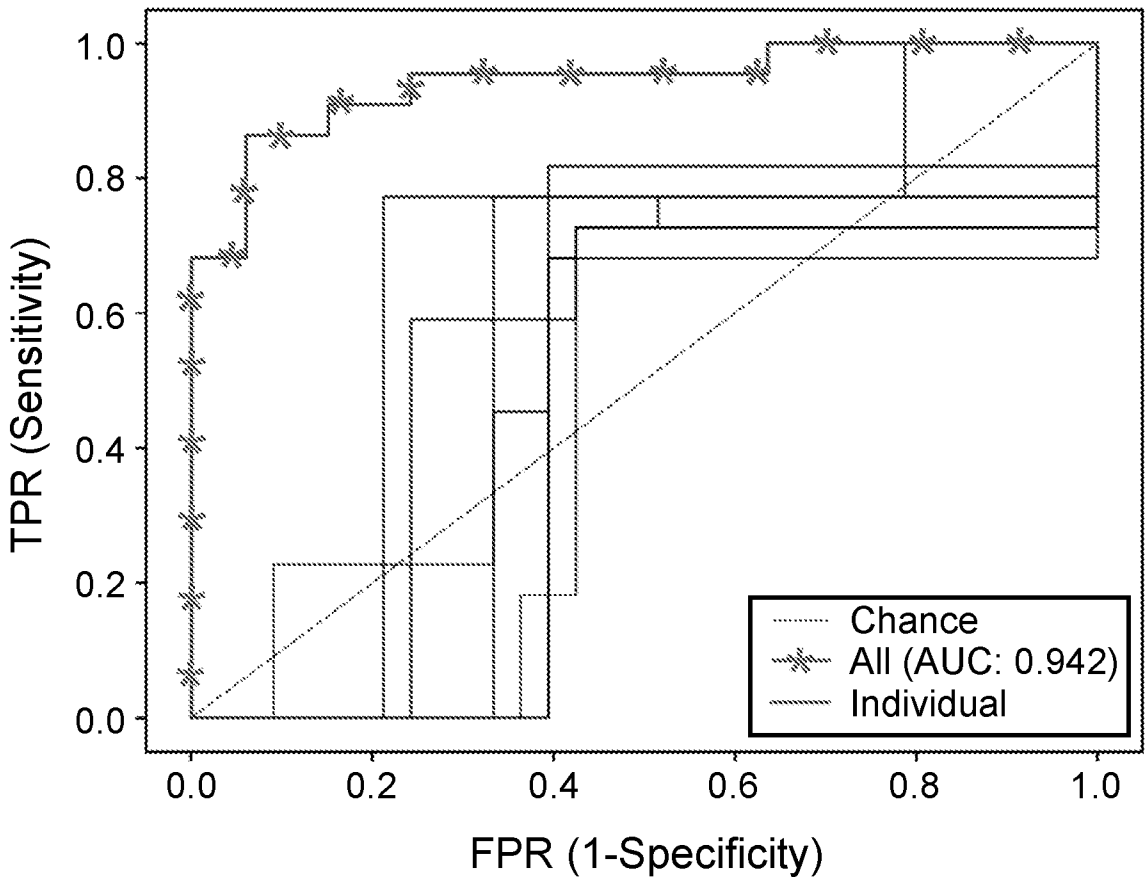
1.0
0.8
0.6
0.4
0.2
0.0
TPR (Sensitivity)
0.0
0.2
0.4
0.6
0.8
1.0
FPR (1-Specificity)
Chance
All (AUC: 0.942)
Individual

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/019557

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61P 35/00*** (2024.01); ***G01N 33/574*** (2024.01); ***G16B 25/10*** (2024.01)

CPC: ***G01N 33/57442; A61P 35/00; G16B 25/10;*** *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 2022/0244263 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 04 August 2022 (04.08.2022) entire document	1-3
A	US 2022/0180972 A1 (BOSTONGENE CORPORATION) 09 June 2022 (09.06.2022) entire document	1-3
A	US 2010/0068724 A1 (FUNG et al.) 18 March 2010 (18.03.2010) entire document	1-3
A	US 2015/0337392 A1 (GEADIC BIOTEC AIE) 26 November 2015 (26.11.2015) entire document	1-3
A	US 2022/0120750 A1 (TYMORA ANALYTICAL OPERATIONS INC.) 21 April 2022 (21.04.2022) 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
09 May 2024 (09.05.2024)	**01 July 2024 (01.07.2024)**

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/US2024/019557

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 endometrial cancer.

Group II+: claims 4, 5, 7, 11-15, 22, and 24-28 are drawn to methods of treating endometrial cancer in a subject, and methods of monitoring for endometrial cancer recurrence in a subject.

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

Group IV+: claim 20 is drawn to a method of evaluating a treatment for endometrial cancer in a subject.

Group V+: claim 21 is drawn to a method of evaluating the efficacy of a treatment for endometrial cancer in a subject.

Group VI+: claim 23 is drawn to a method of adjusting a treatment for endometrial cancer in a subject.

Group VII+: 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 GUCA2A, and methods of evaluating a subject for endometrial 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 and Table 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 GUCA2A, and methods of evaluating a subject for endometrial cancer comprising the same.

The first invention of Group II+ is restricted to a protein selected to be GUCA2A, and methods of treating endometrial cancer in a subject comprising the same, and methods of monitoring for endometrial cancer recurrence in a subject comprising the same.

The first invention of Group III+ is restricted to a protein selected to be GUCA2A, and methods of detecting endometrial cancer in a subject comprising the same.

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

The first invention of Group IV+ is restricted to a protein selected to be GUCA2A, and a method of evaluating a treatment for endometrial cancer in a subject comprising the same.

The first invention of Group V+ is restricted to a protein selected to be GUCA2A, and a method of evaluating the efficacy of a treatment for endometrial cancer in a subject comprising the same.

The first invention of Group VI+ is restricted to a protein selected to be GUCA2A, and a method of adjusting a treatment for endometrial cancer in a subject comprising the same.

The first invention of Group VII+ is restricted to a protein selected to be GUCA2A, 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 SPINK1, and methods of evaluating a subject for endometrial 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+-VII+ 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+, V+, VI+, and VII+ formulas do not share a significant structural element responsible for evaluating a subject for endometrial cancer 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 endometrial cancer, are not present in Groups II+-VII+; the special technical features of Groups II+, methods of treating endometrial cancer in a subject, and methods of monitoring for endometrial cancer recurrence in a subject, are not present in Groups I+, or III+-VII+; the special technical features of Group III+, methods of detecting endometrial cancer in a subject, are not present is Groups I+, II+, or IV+-VII+; the special technical features of Groups IV +, a method of evaluating a treatment for endometrial cancer in a subject, are not present in Groups I+-III+, or V+-VII+; the special technical features of Groups V+, a method of evaluating the efficacy of a treatment for endometrial cancer in a subject, are not present in Groups I+-IV+, VI+, or VII+; the special technical features of Group VI+, a method of adjusting a treatment for endometrial cancer in a subject, are not present in Groups I+-V+ or VII+; and the special technical features of Group VII+, a method of measuring amounts of proteins in a subject, are not present in Groups I+-VI+.

Additionally, even if Groups I+-VII+ were considered to share the technical features of a method of evaluating a subject for endometrial 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 endometrial cancer in a subject, comprising (a) acquiring results; and (b) administering a treatment to the subject; A method of treating endometrial cancer in a subject, the method comprising: (a) acquiring results from an evaluation of the subject that determined the subject has endometrial 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 endometrial cancer; A method of detecting endometrial 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 endometrial cancer is detected; A method of treating endometrial cancer in a subject, comprising (a) acquiring results; and (b) administering a treatment to the subject; A method of treating endometrial 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 endometrial cancer is detected; and

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

administering a treatment to the subject when endometrial cancer is detected; A method of treating endometrial cancer in a subject in whom endometrial cancer was detected, the method comprising administering a treatment for endometrial cancer to the subject, wherein endometrial 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 endometrial cancer is detected; A method of evaluating a treatment for endometrial cancer in a subject, the method comprising: (a) administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising (a) administering a treatment for endometrial 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; A method of treating endometrial cancer in a subject, the method comprising (a) administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising (a) administering a treatment for endometrial 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 endometrial cancer in a subject, the method comprising (a) administering a treatment for endometrial 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 endometrial cancer recurrence in a subject, the method comprising (a) administering a treatment for endometrial 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 endometrial cancer is recurring; A method of treating endometrial cancer in a subject, the method comprising (a) administering a treatment for endometrial 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; 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 2015/0337392 A1 to Geadic Biotec AIE teaches a method of evaluating a subject for endometrial cancer, the method comprising: determining in a biological sample from the subject a concentration of one or more proteins; thereby evaluating the subject for cancer (the present invention relates to an in vitro diagnostic method for the diagnosis of endometrial cancer or an increased likelihood of endometrial comprising detecting the level of (1) from 1 to 17 biomarker(s)… in a sample from a patient, Para. [0017]; the level of protein corresponding to the biomarker is determined, Para. [0068]); A method of detecting endometrial cancer in a subject, the 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 endometrial cancer is detected (the present invention relates to an in vitro diagnostic method for the diagnosis of endometrial cancer or an increased likelihood of endometrial comprising detecting the level of (1) from 1 to 17 biomarker(s)… in a sample from a patient, Para. [0017]; the level of protein corresponding to the biomarker is determined, Para. [0068]; A support vector machine based algorithm was used to identify combinations of markers of Table 1 that are useful for predicting endometrial cancer, Para. [0455]); A method of evaluating a treatment for endometrial cancer in a subject, the method comprising: (a) administering a treatment for endometrial cancer, and (b) determining in a biological sample from the subject a concentration of one or more proteins; thereby evaluating the treatment (the present invention relates to an in vitro diagnostic method for the diagnosis of endometrial cancer or an increased likelihood of endometrial comprising detecting the level of (1) from 1 to 17 biomarker(s)… in a sample from a patient, Para. [0017]; the level of protein corresponding to the biomarker is determined, Para. [0068]; The biomarkers, reagents, targets, assays, tests, inquiries and methodologies described herein can be employed in a variety of contexts, including… efficacy monitoring, Para. [0319]); A method of evaluating the efficacy of a treatment for endometrial cancer in a subject, the method comprising (a) administering a treatment for endometrial 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 (the present invention relates to an in vitro diagnostic method for the diagnosis of endometrial cancer or an increased likelihood of endometrial comprising detecting the level of (1) from 1 to 17 biomarker(s)… in a sample from a patient, Para. [0017]; the level of protein corresponding to the biomarker is determined, Para. [0068]; The biomarkers,

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

reagents, targets, assays, tests, inquiries and methodologies described herein can be employed in a variety of contexts, including… efficacy monitoring, Para. [0319]); A method of monitoring for endometrial cancer recurrence in a subject, the method comprising (a) administering a treatment for endometrial 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 endometrial cancer is recurring (the present invention relates to an in vitro diagnostic method for the diagnosis of endometrial cancer or an increased likelihood of endometrial comprising detecting the level of (1) from 1 to 17 biomarker(s)… in a sample from a patient, Para. [0017]; the level of protein corresponding to the biomarker is determined, Para. [0068]; The biomarkers, reagents, targets, assays, tests, inquiries and methodologies described herein can be employed in a variety of contexts, including… efficacy monitoring, Para. [0319]); A method of treating endometrial cancer in a subject, the method comprising (a) administering a treatment for endometrial 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 (the present invention relates to an in vitro diagnostic method for the diagnosis of endometrial cancer or an increased likelihood of endometrial comprising detecting the level of (1) from 1 to 17 biomarker(s) … in a sample from a patient, Para. [0017]; the level of protein corresponding to the biomarker is determined, Para. [0068]).

Further, US 2010/0068724 A1 to Fung et al. teaches a method of treating endometrial cancer in a subject, comprising (a) acquiring results; and (b) administering a treatment to the subject; A method of treating endometrial cancer in a subject, the method comprising: (a) acquiring results from an evaluation of the subject that determined the subject has endometrial 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 endometrial cancer (a method for treating or reducing the progression or likelihood of a disease, Para. [0170]; this invention provides methods for determining the stage of disease in a subject. Each stage of the disease has a characteristic amount of a biomarker or relative amounts of a set of biomarkers (a pattern). The stage of a disease is determined by measuring the relevant biomarker or biomarkers and then either submitting them to a classification algorithm or comparing them with a reference amount and/or pattern of biomarkers that is associated with the particular stage, Para. [0138]; data derived from the spectra (e.g., mass spectra or time-of-flight spectra) that are generated using samples such as “known samples” can then be used to “train” a classification model, Para. [0143]; It has further been found that hepcidin is a biomarker that is differentially present in subjects having endometrial cancer, Para. [0011]); A method of treating endometrial cancer in a subject, comprising (a) acquiring results; and (b) administering a treatment to the subject (a method for treating or reducing the progression or likelihood of a disease, Para. [0170]; this invention provides methods for determining the stage of disease in a subject. Each stage of the disease has a characteristic amount of a biomarker or relative amounts of a set of biomarkers (a pattern). The stage of a disease is determined by measuring the relevant biomarker or biomarkers and then either submitting them to a classification algorithm or comparing them with a reference amount and/or pattern of biomarkers that is associated with the particular stage, Para. [0138]; data derived from the spectra (e.g., mass spectra or time-of-flight spectra) that are generated using samples such as “known samples” can then be used to “train” a classification model, Para. [0143]; It has further been found that hepcidin is a biomarker that is differentially present in subjects having endometrial cancer, Para. [0011]); A method of treating endometrial 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 endometrial cancer is detected; and administering a treatment to the subject when endometrial cancer is detected (a method for treating or reducing the progression or likelihood of a disease, Para. [0170]; this invention provides methods for determining the stage of disease in a subject. Each stage of the disease has a characteristic amount of a biomarker or relative amounts of a set of biomarkers (a pattern). The stage of a disease is determined by measuring the relevant biomarker or biomarkers and then either submitting them to a classification algorithm or comparing them with a reference amount and/or pattern of biomarkers that is associated with the particular stage, Para. [0138]; data derived from the spectra (e.g., mass spectra or time-of-flight spectra) that are generated using samples such as “known samples” can then be used to “train” a classification model, Para. [0143]; It has further been found that hepcidin is a biomarker that is differentially present in subjects having endometrial cancer, Para. [0011]); A method of treating endometrial cancer in a subject in whom endometrial cancer was detected, the method comprising administering a treatment for endometrial cancer to the subject, wherein endometrial cancer was detected in the subject by a method

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

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 endometrial cancer is detected (a method for treating or reducing the progression or likelihood of a disease, Para. [0170]; this invention provides methods for determining the stage of disease in a subject. Each stage of the disease has a characteristic amount of a biomarker or relative amounts of a set of biomarkers (a pattern). The stage of a disease is determined by measuring the relevant biomarker or biomarkers and then either submitting them to a classification algorithm or comparing them with a reference amount and/or pattern of biomarkers that is associated with the particular stage, Para. [0138]; data derived from the spectra (e.g., mass spectra or time-of-flight spectra) that are generated using samples such as "known samples" can then be used to "train" a classification model, Para. [0143]; It has further been found that hepcidin is a biomarker that is differentially present in subjects having endometrial cancer, Para. [0011]); A method of treating endometrial cancer in a subject, the method comprising (a) administering a treatment for endometrial 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 for treating or reducing the progression or likelihood of a disease, Para. [0170]; this invention provides methods for determining the stage of disease in a subject. Each stage of the disease has a characteristic amount of a biomarker or relative amounts of a set of biomarkers (a pattern). The stage of a disease is determined by measuring the relevant biomarker or biomarkers and then either submitting them to a classification algorithm or comparing them with a reference amount and/or pattern of biomarkers that is associated with the particular stage, Para. [0138]; data derived from the spectra (e.g., mass spectra or time-of-flight spectra) that are generated using samples such as "known samples" can then be used to "train" a classification model, Para. [0143]; It has further been found that hepcidin is a biomarker that is differentially present in subjects having endometrial cancer, Para. [0011]); A method of adjusting a treatment for endometrial cancer in a subject, the method comprising (a) administering a treatment for endometrial 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 of the invention may further comprises reporting the status to the subject, recording the status on a tangible medium, and/or managing subject treatment based on the status. One or more biomarker may be after subject management and the measurement correlated with disease progression, Para. [0020]; For example, hepcidin is increased with disease, while transthyretin is decreased in disease. Therefore, one can follow the course of the amounts of these biomarkers in the subject during the course of treatment. Accordingly, this method involves measuring one or more biomarkers in a subject receiving drug therapy, and correlating the amounts of the biomarkers with the disease status of the subject. One embodiment of this method involves determining the levels of the biomarkers for at least two different time points during a course of drug therapy, e.g., a first time and a second time, and comparing the change in amounts of the biomarkers, if any. For example, the biomarkers can be measured before and after drug administration or at two different time points during drug administration. The effect of therapy is determined based on these comparisons. If a treatment is effective, then the biomarkers will trend toward normal, while if treatment is ineffective, the biomarkers will trend toward disease indications. If a treatment is effective, then the biomarkers will trend toward normal, while if treatment is ineffective, the biomarkers will trend toward disease indications, Para. [0164]); A method of treating endometrial cancer in a subject, the method comprising (a) administering a treatment for endometrial 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 for treating or reducing the progression or likelihood of a disease, Para. [0170]; this invention provides methods for determining the stage of disease in a subject. Each stage of the disease has a characteristic amount of a biomarker or relative amounts of a set of biomarkers (a pattern). The stage of a disease is determined by measuring the relevant biomarker or biomarkers and then either submitting them to a classification algorithm or comparing them with a reference amount and/or pattern of biomarkers that is associated with the particular stage, Para. [0138]; data derived from the spectra (e.g., mass spectra or time-of-flight spectra) that are generated using samples such as "known samples" can then be used to "train" a classification model, Para. [0143]; It has further been found that hepcidin is a biomarker that is differentially present in subjects having endometrial cancer, Para. [0011]).

Further, US 2022/0120750 A1 to Tymora Analytical Operations Inc. teaches a biomarker from Table 1 (Extracellular vesicle biomarkers for endometrial cancer, Title; This disclosure is the first such method to successfully demonstrate the feasibility of developing uterine lavage- and plasma-derived EV proteins for endometrial cancer detection and profiling…. It is envisioned to further apply this innovative procedure to

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

validate and fully develop the disclosed current biomarker panel in Table I for detection and monitoring of endometrial cancer, Para. [0020]; Table I discloses IGFBP3).

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

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.