FOLATE RECEPTOR ALPHA AS A DIAGNOSTIC AND PROGNOSTIC MARKER FOR FOLATE RECEPTOR ALPHA-EXPRESSING CANCERS

RELATED APPLICATIONS

This application claims the benefit of the filing date of U.S. Provisional Application No. 61/410,497, filed on November 5, 2010, and U.S. Provisional Application No. 61/508,444, filed on July 15, 2011, the entire contents of each of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

In humans, the high affinity receptor for folate comes in three isoforms: alpha, beta, and gamma. The alpha and beta forms are typically bound to the membranes of cells by a glycosyl phosphatidylinositol (GPI) anchor. They recycle between extracellular and endocytic compartments and are capable of transporting folate into the cell. Soluble forms of FRα may be derived by the action of proteases or phospholipase on membrane anchored folate receptors.

Folate receptor alpha (also referred to as FRα, FR-alpha, FOLR-1 or FOLR1) is expressed in a variety of epithelial tissues, including those of the choroid plexus, lung, thyroid, kidney, uterus, breast, Fallopian tube, epididymis, and salivary glands. Weitman, SD *et al.* *Cancer Res* 52: 3396-3401 (1992); Weitman SD et al, *Cancer Res* 52: 6708-6711. Overexpression of FRα has been observed in various cancers, including lung cancer (*e.g.*, bronchioalveolar carcinomas, carcinoid tumors, and non-small cell lung cancers, such as adenocarcinomas); mesothelioma; ovarian cancer; renal cancer; brain cancer (*e.g.*, anaplastic ependymoma, cerebellar juvenile pilocytic astrocytoma, and brain metastases); cervical cancer; nasopharyngeal cancer; mesodermally derived tumor; squamous cell carcinoma of the head and neck; endometrial cancer; endometrioid adenocarcinomas of the ovary, serous cystadenocarcinomas, breast cancer; bladder cancer; pancreatic cancer; bone cancer (*e.g.*, high-grade osteosarcoma); pituitary cancer (*e.g.*, pituitary adenomas); colorectal cancer and medullary thyroid cancer. See *e.g.*, U.S. Patent No. 7,754,698; U.S. Patent Application No. 2005/0232919; WO 2009/132081; Bueno R *et al. J of Thoracic and Cardiovascular Surgery*, 121(2) : 225-233 (2001); Elkanat H & Ratnam M. *Frontiers in Bioscience*, 11, 506-519 (2006);

Fisher R.E. J Nucl Med, 49: 899-906 (2008); Franklin, WA *et al. Int J Cancer*, Suppl 8: 89-95 (1994); Hartman L.C. *et al. Int J Cancer* 121: 938-942 (2007); Iwakiri S *et al. Annals of Surgical Oncology*, 15(3): 889-899; Parker N. *et al. Analytical Biochemistry*, 338: 284-293 (2005); Weitman, SD *et al. Cancer Res* 52: 3396-3401 (1992); Saba N.F. *et al. Head Neck*, 31(4): 475-481 (2009); Yang R *et al. Clin Cancer Res* 13: 2557-2567 (2007). In some types of cancers (*e.g.*, squamous cell carcinoma of the head and neck), a higher level of FRα expression is associated with a worse prognosis, whereas in other types of cancers (*e.g.*, non-small-cell lung cancers), a higher level of FRα expression is associated with a better prognosis. See, *e.g.*, Iwakiri S *et al. Annals of Surgical Oncology*, 15(3): 889-899; Saba N.F. *et al. Head Neck*, 31(4): 475-481 (2009).

Earlier detection of cancer improves survival rates and quality of life. To improve the likelihood of early detection and treatment, a pressing need exists for non-invasive methods for diagnosing cancer, for determining the level of risk of developing cancer, and for predicting the progression of cancer. The present invention satisfies these needs for FRα-expressing cancers.

SUMMARY

The present disclosure provides methods of assessing whether a subject is afflicted with FRα-expressing cancers such as lung or ovarian cancer, methods of assessing the progression of FRα-expressing cancers such as lung or ovarian cancer in a subject afflicted with the FRα-expressing cancers, methods of stratifying an FRα-expressing cancer subject into one of at least four cancer therapy groups, methods of assessing the efficacy of MORAb-003 treatment of ovarian cancer or lung cancer and kits for assessing whether a subject is afflicted with FRα-expressing cancers such as lung or ovarian cancer or for assessing the progression of FRα-expressing cancers such as lung or ovarian cancer in a subject.

Methods of Assessing Whether a Subject is Afflicted with an FRα Expressing Cancer

In a first example, the present disclosure provides a method of assessing whether a subject is afflicted with an FRα-expressing cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell, in a sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell with the level of FRα in a control sample, wherein a difference between the level of FRα in

the sample derived from the subject and the level of FRα in the control sample is an indication that the subject is afflicted with an FRα-expressing cancer; wherein the level of FRα in the sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα. In a particular example, the sample is either urine, serum, plasma or ascites.

In one aspect, the present disclosure refers to a method of assessing whether a subject is afflicted with ovarian or lung cancer, the method comprising

contacting a urine sample derived from said subject with an antibody that binds FRα, thereby determining the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample;

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample derived from said subject with the level of FRα in a control sample;

identifying an increase in the level of FRα in the urine sample derived from said subject as compared to the level of FRα in the control sample; and

determining that the subject is afflicted with ovarian or lung cancer based on said increase.

In another example, the present disclosure is directed to a method of assessing whether a subject is afflicted with an FRα-expressing cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a urine sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the urine sample derived from the subject with the level of FRα in a control sample, wherein a difference between the level of FRα in the urine sample derived from the subject and the level of FRα in the control sample is an indication that the subject is afflicted with an FRα-expressing cancer. In a further example, the present disclosure provides a method of assessing whether a subject is afflicted with a cancer that expresses FRα, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a serum sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the serum sample derived from the subject with the level of FRα in a control sample, wherein a difference between the level of FRα in the serum sample derived from the subject and the level of FRα in the control sample is an indication that the subject is afflicted with an FRα-expressing cancer.

In another aspect, the present disclosure refers to a method of assessing whether a subject is afflicted with folate receptor alpha (FRα)-expressing ovarian cancer or lung cancer, the method comprising

determining the level of FRα which is not bound to a cell in a serum or plasma sample derived from said subject; and

comparing the level of FRα which is not bound to a cell with the level of FRα in a control sample,

identifying an increase in the level of FRα in the serum or plasma sample derived from said subject as compared to the level of FRα in the control sample; and

determining that the subject is afflicted with ovarian cancer or lung cancer based upon said increase,

wherein the level of FRα which is not bound to a cell in the serum or plasma sample derived from said subject is assessed by contacting the serum or plasma sample with a pair of antibodies that bind FRα in a two-antibody sandwich assay, wherein the two antibody-sandwich assay is an electrochemiluminescence assay (ECLIA) or enzyme immunoassay (EIA).

In various examples of the foregoing examples of the disclosure, the FRα-expressing cancer is selected from the group consisting of lung cancer, mesothelioma, ovarian cancer, renal cancer, brain cancer, cervical cancer, nasopharyngeal cancer, squamous cell carcinoma of the head and neck, endometrial cancer, breast cancer, bladder cancer, pancreatic cancer, bone cancer, pituitary cancer, colorectal cancer and medullary thyroid cancer. In a particular example, the FRα-expressing cancer is ovarian cancer. In another example, the FRα-expressing cancer is non-small cell lung cancer, such as an adenocarcinoma.

In another example, the present disclosure is directed to methods of assessing whether a subject is afflicted with ovarian cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a urine sample derived from the subject, wherein the presence of FRα which is not bound to a cell in the urine sample at

a concentration of greater than about 9100 pg/ml is an indication that the subject is afflicted with ovarian cancer.

In various examples of the foregoing examples of the disclosure, the presence of FRα in the urine sample at a concentration of greater than about 9500 pg/mL, about 10,000 pg/mL, about 11,000 pg/mL, about 12,000 pg/mL, about 13,000 pg/mL, about 14,000 pg/mL, about 15,000 pg/mL, about 16,000 pg/mL, about 17,000 pg/mL, about 18,000 pg/mL, about 19,000 pg/mL, or about 20,000 pg/mL is an indication that the subject is afflicted with ovarian cancer.

In various examples, the level of FRα is determined by contacting the sample with an antibody that binds FRα. For example, the antibody is selected from the group consisting of:

(a) an antibody that binds the same epitope as the MORAb-003 antibody;

(b) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3;

(c) the MOV18 antibody;

(d) an antibody that binds the same epitope as the MOV18 antibody;

(e) the 548908 antibody;

(f) an antibody that binds the same epitope as the 548908 antibody;

(g) the 6D398 antibody;

(h) an antibody that binds the same epitope as the 6D398 antibody;

(i) an antibody that binds the same epitope as the 26B3 antibody;

(j) an antibody comprising SEQ ID NO:55 (GYFMN) as CDRH1, SEQ ID NO:56 (RIFPYNGDTFYNQKFKG) as CDRH2, SEQ ID NO:57 (GTHYFDY) as CDRH3, SEQ ID NO:51 (RTSENIFSYLA) as CDRL1 , SEQ ID NO:52 (NAKTLAE) as CDRL2 and SEQ ID NO:53 (QHHYAFPWT) as CDRL3;

(k) the 26B3 antibody;

(l) an antibody that binds the same epitope as the 19D4 antibody;

(m) an antibody comprising SEQ ID NO:39 (HPYMH) as CDRH1, SEQ ID NO:40 (RIDPANGNTKYDPKFQG) as CDRH2, SEQ ID NO:41 (EEVADYTMDY) as CDRH3, SEQ ID NO:35 (RASESVDTYGNNFIH) as CDRL1, SEQ ID NO:36 (LASNLES) as CDRL2 and SEQ ID NO:37 (QQNNGDPWT) as CDRL3;

(n) the 19D4 antibody;

(o) an antibody that binds the same epitope as the 9F3 antibody;

(p) an antibody comprising SEQ ID NO:31 (SGYYWN) as CDRH1, SEQ ID NO:32 (YIKSDGSNNYNPSLKN) as CDRH2, SEQ ID NO:33 (EWKAMDY) as CDRH3, SEQ ID NO:27 (RASSTVSYSYLH) as CDRL1, SEQ ID NO:28 (GTSNLAS) as CDRL2 and SEQ ID NO:29 (QQYSGYPLT) as CDRL3;

(q) the 9F3 antibody;

(r) an antibody that binds the same epitope as the 24F12 antibody;

(s) an antibody comprising SEQ ID NO:47 (SYAMS) as CDRH1, SEQ ID NO:48 (EIGSGGSYTYYPDTVTG) as CDRH2, SEQ ID NO:49 (ETTAGYFDY) as CDRH3, SEQ ID NO:43 (SASQGINNFLN) as CDRL1, SEQ ID NO:44 (YTSSLHS) as CDRL2 and SEQ ID NO:45 (QHFSKLPWT) as CDRL3;

(t) the 24F12 antibody;

(u) an antibody that comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16);

(v) an antibody that comprises a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21);

(w) an antibody that comprises the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16);

(x) an antibody that comprises the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); and

(y) an antibody that comprises the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In a particular example, the antibody binds the same epitope as the MORAb-003 antibody. In another example, the antibody includes SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as

CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3. In another example, the antibody is the MOV18 antibody. In yet another example, the antibody binds the same epitope as the MOV18 antibody. In a further example, the antibody comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16). Alternatively or in combination, the antibody includes a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21). In certain examples, the antibody includes (i) the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); or the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In a particular example, the level of FRα in the sample derived from said subject is assessed by contacting the sample with a pair of antibodies selected from the group consisting of (a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody; (b) 9F3 antibody immobilized to a solid support and labeled 24F12 antibody; (c) 26B3 antibody immobilized to a solid support and labeled 19D4 antibody; and (d) 9F3 antibody immobilized to a solid support and labeled 26B3 antibody.

In certain examples, the antibody is selected from the group consisting of a murine antibody, a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, a Fab, Fab'2, ScFv, SMIP, affibody, avimer, versabody, nanobody, and a domain antibody. Alternatively, or in combination, the antibody is labeled, for example, with a label selected from the group consisting of a radio-label, a biotin-label, a chromophore-label, a fluorophore-label, or an enzyme-label.

In certain examples, the level of FRα is determined by using a technique selected from the group consisting of western blot analysis, radioimmunoassay, immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion,

solution phase assay, electrochemiluminescence immunoassay (ECLIA) and ELISA assay.

In various examples of the foregoing examples of the disclosure, the control sample is a standardized control level of FRα in a healthy subject.

In certain examples, the sample is treated with guanidine prior to determining the level of FRα in the sample. Alternatively or in combination, the sample is diluted prior to determining the level of FRα in the sample. Alternatively, or in combination, the sample is centrifuged, vortexed, or both, prior to determining the level of FRα in the sample.

In yet another example, the present disclosure is directed to a method of assessing whether a subject is afflicted with ovarian cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the sample with the level of FRα in a control sample, wherein a difference between the levels of FRα in the sample derived from the subject and in the control sample is an indication that the subject is afflicted with ovarian cancer; wherein the level of FRα in the sample derived from the subject is assessed by contacting the sample with (a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody, (b) 9F3 antibody immobilized to a solid support and labeled 24F12 antibody, (c) 26B3 antibody immobilized to a solid support and labeled 19D4 antibody, and (d) 9F3 antibody immobilized to a solid support and labeled 26B3 antibody. For example, the sample may be urine, serum, plasma or ascites.

Methods of Assessing the Progression of an FRα Expressing Cancer in a Subject

In a further example, the present disclosure is directed to a method of assessing the progression of an FRα-expressing cancer in a subject afflicted with an FRα-expressing cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell, in a sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell with the level of FRα in a control sample, wherein an increase in the level of FRα in the sample derived from the subject as compared with the level of FRα in the control sample is an indication that the cancer will progress rapidly; and wherein a decrease in the level of FRα in the sample derived from the subject as compared with the level of FRα in the control sample is an indication that

the cancer will progress slowly or will regress, thereby assessing the progression of the FRα-expressing cancer in the subject; wherein the level of FRα which is not bound to a cell in the sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα. In a particular example, the sample is urine, serum, plasma or ascites.

In another aspect, the present disclosure refers to a method of assessing the progression of ovarian cancer in a subject afflicted with ovarian cancer, the method comprising

contacting a urine sample derived from said subject with an antibody that binds FRα, thereby determining the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample;

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample derived from said subject with the level of FRα in a control sample; and

(i) identifying an increase in the level of FRα in the urine sample derived from said subject as compared to the level of FRα in the control sample and determining that the ovarian cancer will progress rapidly based on said increase; or

(ii) identifying a decrease in the level of FRα in the urine sample derived from said subject as compared to the level of FRα in the control sample and determining that the ovarian cancer will progress slowly based on said decrease.

In another example, the present disclosure provides a method of assessing the progression of an FRα-expressing cancer in a subject afflicted with an FRα-expressing cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a urine sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the urine sample derived from the subject with the level of FRα in a control sample, wherein an increase in the level of FRα in the urine sample derived from the subject as compared with the level of FRα in the control sample is an indication that the cancer will progress rapidly; and wherein a decrease in the level of FRα in the urine sample derived from the subject as compared with the level of FRα in the control sample is an indication that the cancer will progress slowly or will regress, thereby assessing the progression of the FRα-expressing cancer in the subject.

In a further example, the present disclosure provides methods of assessing the progression of an FRα-expressing cancer in a subject afflicted with an

FRα-expressing cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a serum sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the serum sample derived from the subject with the level of FRα in control sample, wherein an increase in the level of FRα in the serum sample derived from the subject as compared with the level of FRα in the control sample is an indication that the cancer will progress rapidly; and wherein a decrease in the level of FRα in the serum sample derived from the subject as compared with the level of FRα in the control sample is an indication that the cancer will progress slowly or will regress, thereby assessing the progression of the FRα-expressing cancer in the subject.

In another aspect, the present disclosure refers to a method of assessing the progression of ovarian cancer in a subject afflicted with ovarian cancer, the method comprising

determining the level of folate receptor alpha (FRα) which is not bound to a cell in a serum or plasma sample derived from said subject by performing a two antibody sandwich assay, wherein the two antibody-sandwich assay is an electrochemiluminescence assay (ECLIA) or enzyme immunoassay (EIA);

comparing the level of folate receptor alpha (FRα) which is not bound to a cell with the level of FRα in a control sample; and

(i) identifying an increase in the level of FRα in the serum or plasma sample derived from said subject as compared to the level of FRα in the control sample and determining that the ovarian cancer will progress rapidly based on said increase; or

(ii) identifying a decrease in the level of FRα in the serum or plasma sample derived from said subject as compared to the level of FRα in the control sample and determining that the ovarian cancer will progress slowly based on said decrease.

In various examples of the foregoing examples of the disclosure, the FRα-expressing cancer is selected from the group consisting of lung cancer, mesothelioma, ovarian cancer, renal cancer, brain cancer, cervical cancer, nasopharyngeal cancer, squamous cell carcinoma of the head and neck, endometrial cancer, breast cancer,

bladder cancer, pancreatic cancer, bone cancer, pituitary cancer, colorectal cancer and medullary thyroid cancer. In a particular example, the FRα-expressing cancer is ovarian cancer. In another example, the FRα-expressing cancer is non-small cell lung cancer, such as an adenocarcinoma.

In another example, the present disclosure is directed to methods of assessing whether a subject is afflicted with ovarian cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a urine sample derived from the subject, wherein the presence of FRα which is not bound to a cell in the urine sample at a concentration of greater than about 9100 pg/ml is an indication that the subject is afflicted with ovarian cancer.

In various examples of the foregoing examples of the disclosure, the presence of FRα in the urine sample at a concentration of greater than about 9500 pg/mL, about 10,000 pg/mL, about 11,000 pg/mL, about 12,000 pg/mL, about 13,000 pg/mL, about 14,000 pg/mL, about 15,000 pg/mL, about 16,000 pg/mL, about 17,000 pg/mL, about 18,000 pg/mL, about 19,000 pg/mL, or about 20,000 pg/mL is an indication that the subject is afflicted with ovarian cancer.

In various examples, the level of FRα is determined by contacting the sample with an antibody that binds FRα. For example, the antibody is selected from the group consisting of:

(a) an antibody that binds the same epitope as the MORAb-003 antibody;

(b) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3;

(c) the MOV18 antibody;

(d) an antibody that binds the same epitope as the MOV18 antibody;

(e) the 548908 antibody;

(f) an antibody that binds the same epitope as the 548908 antibody;

(g) the 6D398 antibody;

(h) an antibody that binds the same epitope as the 6D398 antibody;

(i) an antibody that binds the same epitope as the 26B3 antibody;

(j) an antibody comprising SEQ ID NO:55 (GYFMN) as CDRH1, SEQ ID NO:56 (RIFPYNGDTFYNQKFKG) as CDRH2, SEQ ID NO:57 (GTHYFDY) as

CDRH3, SEQ ID NO:51 (RTSENIFSYLA) as CDRL1 , SEQ ID NO:52 (NAKTLAE) as CDRL2 and SEQ ID NO:53 (QHHYAFPWT) as CDRL3;

(k) the 26B3 antibody;

(l) an antibody that binds the same epitope as the 19D4 antibody;

(m) an antibody comprising SEQ ID NO:39 (HPYMH) as CDRH1, SEQ ID NO:40 (RIDPANGNTKYDPKFQG) as CDRH2, SEQ ID NO:41 (EEVADYTMDY) as CDRH3, SEQ ID NO:35 (RASESVDTYGNNFIH) as CDRL1, SEQ ID NO:36 (LASNLES) as CDRL2 and SEQ ID NO:37 (QQNNGDPWT) as CDRL3;

(n) the 19D4 antibody;

(o) an antibody that binds the same epitope as the 9F3 antibody;

(p) an antibody comprising SEQ ID NO:31 (SGYYWN) as CDRH1, SEQ ID NO:32 (YIKSDGSNNYNPSLKN) as CDRH2, SEQ ID NO:33 (EWKAMDY) as CDRH3, SEQ ID NO:27 (RASSTVSYSYLH) as CDRL1, SEQ ID NO:28 (GTSNLAS) as CDRL2 and SEQ ID NO:29 (QQYSGYPLT) as CDRL3;

(q) the 9F3 antibody;

(r) an antibody that binds the same epitope as the 24F12 antibody;

(s) an antibody comprising SEQ ID NO:47 (SYAMS) as CDRH1, SEQ ID NO:48 (EIGSGGSYTYYPDTVTG) as CDRH2, SEQ ID NO:49 (ETTAGYFDY) as CDRH3, SEQ ID NO:43 (SASQGINNFLN) as CDRL1, SEQ ID NO:44 (YTSSLHS) as CDRL2 and SEQ ID NO:45 (QHFSKLPWT) as CDRL3;

(t) the 24F12 antibody;

(u) an antibody that comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16);

(v) an antibody that comprises a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21);

(w) an antibody that comprises the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16);

(x) an antibody that comprises the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); and

(y) an antibody that comprises the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In a particular example, the antibody binds the same epitope as the MORAb-003 antibody. In another example, the antibody includes SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3. In another example, the antibody is the MOV18 antibody. In yet another example, the antibody binds the same epitope as the MOV18 antibody. In a further example, the antibody comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16). Alternatively or in combination, the antibody includes a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21). In certain examples, the antibody includes (i) the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); or the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In a particular example, the level of FRα in the sample derived from said subject is assessed by contacting the sample with a pair of antibodies selected from the group consisting of (a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody; (b) 9F3 antibody immobilized to a solid support and labeled 24F12 antibody; (c) 26B3 antibody immobilized to a solid support and labeled 19D4 antibody; and (d) 9F3 antibody immobilized to a solid support and labeled 26B3 antibody.

In certain examples, the antibody is selected from the group consisting of a murine antibody, a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, a Fab, Fab'2, ScFv, SMIP, affibody, avimer, versabody, nanobody, and a domain antibody. Alternatively, or in combination, the antibody is labeled, for example, with a label selected from the group consisting of a radio-label, a biotin-label, a chromophore-label, a fluorophore-label, or an enzyme-label.

In certain examples, the level of FRα is determined by using a technique selected from the group consisting of western blot analysis, radioimmunoassay, immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, solution phase assay, electrochemiluminescence immunoassay (ECLIA) and ELISA assay.

In various examples of the foregoing examples of the disclosure, the control sample is a standardized control level of FRα in a healthy subject. In another example, the control sample is a sample previously obtained from the subject.

In certain examples, the sample is treated with guanidine prior to determining the level of FRα in the sample. Alternatively or in combination, the sample is diluted prior to determining the level of FRα in the sample. Alternatively, or in combination, the sample is centrifuged, vortexed, or both, prior to determining the level of FRα in the sample.

In a further example, the present disclosure provides methods of assessing the progression of ovarian cancer in a subject afflicted with ovarian cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the sample with the level of FRα in a control sample, wherein an increase in the level of FRα in the sample derived from the subject as compared with the level of FRα in the control sample is an indication that the ovarian cancer will progress rapidly; and wherein a decrease in the level of FRα in the sample derived from the subject as compared with the level of FRα in the control sample is an indication that the ovarian cancer will progress slowly or will regress, thereby assessing the progression of ovarian cancer in the subject; wherein the level of FRα in the sample derived from the subject is assessed by contacting the sample with (a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody, (b) 9F3 antibody immobilized to a solid support and labeled 24F12 antibody, (c) 26B3 antibody immobilized to a solid

support and labeled 19D4 antibody, and (d) 9F3 antibody immobilized to a solid support and labeled 26B3 antibody. For example, the sample may be urine, serum, plasma or ascites.

Methods of Stratifying an FRα Expressing Cancer into Cancer Therapy Groups

In a further example, the present disclosure provides a method of stratifying a subject afflicted with an FRα-expressing cancer into one of at least four cancer therapy groups by determining the level of folate receptor alpha (FRα) which is not bound to a cell, in a sample derived from the subject; and stratifying the subject into one of at least four cancer therapy groups based on the level of folate receptor alpha (FRα) which is not bound to a cell; wherein the level of FRα which is not bound to a cell in the sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα. For example, the sample is selected from the group consisting of urine, serum, plasma or ascites.

In another aspect, the present disclosure refers to a method of stratifying a subject afflicted with ovarian cancer into one of at least four cancer therapy groups comprising:

contacting a urine sample derived from said subject with an antibody that binds FRα, thereby determining the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample;

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample derived from said subject with the level of FRα in a control sample; and

stratifying the subject into one of at least four cancer therapy groups based on the level of folate receptor alpha (FRα) which is not bound to a cell.

In another aspect, the present disclosure refers to a method of stratifying a subject afflicted with ovarian cancer into one of at least four cancer therapy groups comprising:

determining the level of folate receptor alpha (FRα) which is not bound to a cell, in a serum or plasma sample derived from said subject by performing a two antibody sandwich assay, wherein the two antibody-sandwich assay is an electrochemiluminescence assay (ECLIA) or enzyme immunoassay (EIA); and

stratifying the subject into one of at least four cancer therapy groups based on the level of folate receptor alpha (FRα) which is not bound to a cell.

In yet another example, the present disclosure provides a method of stratifying a subject afflicted with an FRα-expressing cancer into one of at least four cancer therapy groups by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a urine sample derived from the subject; and stratifying the subject into one of at least four cancer therapy groups based on the level of folate receptor alpha (FRα) which is not bound to a cell in the sample. In a further example, the present disclosure is directed to methods of stratifying a subject afflicted with an FRα-expressing cancer into one of at least four cancer therapy groups by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a serum sample derived from the subject; and stratifying the subject into one of at least four cancer therapy groups based on the level of folate receptor alpha (FRα) which is not bound to a cell in the serum sample.

In various examples of the foregoing examples of the disclosure, the FRα-expressing cancer is selected from the group consisting of lung cancer, mesothelioma, ovarian cancer, renal cancer, brain cancer, cervical cancer, nasopharyngeal cancer, squamous cell carcinoma of the head and neck, endometrial cancer, breast cancer, bladder cancer, pancreatic cancer, bone cancer, pituitary cancer, colorectal cancer and medullary thyroid cancer. In a particular example, the FRα-expressing cancer is ovarian cancer. In another example, the FRα-expressing cancer is non-small cell lung cancer, such as an adenocarcinoma.

In another example, the present disclosure is directed to methods of assessing whether a subject is afflicted with ovarian cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a urine sample derived from the subject, wherein the presence of FRα which is not bound to a cell in the urine sample at a concentration of greater than about 9100 pg/ml is an indication that the subject is afflicted with ovarian cancer.

In various examples of the foregoing examples of the disclosure, the presence of FRα in the urine sample at a concentration of greater than about 9500 pg/mL, about 10,000 pg/mL, about 11,000 pg/mL, about 12,000 pg/mL, about 13,000 pg/mL, about 14,000 pg/mL, about 15,000 pg/mL, about 16,000 pg/mL, about 17,000 pg/mL, about 18,000 pg/mL, about 19,000 pg/mL, or about 20,000 pg/mL is an indication that the subject is afflicted with ovarian cancer.

In various examples, the level of FRα is determined by contacting the sample with an antibody that binds FRα. For example, the antibody is selected from the group consisting of:

(a) an antibody that binds the same epitope as the MORAb-003 antibody;

(b) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3;

(c) the MOV18 antibody;

(d) an antibody that binds the same epitope as the MOV18 antibody;

(e) the 548908 antibody;

(f) an antibody that binds the same epitope as the 548908 antibody;

(g) the 6D398 antibody;

(h) an antibody that binds the same epitope as the 6D398 antibody;

(i) an antibody that binds the same epitope as the 26B3 antibody;

(j) an antibody comprising SEQ ID NO:55 (GYFMN) as CDRH1, SEQ ID NO:56 (RIFPYNGDTFYNQKFKG) as CDRH2, SEQ ID NO:57 (GTHYFDY) as CDRH3, SEQ ID NO:51 (RTSENIFSYLA) as CDRL1 , SEQ ID NO:52 (NAKTLAE) as CDRL2 and SEQ ID NO:53 (QHHYAFPWT) as CDRL3;

(k) the 26B3 antibody;

(l) an antibody that binds the same epitope as the 19D4 antibody;

(m) an antibody comprising SEQ ID NO:39 (HPYMH) as CDRH1, SEQ ID NO:40 (RIDPANGNTKYDPKFQG) as CDRH2, SEQ ID NO:41 (EEVADYTMDY) as CDRH3, SEQ ID NO:35 (RASESVDTYGNNFIH) as CDRL1, SEQ ID NO:36 (LASNLES) as CDRL2 and SEQ ID NO:37 (QQNNGDPWT) as CDRL3;

(n) the 19D4 antibody;

(o) an antibody that binds the same epitope as the 9F3 antibody;

(p) an antibody comprising SEQ ID NO:31 (SGYYWN) as CDRH1, SEQ ID NO:32 (YIKSDGSNNYNPSLKN) as CDRH2, SEQ ID NO:33 (EWKAMDY) as CDRH3, SEQ ID NO:27 (RASSTVSYSYLH) as CDRL1, SEQ ID NO:28 (GTSNLAS) as CDRL2 and SEQ ID NO:29 (QQYSGYPLT) as CDRL3;

(q) the 9F3 antibody;

(r) an antibody that binds the same epitope as the 24F12 antibody;

(s) an antibody comprising SEQ ID NO:47 (SYAMS) as CDRH1, SEQ ID NO:48 (EIGSGGSYTYYPDTVTG) as CDRH2, SEQ ID NO:49 (ETTAGYFDY) as CDRH3, SEQ ID NO:43 (SASQGINNFLN) as CDRL1, SEQ ID NO:44 (YTSSLHS) as CDRL2 and SEQ ID NO:45 (QHFSKLPWT) as CDRL3;

(t) the 24F12 antibody;

(u) an antibody that comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16);

(v) an antibody that comprises a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21);

(w) an antibody that comprises the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16);

(x) an antibody that comprises the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); and

(y) an antibody that comprises the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In a particular example, the antibody binds the same epitope as the MORAb-003 antibody. In another example, the antibody includes SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3. In another example, the antibody is the MOV18 antibody. In yet another example, the antibody binds the same epitope as the MOV18 antibody. In a further example, the antibody comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16). Alternatively or in combination, the antibody includes a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21). In certain examples, the antibody includes (i) the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); or the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In a particular example, the level of FRα in the sample derived from said subject is assessed by contacting the sample with a pair of antibodies selected from the group consisting of (a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody; (b) 9F3 antibody immobilized to a solid support and labeled 24F12 antibody; (c) 26B3 antibody immobilized to a solid support and labeled 19D4 antibody; and (d) 9F3 antibody immobilized to a solid support and labeled 26B3 antibody.

In certain examples, the antibody is selected from the group consisting of a murine antibody, a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, a Fab, Fab'2, ScFv, SMIP, affibody, avimer, versabody, nanobody, and a domain antibody. Alternatively, or in combination, the antibody is labeled, for example, with a label selected from the group consisting of a radio-label, a biotin-label, a chromophore-label, a fluorophore-label, or an enzyme-label.

In certain examples, the level of FRα is determined by using a technique selected from the group consisting of western blot analysis, radioimmunoassay, immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, solution phase assay, electrochemiluminescence immunoassay (ECLIA) and ELISA assay.

In various examples of the foregoing examples of the disclosure, the control sample is a standardized control level of FRα in a healthy subject.

In certain examples, the sample is treated with guanidine prior to determining the level of FRα in the sample. Alternatively or in combination, the sample is diluted prior to determining the level of FRα in the sample. Alternatively, or in combination, the sample is centrifuged, vortexed, or both, prior to determining the level of FRα in the sample.

In a particular example, the subject is stratified in Stage I, Stage II, Stage III or Stage IV ovarian cancer.

In a further example, the present disclosure provides a method of stratifying an ovarian cancer subject into one of at least four cancer therapy groups by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a sample derived from the subject; and stratifying the subject into one of at least four cancer therapy groups based on the level of folate receptor alpha (FRα) which is not bound to a cell in the sample; wherein the level of FRα in the sample derived from the subject is assessed by contacting the sample with (a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody, (b) 9F3 antibody immobilized to a solid support and labeled 24F12 antibody, (c) 26B3 antibody immobilized to a solid support and labeled 19D4 antibody, and (d) 9F3 antibody immobilized to a solid support and labeled 26B3 antibody. For example, the sample may be urine, serum, plasma or ascites.

Methods of Monitoring the Efficacy of MORAb-003 Treatment of Ovarian Cancer or Lung Cancer

In one example, the present disclosure provides a method of monitoring the efficacy of MORAb-003 treatment of ovarian cancer or lung cancer in a subject suffering from ovarian cancer or lung cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell, in a sample derived from the subject, wherein the subject has been previously administered MORAb-003; and comparing the level of folate

receptor alpha (FRα) which is not bound to a cell with the level of FRα in a control sample, wherein an increase in the level of FRα in the sample derived from the subject as compared with the level of FRα in the control sample is an indication that the MORAb-003 treatment is not efficacious; and wherein a decrease in the level of FRα in the sample derived from the subject as compared with the level of FRα in the control sample is an indication that the MORAb-003 treatment is efficacious. In particular examples, the level of FRα which is not bound to a cell in the sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα. For example, the sample may be urine, serum, plasma or ascites.

In another aspect, the present disclosure refers to a method of monitoring the efficacy of MORAb-003 treatment of ovarian cancer in a subject suffering from ovarian cancer, the method comprising

contacting a urine sample derived from said subject with an antibody that binds FRα, thereby determining the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample, wherein said subject has been previously administered MORAb-003;

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample derived from said subject with the level of FRα in a control sample; and

(i) identifying an increase in the level of FRα in the urine sample derived from said subject as compared to the level of FRα in the control sample and determining that the MORAb-003 treatment is not efficacious; or

(ii) identifying a decrease in the level of FRα in the urine sample derived from said subject as compared to the level of FRα in the control sample and determining that the MORAb-003 treatment is efficacious.

In a further example, the present disclosure provides a method of monitoring the efficacy of MORAb-003 treatment of ovarian cancer or lung cancer in a subject suffering from ovarian cancer or lung cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a urine sample derived from the subject, wherein the subject has been previously administered MORAb-003; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the urine sample derived from the subject with the level of FRα in a control sample, wherein an increase in the level of FRα in the urine sample derived from the subject as compared

with the level of FRα in the control sample is an indication that the MORAb-003 treatment is not efficacious; and wherein a decrease in the level of FRα in the urine sample derived from the subject as compared with the level of FRα in the control sample is an indication that the MORAb-003 treatment is efficacious.

In yet another example, the present disclosure is directed to a method of monitoring the efficacy of MORAb-003 treatment of ovarian cancer or lung cancer in a subject suffering from ovarian cancer or lung cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a serum sample derived from the subject, wherein the subject has been previously administered MORAb-003; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the serum sample derived from the subject with the level of FRα in a control sample, wherein an increase in the level of FRα in the serum sample derived from the subject as compared with the level of FRα in the control sample is an indication that the MORAb-003 treatment is not efficacious; and wherein a decrease in the level of FRα in the serum sample derived from the subject as compared with the level of FRα in the control sample is an indication that the MORAb-003 treatment is efficacious.

In another aspect, the present disclosure refers to a method of monitoring the efficacy of MORAb-003 treatment of ovarian cancer in a subject suffering from ovarian cancer, the method comprising

determining the level of folate receptor alpha (FRα) which is not bound to a cell in a serum or plasma sample derived from said subject by performing a two antibody sandwich assay, wherein the two antibody-sandwich assay is an electrochemiluminescence assay (ECLIA) or enzyme immunoassay (EIA), wherein said subject has been previously administered MORAb-003;

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in said serum or plasma sample derived from said subject with the level of FRα in a control sample; and

(i) identifying an increase in the level of FRα in the serum or plasma sample derived from said subject as compared to the level of FRα in the control sample and determining that the MORAb-003 treatment is not efficacious; or

(ii) identifying a decrease in the level of FRα in the serum or plasma sample derived from said subject as compared to the level of FRα in the control sample and determining that the MORAb-003 treatment is efficacious.

In various examples, the level of FRα is determined by contacting the sample with an antibody that binds FRα. For example, the antibody is selected from the group consisting of:

(a) an antibody that binds the same epitope as the MORAb-003 antibody;

(b) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3;

(c) the MOV18 antibody;

(d) an antibody that binds the same epitope as the MOV18 antibody;

(e) the 548908 antibody;

(f) an antibody that binds the same epitope as the 548908 antibody;

(g) the 6D398 antibody;

(h) an antibody that binds the same epitope as the 6D398 antibody;

(i) an antibody that binds the same epitope as the 26B3 antibody;

(j) an antibody comprising SEQ ID NO:55 (GYFMN) as CDRH1, SEQ ID NO:56 (RIFPYNGDTFYNQKFKG) as CDRH2, SEQ ID NO:57 (GTHYFDY) as CDRH3, SEQ ID NO:51 (RTSENIFSYLA) as CDRL1 , SEQ ID NO:52 (NAKTLAE) as CDRL2 and SEQ ID NO:53 (QHHYAFPWT) as CDRL3;

(k) the 26B3 antibody;

(l) an antibody that binds the same epitope as the 19D4 antibody;

(m) an antibody comprising SEQ ID NO:39 (HPYMH) as CDRH1, SEQ ID NO:40 (RIDPANGNTKYDPKFQG) as CDRH2, SEQ ID NO:41 (EEVADYTMDY) as CDRH3, SEQ ID NO:35 (RASESVDTYGNNFIH) as CDRL1, SEQ ID NO:36 (LASNLES) as CDRL2 and SEQ ID NO:37 (QQNNGDPWT) as CDRL3;

(n) the 19D4 antibody;

(o) an antibody that binds the same epitope as the 9F3 antibody;

(p) an antibody comprising SEQ ID NO:31 (SGYYWN) as CDRH1, SEQ ID NO:32 (YIKSDGSNNYNPSLKN) as CDRH2, SEQ ID NO:33 (EWKAMDY) as CDRH3, SEQ ID NO:27 (RASSTVSYSYLH) as CDRL1, SEQ ID NO:28 (GTSNLAS) as CDRL2 and SEQ ID NO:29 (QQYSGYPLT) as CDRL3;

(q) the 9F3 antibody;

(r) an antibody that binds the same epitope as the 24F12 antibody;

(s) an antibody comprising SEQ ID NO:47 (SYAMS) as CDRH1, SEQ ID NO:48 (EIGSGGSYTYYPDTVTG) as CDRH2, SEQ ID NO:49 (ETTAGYFDY) as CDRH3, SEQ ID NO:43 (SASQGINNFLN) as CDRL1, SEQ ID NO:44 (YTSSLHS) as CDRL2 and SEQ ID NO:45 (QHFSKLPWT) as CDRL3;

(t) the 24F12 antibody;

(u) an antibody that comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16);

(v) an antibody that comprises a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21);

(w) an antibody that comprises the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16);

(x) an antibody that comprises the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); and

(y) an antibody that comprises the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In a particular example, the antibody binds the same epitope as the MORAb-003 antibody. In another example, the antibody includes SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3. In another example, the antibody is the MOV18 antibody. In yet another example, the antibody binds the same epitope as the MOV18 antibody. In a further example, the antibody comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16). Alternatively or in combination, the antibody includes a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH

FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21). In certain examples, the antibody includes (i) the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); or the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In a particular example, the level of FRα in the sample derived from said subject is assessed by contacting the sample with a pair of antibodies selected from the group consisting of (a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody; (b) 9F3 antibody immobilized to a solid support and labeled 24F12 antibody; (c) 26B3 antibody immobilized to a solid support and labeled 19D4 antibody; and (d) 9F3 antibody immobilized to a solid support and labeled 26B3 antibody.

In certain examples, the antibody is selected from the group consisting of a murine antibody, a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, a Fab, Fab'2, ScFv, SMIP, affibody, avimer, versabody, nanobody, and a domain antibody. Alternatively, or in combination, the antibody is labeled, for example, with a label selected from the group consisting of a radio-label, a biotin-label, a chromophore-label, a fluorophore-label, or an enzyme-label.

In certain examples, the level of FRα is determined by using a technique selected from the group consisting of western blot analysis, radioimmunoassay, immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, solution phase assay, electrochemiluminescence immunoassay (ECLIA) and ELISA assay.

In various examples of the foregoing examples of the disclosure, the control sample is a standardized control level of FRα in a healthy subject. In another example, the control sample is a sample previously obtained from the subject.

In certain examples, the sample is treated with guanidine prior to determining the level of FRα in the sample. Alternatively or in combination, the sample is diluted prior to determining the level of FRα in the sample. Alternatively, or in

combination, the sample is centrifuged, vortexed, or both, prior to determining the level of FRα in the sample.

Methods of Predicting Whether a Subject Will Respond to MORAb-003 treatment

In one example, the present disclosure provides a method for predicting whether a subject suffering from an FRα expressing cancer, such as ovarian cancer or lung cancer, will respond to treatment with MORAb-003, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the sample derived from the subject with the level of FRα in a control sample, wherein a difference between the level of FRα in the sample derived from the subject and the level of FRα in the control sample is an indication that the subject will respond to treatment with MORAb-003.

In another aspect, the present disclosure refers to a method for predicting whether a subject suffering from ovarian cancer will respond to treatment with MORAb-003, the method comprising

contacting a urine sample derived from said subject with an antibody that binds FRα, thereby determining the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample;

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample derived from said subject with the level of FRα in a control sample; and

identifying an increase in the level of FRα in the urine sample derived from said subject as compared to the level of FRα in the control sample and determining that the subject will respond to treatment with MORAb-003.

In one example, the present disclosure provides a method for predicting whether a subject suffering from an FRα expressing cancer, such as ovarian cancer or lung cancer, will respond to treatment with MORAb-003, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a urine sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the urine sample derived from the subject with the level of FRα in a control sample, wherein a difference between the level of FRα in the urine sample

derived from the subject and the level of FRα in the control sample is an indication that the subject will respond to treatment with MORAb-003.

In a further example, the present disclosure provides a method for predicting whether a subject suffering from an FRα expressing cancer, such as ovarian cancer or lung cancer, will respond to treatment with MORAb-003, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a serum sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the serum sample derived from the subject with the level of FRα in a control sample, wherein a difference between the level of FRα in the serum sample derived from the subject and the level of FRα in the control sample is an indication that the subject will respond to treatment with MORAb-003.

In another aspect, the present disclosure refers to a method for predicting whether a subject suffering from ovarian cancer will respond to treatment with MORAb-003, the method comprising

determining the level of folate receptor alpha (FRα) which is not bound to a cell in a serum or plasma sample derived from said subject,

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the serum or plasma sample derived from said subject with the level of FRα in a control sample,

identifying an increase in the level of FRα in the serum or plasma sample derived from said subject as compared to the level of FRα in the control sample and determining that the subject will respond to treatment with MORAb-003,

wherein the level of FRα which is not bound to a cell in the serum or plasma sample derived from said subject is assessed by contacting the serum or plasma sample with a pair of antibodies that bind FRα in a two-antibody sandwich assay, wherein the two antibody-sandwich assay is an electrochemiluminescence assay (ECLIA) or enzyme immunoassay (EIA).

In further examples, the FRα-expressing cancer is selected from the group consisting of lung cancer, mesothelioma, ovarian cancer, renal cancer, brain cancer,

cervical cancer, nasopharyngeal cancer, squamous cell carcinoma of the head and neck, endometrial cancer, breast cancer, bladder cancer, pancreatic cancer, bone cancer, pituitary cancer, colorectal cancer and medullary thyroid cancer. In a particular example, the FRα-expressing cancer is ovarian cancer. In another example, the FRα-expressing lung cancer is non-small cell lung cancer, such as adenocarcinoma.

In a further example, the present disclosure provides methods for predicting whether a subject suffering from ovarian cancer will respond to treatment with MORAb-003, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a urine sample derived from the subject, wherein the presence of FRα which is not bound to a cell in the urine sample at a concentration of greater than about 9100 pg/ml is an indication that the subject will respond to treatment with MORAb-003.

In various examples of the foregoing examples of the disclosure, the presence of FRα in the urine sample at a concentration of greater than about 9500 pg/mL, about 10,000 pg/mL, about 11,000 pg/mL, about 12,000 pg/mL, about 13,000 pg/mL, about 14,000 pg/mL, about 15,000 pg/mL, about 16,000 pg/mL, about 17,000 pg/mL, about 18,000 pg/mL, about 19,000 pg/mL, or about 20,000 pg/mL is an indication that the subject is afflicted with ovarian cancer.

In various examples of the foregoing examples of the disclosure, the level of FRα is determined by contacting the sample with an antibody that binds FRα. For example, the antibody is selected from the group consisting of:

(a) an antibody that binds the same epitope as the MORAb-003 antibody;

(b) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3;

(c) the MOV18 antibody;

(d) an antibody that binds the same epitope as the MOV18 antibody;

(e) the 548908 antibody;

(f) an antibody that binds the same epitope as the 548908 antibody;

(g) the 6D398 antibody;

(h) an antibody that binds the same epitope as the 6D398 antibody;

(i) an antibody that binds the same epitope as the 26B3 antibody;

(j) an antibody comprising SEQ ID NO:55 (GYFMN) as CDRH1, SEQ ID NO:56 (RIFPYNGDTFYNQKFKG) as CDRH2, SEQ ID NO:57 (GTHYFDY) as CDRH3, SEQ ID NO:51 (RTSENIFSYLA) as CDRL1 , SEQ ID NO:52 (NAKTLAE) as CDRL2 and SEQ ID NO:53 (QHHYAFPWT) as CDRL3;

(k) the 26B3 antibody;

(l) an antibody that binds the same epitope as the 19D4 antibody;

(m) an antibody comprising SEQ ID NO:39 (HPYMH) as CDRH1, SEQ ID NO:40 (RIDPANGNTKYDPKFQG) as CDRH2, SEQ ID NO:41 (EEVADYTMDY) as CDRH3, SEQ ID NO:35 (RASESVDTYGNNFIH) as CDRL1, SEQ ID NO:36 (LASNLES) as CDRL2 and SEQ ID NO:37 (QQNNGDPWT) as CDRL3;

(n) the 19D4 antibody;

(o) an antibody that binds the same epitope as the 9F3 antibody;

(p) an antibody comprising SEQ ID NO:31 (SGYYWN) as CDRH1, SEQ ID NO:32 (YIKSDGSNNYNPSLKN) as CDRH2, SEQ ID NO:33 (EWKAMDY) as CDRH3, SEQ ID NO:27 (RASSTVSYSYLH) as CDRL1, SEQ ID NO:28 (GTSNLAS) as CDRL2 and SEQ ID NO:29 (QQYSGYPLT) as CDRL3;

(q) the 9F3 antibody;

(r) an antibody that binds the same epitope as the 24F12 antibody;

(s) an antibody comprising SEQ ID NO:47 (SYAMS) as CDRH1, SEQ ID NO:48 (EIGSGGSYTYYPDTVTG) as CDRH2, SEQ ID NO:49 (ETTAGYFDY) as CDRH3, SEQ ID NO:43 (SASQGINNFLN) as CDRL1, SEQ ID NO:44 (YTSSLHS) as CDRL2 and SEQ ID NO:45 (QHFSKLPWT) as CDRL3;

(t) the 24F12 antibody;

(u) an antibody that comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16);

(v) an antibody that comprises a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21);

(w) an antibody that comprises the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16);

(x) an antibody that comprises the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); and

(y) an antibody that comprises the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In a particular example, the antibody binds the same epitope as the MORAb-003 antibody. In another example, the antibody includes SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3. In another example, the antibody is the MOV18 antibody. In yet another example, the antibody binds the same epitope as the MOV18 antibody. In a further example, the antibody comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16). Alternatively or in combination, the antibody includes a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21). In certain examples, the antibody includes (i) the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); or the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In a particular example, the level of FRα in the sample derived from said subject is assessed by contacting the sample with a pair of antibodies selected from the group consisting of (a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody; (b) 9F3 antibody immobilized to a solid support and labeled 24F12 antibody; (c) 26B3 antibody immobilized to a solid support and labeled 19D4 antibody; and (d) 9F3 antibody immobilized to a solid support and labeled 26B3 antibody.

In certain examples, the antibody is selected from the group consisting of a murine antibody, a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, a Fab, Fab'2, ScFv, SMIP, affibody, avimer, versabody, nanobody, and a domain antibody. Alternatively, or in combination, the antibody is labeled, for example, with a label selected from the group consisting of a radio-label, a biotin-label, a chromophore-label, a fluorophore-label, or an enzyme-label.

In certain examples, the level of FRα is determined by using a technique selected from the group consisting of western blot analysis, radioimmunoassay, immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, solution phase assay, electrochemiluminescence immunoassay (ECLIA) and ELISA assay.

In various examples of the foregoing examples of the disclosure, the control sample is a standardized control level of FRα in a healthy subject.

In certain examples, the sample is treated with guanidine prior to determining the level of FRα in the sample. Alternatively or in combination, the sample is diluted prior to determining the level of FRα in the sample. Alternatively, or in combination, the sample is centrifuged, vortexed, or both, prior to determining the level of FRα in the sample.

In a further example, the present disclosure provides a method for predicting whether a subject suffering from an FRα expressing cancer, such as ovarian cancer or lung cancer, will respond to treatment with MORAb-003, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the sample with the level of FRα in a control sample, wherein a difference between the levels of FRα in the sample derived from the subject and in the control sample is an indication that the subject will respond to treatment with MORAb-003; wherein the level of FRα in the sample derived from the subject is assessed by contacting the sample with (a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody, (b) 9F3 antibody immobilized to a solid support and labeled 24F12 antibody, (c) 26B3 antibody immobilized to a solid support and labeled 19D4 antibody, and (d) 9F3 antibody immobilized to a solid support and labeled 26B3 antibody. For example, the sample may be urine, serum, plasma or ascites.

In various examples of the foregoing examples of the disclosure, the MORAb-003 for treatment is (a) an antibody that comprises the heavy chain amino acid sequence as set forth in SEQ ID NO:7 and the light chain amino acid sequence as set forth in SEQ ID NO:8; (b) an antibody that binds the same epitope as the MORAb-003 antibody; or (c) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3.

Methods of Treating a Subject Having Ovarian Cancer or Lung Cancer

In another example, the present disclosure provides methods of treating a subject having ovarian cancer or lung cancer by determining the level of folate receptor alpha (FRα) which is not bound to a cell, in a sample derived from said subject (for example, urine, serum, plasma or ascites); and comparing the level of folate receptor alpha (FRα) which is not bound to a cell with the level of FRα in a control sample, wherein a difference between the level of FRα in the sample derived from said subject and the level of FRα in the control sample is an indication that the subject is afflicted with ovarian cancer or lung cancer; and administering a therapeutically effective amount of MORAb-003 to said subject.

In another example, the present disclosure provides methods of treating a subject having ovarian cancer or lung cancer by determining the level of folate receptor alpha (FRα) which is not bound to a cell, in a urine sample derived from said subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell with the level of FRα in a control sample, wherein a difference between the level of FRα in the urine sample derived from said subject and the level of FRα in the control sample is an indication that the subject is afflicted with ovarian cancer or lung cancer; and administering a therapeutically effective amount of MORAb-003 to said subject.

In another example, the present disclosure provides methods of treating a subject having ovarian cancer or lung cancer by determining the level of folate receptor alpha (FRα) which is not bound to a cell, in a serum sample derived from said subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell with the level of FRα in a control sample, wherein a difference between the level of FRα in the serum sample derived from said subject and the level of FRα in the control sample is an

indication that the subject is afflicted with ovarian cancer or lung cancer; and administering a therapeutically effective amount of MORAb-003 to said subject.

In a further example, the present disclosure provides methods for treating a subject suffering from ovarian cancer by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a urine sample derived from the subject, wherein the presence of FRα which is not bound to a cell in the urine sample at a concentration of greater than about 9100 pg/ml is an indication that the subject will respond to treatment with MORAb-003; and administering a therapeutically effective amount of MORAb-003 to said subject.

In particular examples, the level of FRα which is not bound to a cell in the sample derived from said subject is assessed by contacting the sample with an antibody that binds FRα.

In various examples of the foregoing examples of the disclosure, the presence of FRα in the urine sample at a concentration of greater than about 9500 pg/mL, about 10,000 pg/mL, about 11,000 pg/mL, about 12,000 pg/mL, about 13,000 pg/mL, about 14,000 pg/mL, about 15,000 pg/mL, about 16,000 pg/mL, about 17,000 pg/mL, about 18,000 pg/mL, about 19,000 pg/mL, or about 20,000 pg/mL is an indication that the subject is afflicted with ovarian cancer.

In various examples of the foregoing examples of the disclosure, the level of FRα is determined by contacting the sample with an antibody that binds FRα. For example, the antibody is selected from the group consisting of:

(a) an antibody that binds the same epitope as the MORAb-003 antibody;

(b) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3;

(c) the MOV18 antibody;

(d) an antibody that binds the same epitope as the MOV18 antibody;

(e) the 548908 antibody;

(f) an antibody that binds the same epitope as the 548908 antibody;

(g) the 6D398 antibody;

(h) an antibody that binds the same epitope as the 6D398 antibody;

(i) an antibody that binds the same epitope as the 26B3 antibody;

(j) an antibody comprising SEQ ID NO:55 (GYFMN) as CDRH1, SEQ ID NO:56 (RIFPYNGDTFYNQKFKG) as CDRH2, SEQ ID NO:57 (GTHYFDY) as CDRH3, SEQ ID NO:51 (RTSENIFSYLA) as CDRL1 , SEQ ID NO:52 (NAKTLAE) as CDRL2 and SEQ ID NO:53 (QHHYAFPWT) as CDRL3;

(k) the 26B3 antibody;

(l) an antibody that binds the same epitope as the 19D4 antibody;

(m) an antibody comprising SEQ ID NO:39 (HPYMH) as CDRH1, SEQ ID NO:40 (RIDPANGNTKYDPKFQG) as CDRH2, SEQ ID NO:41 (EEVADYTMDY) as CDRH3, SEQ ID NO:35 (RASESVDTYGNNFIH) as CDRL1, SEQ ID NO:36 (LASNLES) as CDRL2 and SEQ ID NO:37 (QQNNGDPWT) as CDRL3;

(n) the 19D4 antibody;

(o) an antibody that binds the same epitope as the 9F3 antibody;

(p) an antibody comprising SEQ ID NO:31 (SGYYWN) as CDRH1, SEQ ID NO:32 (YIKSDGSNNYNPSLKN) as CDRH2, SEQ ID NO:33 (EWKAMDY) as CDRH3, SEQ ID NO:27 (RASSTVSYSYLH) as CDRL1, SEQ ID NO:28 (GTSNLAS) as CDRL2 and SEQ ID NO:29 (QQYSGYPLT) as CDRL3;

(q) the 9F3 antibody;

(r) an antibody that binds the same epitope as the 24F12 antibody;

(s) an antibody comprising SEQ ID NO:47 (SYAMS) as CDRH1, SEQ ID NO:48 (EIGSGGSYTYYPDTVTG) as CDRH2, SEQ ID NO:49 (ETTAGYFDY) as CDRH3, SEQ ID NO:43 (SASQGINNFLN) as CDRL1, SEQ ID NO:44 (YTSSLHS) as CDRL2 and SEQ ID NO:45 (QHFSKLPWT) as CDRL3;

(t) the 24F12 antibody;

(u) an antibody that comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16);

(v) an antibody that comprises a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21);

(w) an antibody that comprises the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16);

(x) an antibody that comprises the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); and

(y) an antibody that comprises the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In a particular example, the antibody binds the same epitope as the MORAb-003 antibody. In another example, the antibody includes SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3. In another example, the antibody is the MOV18 antibody. In yet another example, the antibody binds the same epitope as the MOV18 antibody. In a further example, the antibody comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16). Alternatively or in combination, the antibody includes a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21). In certain examples, the antibody includes (i) the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); or the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In a particular example, the level of FRα in the sample derived from said subject is assessed by contacting the sample with a pair of antibodies selected from the group consisting of (a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody; (b) 9F3 antibody immobilized to a solid support and labeled 24F12 antibody; (c) 26B3 antibody immobilized to a solid support and labeled 19D4 antibody; and (d) 9F3 antibody immobilized to a solid support and labeled 26B3 antibody.

In certain examples, the antibody is selected from the group consisting of a murine antibody, a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, a Fab, Fab'2, ScFv, SMIP, affibody, avimer, versabody, nanobody, and a domain antibody. Alternatively, or in combination, the antibody is labeled, for example, with a label selected from the group consisting of a radio-label, a biotin-label, a chromophore-label, a fluorophore-label, or an enzyme-label.

In certain examples, the level of FRα is determined by using a technique selected from the group consisting of western blot analysis, radioimmunoassay, immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, solution phase assay, electrochemiluminescence immunoassay (ECLIA) and ELISA assay.

In various examples of the foregoing examples of the disclosure, the control sample is a standardized control level of FRα in a healthy subject.

In certain examples, the sample is treated with guanidine prior to determining the level of FRα in the sample. Alternatively or in combination, the sample is diluted prior to determining the level of FRα in the sample. Alternatively, or in combination, the sample is centrifuged, vortexed, or both, prior to determining the level of FRα in the sample.

In a further example, the present disclosure provides a method for treating a subject suffering from ovarian cancer or lung cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a sample derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the sample with the level of FRα in a control sample, wherein a difference between the levels of FRα in the sample derived from the subject and in the control sample is an indication that the subject will respond to treatment with MORAb-003; wherein the level of FRα in the sample derived from the subject is assessed by contacting the sample with (a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody, (b) 9F3 antibody immobilized to a solid support and labeled 24F12 antibody, (c) 26B3 antibody immobilized to a solid support and labeled 19D4 antibody, and (d) 9F3 antibody immobilized to a solid support and labeled 26B3 antibody. For example, the sample may be urine, serum, plasma or ascites.

In various examples of the foregoing examples of the disclosure, the MORAb-003 for treatment is (a) an antibody that comprises the heavy chain amino acid sequence

as set forth in SEQ ID NO:7 and the light chain amino acid sequence as set forth in SEQ ID NO:8; (b) an antibody that binds the same epitope as the MORAb-003 antibody; or (c) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3.

Kits of the Disclosure

In one example, the present disclosure provides a kit for assessing whether a subject is afflicted with an FRα-expressing cancer or for assessing the progression of an FRα-expressing cancer in a subject, the kit including means for determining the level of folate receptor alpha (FRα) which is not bound to a cell in a sample derived from the subject; and instructions for use of the kit to assess whether the subject is afflicted with an FRα-expressing cancer or to assess the progression of an FRα-expressing cancer. For example, the FRα-expressing cancer is selected from the group consisting of lung cancer, mesothelioma, ovarian cancer, renal cancer, brain cancer, cervical cancer, nasopharyngeal cancer, squamous cell carcinoma of the head and neck, endometrial cancer, breast cancer, bladder cancer, pancreatic cancer, bone cancer, pituitary cancer, colorectal cancer and medullary thyroid cancer. In a particular example, the FRα-expressing cancer is ovarian cancer. In yet another example, the FRα-expressing cancer is non-small cell lung cancer, for example, adenocarcinoma. In a further example, the sample is either urine, serum, plasma or ascites.

In a further example, the means includes a folate receptor alpha (FRα) binding agent, for example, an antibody. In a further example, the antibody is selected from the group consisting of

(a) an antibody that binds the same epitope as the MORAb-003 antibody;

(b) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3;

(c) the MOV18 antibody;

(d) an antibody that binds the same epitope as the MOV18 antibody;

(e) the 548908 antibody;

(f) an antibody that binds the same epitope as the 548908 antibody;

(g) the 6D398 antibody;

(h) an antibody that binds the same epitope as the 6D398 antibody;

(i) an antibody that binds the same epitope as the 26B3 antibody;

(j) an antibody comprising SEQ ID NO:55 (GYFMN) as CDRH1, SEQ ID NO:56 (RIFPYNGDTFYNQKFKG) as CDRH2, SEQ ID NO:57 (GTHYFDY) as CDRH3, SEQ ID NO:51 (RTSENIFSYLA) as CDRL1 , SEQ ID NO:52 (NAKTLAE) as CDRL2 and SEQ ID NO:53 (QHHYAFPWT) as CDRL3;

(k) the 26B3 antibody;

(l) an antibody that binds the same epitope as the 19D4 antibody;

(m) an antibody comprising SEQ ID NO:39 (HPYMH) as CDRH1, SEQ ID NO:40 (RIDPANGNTKYDPKFQG) as CDRH2, SEQ ID NO:41 (EEVADYTMDY) as CDRH3, SEQ ID NO:35 (RASESVDTYGNNFIH) as CDRL1, SEQ ID NO:36 (LASNLES) as CDRL2 and SEQ ID NO:37 (QQNNGDPWT) as CDRL3;

(n) the 19D4 antibody;

(o) an antibody that binds the same epitope as the 9F3 antibody;

(p) an antibody comprising SEQ ID NO:31 (SGYYWN) as CDRH1, SEQ ID NO:32 (YIKSDGSNNYNPSLKN) as CDRH2, SEQ ID NO:33 (EWKAMDY) as CDRH3, SEQ ID NO:27 (RASSTVSYSYLH) as CDRL1, SEQ ID NO:28 (GTSNLAS) as CDRL2 and SEQ ID NO:29 (QQYSGYPLT) as CDRL3;

(q) the 9F3 antibody;

(r) an antibody that binds the same epitope as the 24F12 antibody;

(s) an antibody comprising SEQ ID NO:47 (SYAMS) as CDRH1, SEQ ID NO:48 (EIGSGGSYTYYPDTVTG) as CDRH2, SEQ ID NO:49 (ETTAGYFDY) as CDRH3, SEQ ID NO:43 (SASQGINNFLN) as CDRL1, SEQ ID NO:44 (YTSSLHS) as CDRL2 and SEQ ID NO:45 (QHFSKLPWT) as CDRL3;

(t) the 24F12 antibody;

(u) an antibody that comprises a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13); LK26HuVKY (SEQ ID NO: 14); LK26HuVKPW (SEQ ID NO: 15); and LK26HuVKPW,Y (SEQ ID NO: 16);

(v) an antibody that comprises a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO:

18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21);

(w) an antibody that comprises the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16);

(x) an antibody that comprises the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16); and

(y) an antibody that comprises the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In certain examples, the antibody is labeled including, but not limited to, a radio-label, a biotin-label, a chromophore-label, a fluorophore-label, or an enzyme-label.

In yet another example, the kit includes a means for obtaining a sample from the subject.

The present disclosure is further illustrated by the following detailed description and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic depiction of an electrochemiluminescence immunoassay (ECLIA) method for assessing the FRα in urine as described in the Examples. MOV18 antibody attached to solid supports bound FRα in urine. The FRα was subsequently detected by binding to Ru-labeled MORAb-003 antibody.

Figure 2 shows the distribution of FRα levels in the urine of ovarian cancer subjects and normal control subjects as measured by ECLIA (see Example 1).

Figure 3 depicts the detection of FRα in urine of ovarian cancer patients (pale band on lane 1, clear band on lane 2) using immunoblotting (see Example 5).

Figure 4 shows the distribution of FRα levels in the urine of ovarian cancer subjects and normal control subjects as measured by ECLIA after the urine was treated with guanidine, as described in Example 7.

Figure 5 depicts an ROC curve showing the sensitivity and specificity of the ECLIA measurement of FRα levels in urine after the urine was treated with guanidine,

as described in Example 7. The area under the curve (AUC) measures the accuracy of the test in separating ovarian cancer from control subjects. A cutoff value (above which the test results were considered abnormal) of 9100 pg/mL was used.

Figure 6 shows the distribution of FRα levels in ovarian cancer (OC) and normal control subjects after correction for creatinine levels. There is a statistically significant difference between ovarian cancer patients and controls in creatinine-corrected levels of FRα ($p=0.007$) (see Example 8).

Figure 7 depicts an ROC analysis of creatinine-corrected FRα levels determined using electrochemiluminescence assay (ECLIA) of guanidine-treated urine samples (see Example 8).

Figure 8 is a schematic depiction of the enzyme immunoassay (EIA) method used for assessing the level of FRα (*i.e.*, FRα) in samples, as described in Example 9. MOV-18 served as the capture antibody, which bound FRα from biological fluids. The FRα was detected by binding to biotinylated MORAb-003, which was detected using avidin conjugated to horseradish peroxidase (avidin-HRP).

Figure 9 depicts results obtained for the measurement of FRα in serum using one- and two-step incubation procedures, as described in Example 9.

Figure 10 is a schematic depiction of the three different combinations of capture and detector antibodies that were used with the enzyme immunoassay (EIA) method for assessing the level of FRα in human plasma, as described in Example 11.

Figure 11 shows the plasma concentrations of FRα (pg/mL) for individual subjects determined using EIA with three combinations of capture and detector antibodies, as described in Example 11.

Figure 12 depicts the relationship between OD values and FRα concentrations (see Example 11).

Figure 13 shows the distribution of plasma FRα concentrations in subjects with ovarian cancer and normal control subjects as determined using EIA (see Example 12).

Figure 14 depicts the correlation between FRα plasma concentrations determined using EIA and ECLIA (see Example 12).

Figure 15 shows correlations between ECLIA measures of FRα levels in serum and urine. The correlation for lung cancer patients was $r=0.24$ (upper panel) and the correlation for ovarian cancer patients was $r=-0.76$ (lower panel) (see Example 13).

Figure 16 shows the correlation of serum versus plasma FRα levels for assays conducted using pair 1 (see Example 16).

Figure 17 shows the correlation of serum versus plasma FRα levels for assays conducted using pair 2 (see Example 16).

Figure 18 shows the correlation of serum FRα levels for assays conducted using pair 1 versus pair 2 (see Example 16).

Figure 19 shows the correlation of plasma FRα levels for assays conducted using pair 1 versus pair 2 (see Example 16).

Figure 20 shows the intraday correlation of serum FRα levels for assays conducted using pair 2 (Example 16).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based, at least in part, on the unexpected discovery that folate receptor alpha (FRα), not bound to a cell, is found at elevated levels in the body fluids, for example, urine or serum, of a subject having an FRα-expressing cancer such as lung or ovarian cancer as compared to a control sample. Moreover, the present invention is based, at least in part, on the identification of an immunological assay that exhibits the necessary sensitivity for assessing FRα levels in samples, where prior attempts to do so repeatedly failed. As a result, the present invention provides methods for diagnosing an FRα-expressing cancer by assessing levels of an FRα not bound to a cell in samples derived from the subject. Indeed, the present invention overcomes the challenges observed during prior attempts to develop an FRα based diagnostic assay for FRα-expressing cancers such as ovarian cancer by providing an immunological assay capable of accurately assessing levels of FRα not bound to a cell in samples.

Accordingly, methods and kits for assessing whether a subject has or is at risk for developing an FRα-expressing cancer and, further, for assessing the progression of FRα-expressing cancer are provided. In various embodiments, the methods involve the comparison of levels of FRα not bound to a cell in samples, for example, urine and serum, as compared to control levels, in assessing the presence, degree or risk of development of ovarian cancer in the subject. In particular embodiments, the methods involve the use of the MORAb-003 antibody, antibodies that bind the same epitope as the MORAb-003 antibody or antibodies having SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3

(HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3, in assessing the levels of FRα not bound to a cell in a sample, *e.g.*, urine or serum. Alternatively or in addition, the MOV18 antibody or an antibody that binds the same epitope of the MOV18 antibody, the 548908 antibody, an antibody that binds the same epitope of the 548908 antibody, the 6D398 antibody or an antibody that binds the same epitope of the 548908 antibody may be used in accordance with the methods of the present invention.

Various aspects of the invention are described in further detail in the following subsections:

I. Definitions

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

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.* to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, the term "subject" refer to human and non-human animals, including veterinary subjects. The term "non-human animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, mice, rabbits, sheep, dog, cat, horse, cow, chickens, amphibians, and reptiles. In a preferred embodiment, the subject is a human.

The terms "cancer" or "tumor" are well known in the art and refer to the presence, *e.g.*, in a subject, of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. Cancer cells are often in the form of a tumor, but such cells may exist alone within a subject, or may be non-tumorigenic cancer cells, such as leukemia cells. As used herein, the term "cancer" includes pre-malignant as well as malignant cancers.

As used herein, an "FRα-expressing cancer" includes any type of cancer characterized in that the cancer cells express FRα. In particular embodiments, the FRα expressing cancer includes cancerous conditions characterized in that the cancer cells are

capable of secreting, shedding, exporting or releasing FRα in such a manner that elevated levels of FRα are detectable in a biological sample from the subject. FRα-expressing cancers include, but are not limited to, lung cancer (*e.g.*, bronchioalveolar carcinomas, carcinoid tumors, and non-small cell lung cancers, such as adenocarcinomas); mesothelioma; ovarian cancer; renal cancer; brain cancer (*e.g.*, anaplastic ependymoma and cerebellar juvenile pilocytic astrocytoma); cervical cancer; nasopharyngeal cancer; mesodermally derived tumor; squamous cell carcinoma of the head and neck; endometrial cancer; endometrioid adenocarcinomas of the ovary, serous cystadenocarcinomas, breast cancer; bladder cancer; pancreatic cancer; bone cancer (*e.g.*, high-grade osteosarcoma); pituitary cancer (*e.g.*, pituitary adenoma). See *e.g.*, U.S. Patent No. 7,754,698; U.S. Patent Application No. 2005/0232919; WO 2009/132081; Bueno R *et al. J of Thoracic and Cardiovascular Surgery*, 121(2) : 225-233 (2001); Elkanat H & Ratnam M. Frontiers in Bioscience, 11, 506-519 (2006); Franklin, WA *et al. Int J Cancer*, Suppl 8: 89-95 (1994); Hartman L.C. *et al. Int J Cancer* 121: 938-942 (2007); Iwakiri S *et al. Annals of Surgical Oncology*, 15(3): 889-899; Weitman, SD *et al. Cancer Res* 52: 3396-3401 (1992); Saba N.F. *et al. Head Neck*, 31(4): 475-481 (2009); Yang R *et al. Clin Cancer Res* 13: 2557-2567 (2007). In a particular embodiment, the FRα-expressing cancer is ovarian cancer. In another embodiment, the FRα-expressing cancer is lung cancer such as non-small cell lung cancer. In other embodiments, the FRα-expressing cancer is colorectal cancer and medullary thyroid cancer.

As used herein, a subject who is "afflicted with an FRα-expressing cancer " is one who is clinically diagnosed with such a cancer by a qualified clinician (for example, by the methods of the present invention), or one who exhibits one or more signs or symptoms (for example, elevated levels of FRα in biological fluids) of such a cancer and is subsequently clinically diagnosed with such a cancer by a qualified clinician (for example, by the methods of the present invention). A non-human subject that serves as an animal model of FRα-expressing cancer may also fall within the scope of the term a subject "afflicted with an FRα-expressing cancer."

The term "ovarian cancer" refers to the art recognized disease and includes each of epithelial ovarian cancer (EOC; >90% of ovarian cancer in Western countries), germ cell tumors (*circa* 2-3% of ovarian cancer), and stromal ovarian cancer. Ovarian cancer is stratified into different groups based on the differentiation of the tumor tissue. In

grade I, the tumor tissue is well differentiated. In grade II, tumor tissue is moderately well differentiated. In grade III, the tumor tissue is poorly differentiated. This grade correlates with a less favorable prognosis than grades I and II.

Ovarian cancer is stratified into different stages based on the spread of the cancer. Stage I is generally confined within the capsule surrounding one (stage IA) or both (stage IB) ovaries, although in some stage I (*i.e.* stage IC) cancers, malignant cells may be detected in ascites, in peritoneal rinse fluid, or on the surface of the ovaries. Stage II involves extension or metastasis of the tumor from one or both ovaries to other pelvic structures. In stage IIA, the tumor extends or has metastasized to the uterus, the fallopian tubes, or both. Stage IIB involves extension of the tumor to the pelvis. Stage IIC is stage IIA or IIB in which malignant cells may be detected in ascites, in peritoneal rinse fluid, or on the surface of the ovaries. In stage III, the tumor comprises at least one malignant extension to the small bowel or the omentum, has formed extrapelvic peritoneal implants of microscopic (stage IIIA) or macroscopic (< 2 centimeter diameter, stage IIIB; > 2 centimeter diameter, stage IIIC) size, or has metastasized to a retroperitoneal or inguinal lymph node (an alternate indicator of stage IIIC). In stage IV, distant (*i.e.* non-peritoneal) metastases of the tumor can be detected.

The durations of the various stages of ovarian cancer are not presently known, but are believed to be at least about a year each (Richart *et al.*, 1969, *Am. J. Obstet. Gynecol.* 105:386). Prognosis declines with increasing stage designation. For example, 5-year survival rates for human subjects diagnosed with stage I, II, III, and IV ovarian cancer are 80%, 57%, 25%, and 8%, respectively.

Each of the foregoing types, groups and stages of ovarian cancer are encompassed by the term "ovarian cancer" as used herein.

As used herein, the term "lung cancer" refers to a disease in tissues of the lung involving uncontrolled cell growth, which, in some cases, leads to metastasis. Lung cancer is the most common cause of cancer-related death in men and women. The majority of primary lung cancers are carcinomas of the lung, derived from epithelial cells. The main types of lung cancer are small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC). In a particular embodiment, the FRα-expressing cancer is a non-small cell lung cancer.

Small cell lung cancer or small cell lung carcinoma (SCLC) is a malignant cancer of the lung, wherein the cancer cells have a flat shape and scanty cytoplasm;

therefore, SCLC is sometimes called "oat cell carcinoma." SCLC is generally more metastatic than NSCLC and is sometimes seen in combination with squamous cell carcinomas.

As used herein, the term "non-small cell lung cancer," also known as non-small cell lung carcinoma (NSCLC), refers to epithelial lung cancer other than small cell lung carcinoma (SCLC). There are three main sub-types: adenocarcinoma, squamous cell lung carcinoma, and large cell lung carcinoma. Other less common types of non-small cell lung cancer include pleomorphic, carcinoid tumor, salivary gland carcinoma, and unclassified carcinoma. Adenocarcinomas account for approximately 40% of lung cancers, and are the most common type of lung cancer in people who have never smoked. Squamous cell carcinomas account for about 25% of lung cancers. Squamous cell carcinoma of the lung is more common in men than in women and is even more highly correlated with a history of tobacco smoking than are other types of lung carcinoma. There are at least four variants (papillary, small cell, clear cell, and basaloid) of squamous cell carcinoma of the lung. Large cell lung carcinomas are a heterogeneous group of malignant neoplasms originating from transformed epithelial cells in the lung. Large cell lung carcinomas are carcinomas that lack light microscopic characteristics of small cell carcinoma, squamous cell carcinoma, or adenocarcinoma.

Different staging systems are used for SCLC and NSCLC. SCLC is categorized as limited disease confined to the ipsilateral hemithorax or as extensive disease with metastasis beyond the ipsilateral hemithorax.

NSCLC may be categorized using the tumor-nodes-metastasis (TNM) staging system. See Spira J & Ettinger, D.S. Multidisciplinary management of lung cancer, N Engl J Med, 350:382- (2004) (hereinafter Spira); Greene FL, Page DL, Fleming ID, Fritz AG, Balch CM, Haller DG, et al (eds). *AJCC Cancer Staging Manual.* 6th edition. New York: Springer-Verlag, 2002:167-77 (hereinafter Greene); Sobin LH, Wittekind CH (eds). International Union Against Cancer. *TNM classification of malignant tumours.* 6th edition. New York: Wiley-Liss (2002) (hereinafter Sobin). In addition, NSCLC is typically treated according to the stage of cancer determined by the following classification scheme (see http://www.cancer.gov/cancertopics/pdq/treatment/non-small-cell-lung/Patient/page2#Keypoint10).

In the *occult (hidden) stage*, cancer cells are found in sputum (mucus coughed up from the lungs), but no tumor can be found in the lung by imaging or bronchoscopy, or the tumor is too small to be checked.

In *stage 0 (carcinoma in situ)*, abnormal cells are found in the lining of the airways. These abnormal cells may become cancer and spread into nearby normal tissue. Stage 0 is also called carcinoma in situ.

Stage I, in which cancer has formed, is divided into stages IA and IB.

In *Stage IA*, the tumor is in the lung only and is 3 centimeters or smaller.

In *Stage IB*, the cancer has not spread to the lymph nodes and one or more of the following is true: (i) The tumor is larger than 3 centimeters but not larger than 5 centimeters; (ii) cancer has spread to the main bronchus and is at least 2 centimeters below where the trachea joins the bronchus; (iii) cancer has spread to the innermost layer of the membrane that covers the lung; (iv) part of the lung has collapsed or developed pneumonitis (inflammation of the lung) in the area where the trachea joins the bronchus.

In *Stage IIA*, cancer has spread to certain lymph nodes on the same side of the chest as the primary tumor; the cancer is (a) 5 cm or smaller, (b) has spread to the main bronchus, and/or (c) has spread to the innermost layer of the lung lining. OR, cancer has not spread to lymph nodes; the cancer is (d) larger than 5 cm but not larger than 7 cm, (e) has spread to the main bronchus, and/or (f) has spread to the innermost layer of the lung lining. Part of the lung may have collapsed or become inflamed. Stage IIA is divided into two sections depending on the size of the tumor, where the tumor is found, and whether there is cancer in the lymph nodes. In the first section, cancer has spread to lymph nodes on the same side of the chest as the tumor. The lymph nodes with cancer are within the lung or near the bronchus. Also, one or more of the following is true: (i) the tumor is not larger than 5 centimeters, (ii) cancer has spread to the main bronchus and is at least 2 centimeters below where the trachea joins the bronchus, (iii) cancer has spread to the innermost layer of the membrane that covers the lung, (iv) part of the lung has collapsed or developed pneumonitis (inflammation of the lung) in the area where the trachea joins the bronchus. In the second section, cancer has not spread to lymph nodes and one or more of the following is true: (i) the tumor is larger than 5 centimeters but not larger than 7 centimeters, (ii) cancer has spread to the main bronchus and is at least 2 centimeters below where the trachea joins the bronchus, (iii) cancer has spread to the

innermost layer of the membrane that covers the lung, (iv) part of the lung has collapsed or developed pneumonitis (inflammation of the lung) in the area where the trachea joins the bronchus.

In *Stage IIB*, cancer has spread to certain lymph nodes on the same side of the chest as the primary tumor; the cancer is (a) larger than 5 cm but not larger than 7 cm, (b) has spread to the main bronchus, and/or (c) has spread to the innermost layer of the lung lining. Part of the lung may have collapsed or become inflamed. Alternatively, (d) the cancer is larger than 7 cm; (e) has spread to the main bronchus, (f) the diaphragm, (g) the chest wall or the lining of the chest wall; and/or (h) has spread to the membrane around the heart. There may be one or more separate tumors in the same lobe of the lung; cancer may have spread to the nerve that controls the diaphragm; the whole lung may have collapsed or become inflamed. Stage IIB is divided into two sections depending on the size of the tumor, where the tumor is found, and whether there is cancer in the lymph nodes. In the first section, cancer has spread to nearby lymph nodes on the same side of the chest as the tumor. The lymph nodes with cancer are within the lung or near the bronchus. Also, one or more of the following is true: (i) the tumor is larger than 5 centimeters but not larger than 7 centimeters, (ii) cancer has spread to the main bronchus and is at least 2 centimeters below where the trachea joins the bronchus, (iii) cancer has spread to the innermost layer of the membrane that covers the lung, (iv) part of the lung has collapsed or developed pneumonitis (inflammation of the lung) in the area where the trachea joins the bronchus. In the second section, cancer has not spread to lymph nodes and one or more of the following is true: (i) the tumor is larger than 7 centimeters, (ii) cancer has spread to the main bronchus (and is less than 2 centimeters below where the trachea joins the bronchus), the chest wall, the diaphragm, or the nerve that controls the diaphragm, (iii) cancer has spread to the membrane around the heart or lining the chest wall, (iv) the whole lung has collapsed or developed pneumonitis (inflammation of the lung), (v) there are one or more separate tumors in the same lobe of the lung.

Stage IIIA is divided into three sections depending on the size of the tumor, where the tumor is found, and which lymph nodes have cancer (if any). In the first section of Stage IIIA, cancer has spread to lymph nodes on the same side of the chest as the tumor. The lymph nodes with cancer are near the sternum (chest bone) or where the bronchus enters the lung. Also, the tumor may be any size; part of the lung (where the

trachea joins the bronchus) or the whole lung may have collapsed or developed pneumonitis (inflammation of the lung); there may be one or more separate tumors in the same lobe of the lung; and cancer may have spread to any of the following: (i) main bronchus, but not the area where the trachea joins the bronchus, (ii) chest wall, (iii) diaphragm and the nerve that controls it, (iv) membrane around the lung or lining the chest wall, (iv) membrane around the heart. In the second section of Stage IIIA, cancer has spread to lymph nodes on the same side of the chest as the tumor. The lymph nodes with cancer are within the lung or near the bronchus. Also, the tumor may be any size; the whole lung may have collapsed or developed pneumonitis (inflammation of the lung); there may be one or more separate tumors in any of the lobes of the lung with cancer; and cancer may have spread to any of the following: (i) main bronchus, but not the area where the trachea joins the bronchus, (ii) chest wall, (iii) diaphragm and the nerve that controls it, (iv) membrane around the lung or lining the chest wall, (v) heart or the membrane around it, (vi) major blood vessels that lead to or from the heart, (vi) trachea, (vii) esophagus, (viii) nerve that controls the larynx (voice box), (ix) sternum (chest bone) or backbone, (x) carina (where the trachea joins the bronchi). In the third section of Stage IIIA, cancer has not spread to the lymph nodes and the tumor may be any size, and cancer has spread to any of the following: (i) heart, (ii) major blood vessels that lead to or from the heart, (iii) trachea, (iv) esophagus, (v) nerve that controls the larynx (voice box), (vi) sternum (chest bone) or backbone, (vi) carina (where the trachea joins the bronchi).

Stage IIIB is divided into two sections depending on the size of the tumor, where the tumor is found, and which lymph nodes have cancer. In the first section, cancer has spread to lymph nodes above the collarbone or to lymph nodes on the opposite side of the chest as the tumor; the tumor may be any size; part of the lung (where the trachea joins the bronchus) or the whole lung may have collapsed or developed pneumonitis (inflammation of the lung); there may be one or more separate tumors in any of the lobes of the lung with cancer; and cancer may have spread to any of the following: (i) main bronchus, (ii) chest wall, (iii) diaphragm and the nerve that controls it, (iv) membrane around the lung or lining the chest wall, (iv) heart or the membrane around it, (v) major blood vessels that lead to or from the heart, (vi) trachea, (vii) esophagus, (viii) nerve that controls the larynx (voice box), (ix) sternum (chest bone) or backbone, (x) carina (where the trachea joins the bronchi). In the second section of Stage IIIB, cancer has spread to

lymph nodes on the same side of the chest as the tumor; the lymph nodes with cancer are near the sternum (chest bone) or where the bronchus enters the lung; the tumor may be any size; there may be separate tumors in different lobes of the same lung; and cancer has spread to any of the following: (i) heart, (ii) major blood vessels that lead to or from the heart, (iii) trachea, (iv) esophagus, (v) nerve that controls the larynx (voice box), (vi) sternum (chest bone) or backbone, (vii) carina (where the trachea joins the bronchi).

In *Stage IV*, the tumor may be any size and cancer may have spread to lymph nodes. One or more of the following is true: there are one or more tumors in both lungs; cancer is found in fluid around the lungs or the heart; cancer has spread to other parts of the body, such as the brain, liver, adrenal glands, kidneys, or bone.

Accordingly, in various embodiments of the foregoing invention, the lung cancer may be stratified into any of the preceding stages (*e.g.*, occult, stage 0, stage IA, stage IB, stage IIA, stage IIB, stage IIIA, stage IIIB or stage IV) based on assessing of the levels of FRα not bound to a cell, such as a normal or cancerous cell, in a sample (for example, urine or serum) of a subject.

As used herein, the term "folate receptor alpha" (also referred to as FRα, FR-alpha, FOLR-1 or FOLR1) refers to the alpha isoform of the high affinity receptor for folate. Membrane bound FRα is attached to the cell surface by a glycosyl phosphatidylinositol (GPI) anchor), recycles between extracellular and endocytic compartments and is capable of transporting folate into the cell. FRα is expressed in a variety of epithelial tissues including those of the female reproductive tract, placenta, breast, kidney proximal tubules, choroid plexus, lung and salivary glands. Soluble forms of FRα may be derived by the action of proteases or phospholipase on membrane anchored folate receptors.

The consensus nucleotide and amino acid sequences for human FRα are set forth herein as SEQ ID NOs: 24 and 25, respectively. Variants, for example, naturally occurring allelic variants or sequences containing at least one amino acid substitution, are encompassed by the terms as used herein.

As used herein, the term "not bound to a cell" refers to FRα that is not attached to the cellular membrane of a cell, such as a cancerous cell. In a particular embodiment, the FRα not bound to a cell is unbound to any cell and is freely floating or solubilized in biological fluids, *e.g.*, urine or serum. For example, the FRα may be shed, secreted or

exported from normal or cancerous cells, for example, from the surface of cancerous cells, into biological fluids.

The "level" of folate receptor alpha not bound to a cell, as used herein, refers to the level of folate receptor alpha protein as determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), solution phase assay, immunoelectrophoresis, Western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, and electrochemiluminescence immunoassay (exemplified below), and the like. In a preferred embodiment, the level is determined using antibody-based techniques, as described in more detail herein.

It is generally preferable to immobilize either an antibody or binding protein specific for FRα not bound to a cell on a solid support for Western blots and immunofluorescence techniques. Suitable solid phase supports or carriers include any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.

One skilled in the art will know many other suitable carriers for binding antibody or antigen, and will be able to adapt such support for use with the present invention. For example, protein isolated from a subject sample (*e.g.*, urine or serum) can be run on a polyacrylamide gel electrophoresis and immobilized onto a solid phase support such as nitrocellulose. The support can then be washed with suitable buffers followed by treatment with the labeled antibody. The solid phase support can then be washed with the buffer a second time to remove unbound antibody. The amount of bound label on the solid support can then be detected by conventional means. Means of detecting proteins using electrophoretic techniques are well known to those of skill in the art (see generally, R. Scopes (1982) *Protein Purification*, Springer-Verlag, N.Y.; Deutscher, (1990) *Methods in Enzymology* Vol. 182: *Guide to Protein Purification*, Academic Press, Inc., N.Y.).

Other standard methods include immunoassay techniques which are well known to one of ordinary skill in the art and may be found in Principles And Practice Of Immunoassay, 2nd Edition, Price and Newman, eds., MacMillan (1997) and Antibodies, A Laboratory Manual, Harlow and Lane, eds., Cold Spring Harbor Laboratory, Ch. 9 (1988), each of which is incorporated herein by reference in its entirety.

Antibodies used in immunoassays to determine the level of expression of folate receptor alpha may be labeled with a detectable label. The term "labeled", with regard to the binding agent or antibody, is intended to encompass direct labeling of the binding agent or antibody by coupling (*i.e.*, physically linking) a detectable substance to the binding agent or antibody, as well as indirect labeling of the binding agent or antibody by reactivity with another reagent that is directly labeled. An example of indirect labeling includes detection of a primary antibody using a fluorescently labeled secondary antibody. In one embodiment, the antibody is labeled, *e.g.*, radio-labeled, chromophore-labeled, fluorophore-labeled, or enzyme-labeled. In another embodiment, the antibody is an antibody derivative (*e.g.*, an antibody conjugated with a substrate or with the protein or ligand of a protein-ligand pair (*e.g.*, biotin-streptavidin), or an antibody fragment (*e.g.*, a single-chain antibody, an isolated antibody hypervariable domain) which binds specifically with FRα not bound to a cell.

In one embodiment of the invention, proteomic methods, *e.g.*, mass spectrometry, are used. Mass spectrometry is an analytical technique that consists of ionizing chemical compounds to generate charged molecules (or fragments thereof) and measuring their mass-to-charge ratios. In a typical mass spectrometry procedure, a sample is obtained from a subject, loaded onto the mass spectrometry, and its components (*e.g.*, FRα) are ionized by different methods (*e.g.*, by impacting them with an electron beam), resulting in the formation of charged particles (ions). The mass-to-charge ratio of the particles is then calculated from the motion of the ions as they transit through electromagnetic fields.

For example, matrix-associated laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) or surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) which involves the application of a sample, such as urine or serum, to a protein-binding chip (Wright, G.L., Jr., *et al.* (2002) *Expert Rev Mol Diagn* 2:549; Li, J., *et al.* (2002) *Clin Chem* 48:1296; Laronga, C., *et al.* (2003) *Dis Markers* 19:229; Petricoin, E.F., *et al.* (2002) 359:572; Adam, B.L., *et al.*

(2002) *Cancer Res* 62:3609; Tolson, J., *et al.* (2004) *Lab Invest* 84:845; Xiao, Z., *et al.* (2001) *Cancer Res* 61:6029) can be used to determine the level of FRα.

Furthermore, *in vivo* techniques for determination of the level of FRα not bound to a cell include introducing into a subject a labeled antibody directed against FRα, which binds to and transforms FRα into a detectable molecule. The presence, level, or location of the detectable FRα not bound to a cell in a subject may be determined using standard imaging techniques.

The term "sample" as used herein refers to a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. In preferred embodiments the sample is a biological fluid containing FRα not bound to a cancerous cell. Biological fluids are typically liquids at physiological temperatures and may include naturally occurring fluids present in, withdrawn from, expressed or otherwise extracted from a subject or biological source. Certain biological fluids derive from particular tissues, organs or localized regions and certain other biological fluids may be more globally or systemically situated in a subject or biological source. Examples of biological fluids include blood, serum and serosal fluids, plasma, lymph, urine, cerebrospinal fluid, saliva, ocular fluids, cystic fluid, tear drops, feces, sputum, mucosal secretions of the secretory tissues and organs, vaginal secretions, gynecological fluids, ascites fluids such as those associated with non-solid tumors, fluids of the pleural, pericardial, peritoneal, abdominal and other body cavities, fluids collected by bronchial lavage and the like. In a particular embodiment, the sample is urine or serum. In another embodiment, the sample does not include ascites or is not an ascite sample. In another embodiment, the sample does not include peritoneal fluid or is not peritoneal fluid.

In one embodiment, the sample is removed from the subject. In another embodiment, the sample is present within the subject. Biological fluids may also include liquid solutions contacted with a subject or biological source, for example, cell and organ culture medium including cell or organ conditioned medium, lavage fluids and the like.

In some embodiments, only a portion of the sample is subjected to an assay for determining the level of FRα not bound to a cell, or various portions of the sample are subjected to various assays for determining the level of FRα not bound to a cell. Also, in many embodiments, the sample may be pre-treated by physical or chemical means

prior to the assay. For example, in embodiments discussed in more detail in the Examples section, samples, for example, urine samples, were subjected to centrifugation, dilution and/or treatment with a solubilizing substance (*e.g.*, guanidine treatment) prior to assaying the samples for FRα not bound to a cell. Such techniques serve to enhance the accuracy, reliability and reproducibility of the assays of the present invention.

The term "control sample," as used herein, refers to any clinically relevant control sample, including, for example, a sample from a healthy subject not afflicted with ovarian cancer, a sample from a subject having a less severe or slower progressing ovarian cancer than the subject to be assessed, a sample from a subject having some other type of cancer or disease, and the like. A control sample may include a sample derived from one or more subjects. A control sample may also be a sample made at an earlier timepoint from the subject to be assessed. For example, the control sample could be a sample taken from the subject to be assessed before the onset of the FRα expressing cancer such as lung or ovarian cancer, at an earlier stage of disease, or before the administration of treatment or of a portion of treatment. The control sample may also be a sample from an animal model, or from a tissue or cell lines derived from the animal model, of the FRα expressing cancer such as lung or ovarian cancer. The level of FRα not bound to a cell in a control sample that consists of a group of measurements may be determined based on any appropriate statistical measure, such as, for example, measures of central tendency including average, median, or modal values.

The term "control level" refers to an accepted or pre-determined level of FRα which is used to compare with the level of FRα in a sample derived from a subject. In one embodiment, the control level of FRα is based on the level of FRα not bound to a cell in sample(s) from a subject(s) having slow disease progression. In another embodiment, the control level of FRα not bound to a cell is based on the level in a sample from a subject(s) having rapid disease progression. In another embodiment, the control level of FRα is based on the level of FRα not bound to a cell in a sample(s) from an unaffected, *i.e.*, non-diseased, subject(s), *i.e.*, a subject who does not have an FRα expressing cancer such as lung or ovarian cancer. In yet another embodiment, the control level of FRα is based on the level of FRα not bound to a cell in a sample from a subject(s) prior to the administration of a therapy for ovarian cancer. In another embodiment, the control level of FRα is based on the level of FRα not bound to a cell in

a sample(s) from a subject(s) having an FRα expressing cancer such as lung or ovarian cancer that is not contacted with a test compound. In another embodiment, the control level of FRα is based on the level of FRα not bound to a cell in a sample(s) from a subject(s) not having an FRα expressing cancer such as lung or ovarian cancer that is contacted with a test compound. In one embodiment, the control level of FRα is based on the level of FRα not bound to a cell in a sample(s) from an animal model of an FRα expressing cancer such as lung or ovarian cancer, a cell, or a cell line derived from the animal model of an FRα expressing cancer such as lung or ovarian cancer.

In one embodiment, the control is a standardized control, such as, for example, a control which is predetermined using an average of the levels of FRα not bound to a cell from a population of subjects having no FRα expressing cancer such as lung or ovarian cancer. In still other embodiments of the invention, a control level of FRα is based on the level of FRα not bound to a cell in a non-cancerous sample(s) derived from the subject having an FRα expressing cancer such as lung or ovarian cancer. For example, when a laparotomy or other medical procedure reveals the presence of ovarian cancer in one portion of the ovaries, the control level of FRα may be determined using the non-affected portion of the ovaries, and this control level may be compared with the level of FRα in an affected portion of the ovaries. Similarly, when a biopsy or other medical procedure reveals the presence of a lung cancer in one portion of the lungs, the control level of FRα may be determined using the non-affected portion of the lungs, and this control level may be compared with the level of FRα in an affected portion of the lungs.

As used herein, "a difference" between the level of folate receptor alpha not bound to a cell in a sample from a subject (*i.e.*, a test sample) and the level of folate receptor alpha not bound to a cell in a control sample refers broadly to any clinically relevant and/or statistically significant difference in the level of folate receptor alpha in the two samples. In an exemplary embodiment, the difference is selected based on a cutoff value determined using a receiver operating characteristic (ROC) analysis, an example of which is presented in Example 6.

In other embodiments, the difference must be greater than the limits of detection of the method for determining the level of FRα not bound to a cell. It is preferred that the difference be at least greater than the standard error of the assessment method, and preferably a difference of at least about 2-, about 3-, about 4-, about 5-, about 6-, about 7-, about 8-, about 9-, about 10-, about 15-, about 20-, about 25-, about 100-, about 500-,

about 1000-fold or greater than the standard error of the assessment method. The difference may be assessed by any appropriate comparison, including any appropriate parametric or nonparametric descriptive statistic or comparison. For example, "an increase" in the level of FRα not bound to a cell may refer to a level in a test sample that is about two, and more preferably about three, about four, about five, about six, about seven, about eight, about nine, about ten or more times more than the level of FRα in the control sample. An increase may also refer to a level in a test sample that is preferably at least about 1.5, and more preferably about two, about three, about four, about five or more standard deviations above the average level of FRα in the control sample. Likewise, "a decrease" in the level of FRα not bound to a cell may refer to a level in a test sample that is preferably at least about two, and more preferably about three, about four, about five, about six, about seven, about eight, about nine, about ten or more times less than the level of FRα in the control sample. A decrease may also refer to a level in a test sample that is preferably at least about 1.5, and more preferably about two, about three, about four, about five or more standard deviations below the average level of FRα in the control sample.

As used herein, the term "contacting the sample" with an FRα binding agent, *e.g.*, an anti-FRα antibody, includes exposing the sample, or any portion thereof with the agent or antibody, such that at least a portion of the sample comes into contact with the agent or antibody. The sample or portion thereof may be altered in some way, such as by subjecting it to physical or chemical treatments (*e.g.*, dilution or guanidine treatment), prior to the act of contacting it with the agent or antibody.

The term "antibody" as used herein, comprises four polypeptide chains, two heavy (H) chains and two light (L) chains, interconnected by disulfide bonds, as well as any functional (*i.e.*, antigen-binding) fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art, and include molecules such as Fab fragments, Fab' fragments, $F(ab')_2$ fragments, Fd fragments, Fabc fragments, Sc antibodies (single chain antibodies), diabodies, individual antibody light chains, individual antibody heavy chains, chimeric fusions between antibody chains and the like. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass.

Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively.

The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

The term "antigen-binding portion" of an antibody, as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, FRα not bound to a cell). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and CH1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb including VH and VL domains; (vi) a dAb fragment (Ward *et al.* (1989) *Nature* 341, 544-546), which consists of a V_H domain; (vii) a dAb which consists of a VH or a VL domain; and (viii) an isolated complementarity determining region (CDR) or (ix) a combination of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined,

using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242, 423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85, 5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Antigen-binding portions can be produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins.

The term "antibody", as used herein, includes polyclonal antibodies, monoclonal antibodies, murine antibodies, chimeric antibodies, humanized antibodies, and human antibodies, and those that occur naturally or are recombinantly produced according to methods well known in the art.

In one embodiment, an antibody for use in the methods of the invention is a bispecific antibody. A "bispecific antibody" is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, *e.g.*, Songsivilai & Lachmann, (1990) *Clin. Exp. Immunol.* 79, 315-321; Kostelny *et al.* (1992) *J. Immunol.* 148, 1547-1553.

In another embodiment, an antibody for use in the methods of the invention is a camelid antibody as described in, for example, PCT Publication WO 94/04678, the entire contents of which are incorporated herein by reference.

A region of the camelid antibody that is the small, single variable domain identified as V_{HH} can be obtained by genetic engineering to yield a small protein having high affinity for a target, resulting in a low molecular weight, antibody-derived protein known as a "camelid nanobody". See U.S. Pat. No. 5,759,808; see also Stijlemans *et al.*, 2004 J. Biol. Chem. 279: 1256-1261; Dumoulin *et al.*, 2003 Nature 424: 783-788; Pleschberger *et al.*, 2003 Bioconjugate Chem. 14: 440-448; Cortez-Retamozo *et al.*, 2002 Int. J. Cancer 89: 456-62; and Lauwereys *et al.*, 1998 EMBO J. 17: 3512-3520. Engineered libraries of camelid antibodies and antibody fragments are commercially available, for example, from Ablynx, Ghent, Belgium. Accordingly, a feature of the present invention is a camelid nanobody having high affinity for FRα.

In other embodiments of the invention, an antibody for use in the methods of the invention is a diabody, a single chain diabody, or a di-diabody.

Diabodies are bivalent, bispecific molecules in which V_H and V_L domains are expressed on a single polypeptide chain, connected by a linker that is too short to allow for pairing between the two domains on the same chain. The V_H and V_L domains pair with complementary domains of another chain, thereby creating two antigen binding sites (see *e.g.*, Holliger *et al.*, 1993 Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak *et al.*, 1994 Structure 2:1121-1123). Diabodies can be produced by expressing two polypeptide chains with either the structure V_{HA}-V_{LB} and V_{HB}-V_{LA} (V_H-V_L configuration), or V_{LA}-V_{HB} and V_{LB}-V_{HA} (V_L-V_H configuration) within the same cell. Most of them can be expressed in soluble form in bacteria.

Single chain diabodies (scDb) are produced by connecting the two diabody-forming polypeptide chains with linker of approximately 15 amino acid residues (see Holliger and Winter, 1997 Cancer Immunol. Immunother., 45(3-4):128-30; Wu *et al.*, 1996 Immunotechnology, 2(1):21-36). scDb can be expressed in bacteria in soluble, active monomeric form (see Holliger and Winter, 1997 Cancer Immunol. Immunother., 45(34): 128-30; Wu *et al.*, 1996 Immunotechnology, 2(1):21-36; Pluckthun and Pack, 1997 Immunotechnology, 3(2): 83-105; Ridgway *et al.*, 1996 Protein Eng., 9(7):617-21).

A diabody can be fused to Fc to generate a "di-diabody" (see Lu *et al.*, 2004 J. Biol. Chem., 279(4):2856-65).

FRα binding molecules that exhibit functional properties of antibodies but derive their framework and antigen binding portions from other polypeptides (*e.g.*, polypeptides other than those encoded by antibody genes or generated by the recombination of antibody genes *in vivo*) may also be used in the methods of the present invention. The antigen binding domains (*e.g.*, FRα binding domains) of these binding molecules are generated through a directed evolution process. See U.S. Pat. No. 7,115,396. Molecules that have an overall fold similar to that of a variable domain of an antibody (an "immunoglobulin-like" fold) are appropriate scaffold proteins. Scaffold proteins suitable for deriving antigen binding molecules include fibronectin or a fibronectin dimer, tenascin, N-cadherin, E-cadherin, ICAM, titin, GCSF-receptor, cytokine receptor, glycosidase inhibitor, antibiotic chromoprotein, myelin membrane adhesion molecule P0, CD8, CD4, CD2, class I MHC, T-cell antigen receptor, CD1, C2 and I-set domains of VCAM-1, I-set immunoglobulin domain of myosin-binding protein

C, I-set immunoglobulin domain of myosin-binding protein H, I-set immunoglobulin domain of telokin, NCAM, twitchin, neuroglian, growth hormone receptor, erythropoietin receptor, prolactin receptor, interferon-gamma receptor, β-galactosidase/glucuronidase, β-glucuronidase, transglutaminase, T-cell antigen receptor, superoxide dismutase, tissue factor domain, cytochrome F, green fluorescent protein, GroEL, and thaumatin.

"Specific binding" when used in the context of antibodies, or antibody fragments, represents binding via domains encoded by immunoglobulin genes or fragments of immunoglobulin genes to one or more epitopes of a protein of interest, but which do not substantially recognize and bind other molecules in a sample containing a mixed population of antigenic molecules. Typically, an antibody binds to a cognate antigen with a Kd of less than about $1x10^{-8}$ M, as measured by a surface plasmon resonance assay or a cell binding assay.

As used herein, a folate receptor alpha "binding agent" includes an antibody that binds FRα not bound to a cell as well as non-antibody binding agents. To generate non-antibody binding agents or binding molecules, a library of clones can be created in which sequences in regions of the scaffold protein that form antigen binding surfaces (*e.g.*, regions analogous in position and structure to CDRs of an antibody variable domain immunoglobulin fold) are randomized. Library clones are tested for specific binding to the antigen of interest (*e.g.*, FRα) and for other functions (*e.g.*, inhibition of biological activity of FRα). Selected clones can be used as the basis for further randomization and selection to produce derivatives of higher affinity for the antigen.

High affinity binding molecules are generated, for example, using the tenth module of fibronectin III (^{10}Fn3) as the scaffold, described in U.S. Pat. Nos. 6,818,418 and 7,115,396; Roberts and Szostak, 1997 Proc. Natl. Acad. Sci USA 94:12297; U.S. Pat. No. 6,261,804; U.S. Pat. No. 6,258,558; and Szostak *et al.* WO98/31700, the entire contents of each of which are incorporated herein by reference.

Non-antibody binding molecules can be produced as dimers or multimers to increase avidity for the target antigen. For example, the antigen binding domain is expressed as a fusion with a constant region (Fc) of an antibody that forms Fc-Fc dimers. See, *e.g.*, U.S. Pat. No. 7,115,396, the entire contents of which are incorporated herein by reference.

An "antigen" is a molecule recognized by the immune system; the term originally came from "antibody generator" and includes a molecule that binds specifically to an antibody. At the molecular level, an antigen is characterized by its ability to be bound at the antigen-binding site of an antibody. In the present invention, the antigen is FRα, such as FRα which is not bound to a cell FRα or a portion thereof.

As used herein, the term "epitope" refers to the molecular surface features of an antigen, *e.g.*, FRα, capable of being bound by an antibody. Antigenic molecules, normally being "large" biological polymers, usually present several surface features that can act as points of interaction for specific antibodies. Any such distinct molecular feature constitutes an epitope. Most antigens therefore have the potential to be bound by several distinct antibodies, each of which is typically specific to a particular epitope. In one embodiment of the present invention, a binding agent, *e.g.*, antibody, binds to an epitope on FRα which is available in the form of the receptor which is not bound to a cell but not in the membrane bound form of the receptor. For example, the antibody may bind to the same epitope on FRα to which MORAB-003 binds.

As used herein, the phrase "progression of an FRα-expressing cancer in a subject afflicted with an FRα-expressing cancer" includes the progression of such a cancer from a less severe to a more severe state. This could include an increase in the number or severity of tumors, the degree of metastasis, the speed with which the cancer is growing and spreading, and the like. For example, "the progression of ovarian cancer" includes the progression of such a cancer from a less severe to a more severe state, such as the progression from Stage I to Stage II, from Stage II to Stage III, etc. Alternatively, the phrase "progression of an FRα-expressing cancer in a subject afflicted with an FRα-expressing cancer" may refer to the regression of an FRα-expressing cancer from a more severe state to a less severe state. For example, in one embodiment, "the progression of ovarian cancer" refers to the regression from Stage IV to Stage III, from Stage III to Stage II, etc. In other embodiments, the "progression of an FRα-expressing cancer in a subject afflicted with an FRα-expressing cancer" may refer to the survival rate determined from the beginning of symptoms of the FRα-expressing cancer, or to the survival rate from the time of diagnosis of the FRα-expressing cancer.

As used herein, the term "stratifying" refers to characterizing an FRα expressing cancer, for example, ovarian or lung cancer, into an appropriate stage based, for example, on the degree of the spread of the cancer, as well accepted stratifications in the

art. For example, stratifying includes characterizing the FRα expressing cancer into Stage I, Stage II, Stage III or Stage IV. In certain embodiments, Stage I refers to cancers that are localized to one part of the body. In certain embodiments, Stages II and III refer to cancers that are locally advanced, wherein a distinction between the stages are often specific to the particular cancer. Finally, Stage IV refers to cancers that have often metastasized, or spread to other organs or throughout the body.

As used herein, the term "survival" refers to the continuation of life of a subject which has been treated for cancer. In one embodiment, survival refers to the failure of a tumor to recur. As used herein, the term "recur" refers to the re-growth of tumor or cancerous cells in a subject in whom primary treatment for the tumor has been administered. The tumor may recur in the original site or in another part of the body. In one embodiment, a tumor that recurs is of the same type as the original tumor for which the subject was treated. For example, if a subject had an ovarian cancer tumor, was treated and subsequently developed another ovarian cancer tumor, the tumor has recurred. In addition, a cancer can recur in a different organ or tissue than the one where it originally occurred.

II. Methods and Kits of the Invention

The present invention is based, at least in part, on the unexpected discovery that folate receptor alpha (FRα), not bound to a cell, is found at elevated levels in the body fluids, for example, urine or serum, of a subject having an FRα-expressing cancer as compared to a control sample. Moreover, the present invention is based, at least in part, on the identification of an immunological assay that exhibits the necessary sensitivity for assessing levels of FRα not bound to a cells in samples, where prior attempts to do so had repeatedly failed. Indeed, the present invention overcomes the challenges observed during prior attempts to develop an FRα-based diagnostic assay for FRα-expressing cancer such as lung or ovarian cancer by providing an immunological assay capable of accurately assessing levels of FRα not bound to a cell in a sample, *e.g.*, urine or serum.

Accordingly, methods and kits for assessing whether a subject has or is at risk for developing an FRα-expressing cancer and, further, for assessing the progression of an FRα-expressing cancer are provided. In various embodiments, the methods involve the comparison of levels of FRα not bound to a cell in samples, for example, urine and

serum, as compared to control levels, in assessing the presence, degree or risk of development of ovarian cancer in the subject.

A. Diagnostic Methods, Prognostic Methods, Risk Assessment Methods, and Stratification Methods

Specifically, the present invention provides diagnostic methods for assessing whether a subject is afflicted with an FRα-expressing cancer, such as lung or ovarian cancer, prognostic methods for predicting the progression of an FRα-expressing cancer such as lung or ovarian cancer, and risk assessment methods for assessing the level of risk that a subject will develop the FRα-expressing cancer. Furthermore, the invention provides stratification methods for stratifying an FRα-expressing cancer such as lung or ovarian cancer subject into cancer therapy groups. The various aspects and embodiments of the invention discussed here are intended to be non-limiting and to encompass all possible combinations of the specific embodiments mentioned, which may apply to any of the methods and kits discussed herein or claimed below.

The methods of the present invention can be practiced in conjunction with any other method used by the skilled practitioner to diagnose an FRα-expressing cancer, predict the progression of an FRα-expressing cancer, or to assess the level of risk that a subject will develop an FRα-expressing cancer.

In one aspect, the invention provides a method of assessing whether a subject is afflicted with an FRα-expressing cancer, by determining the level of folate receptor alpha (FRα) which is not bound to a cell, in a sample (such as urine or serum) derived from the subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell with the level of FRα in a control sample, wherein a difference between the level of FRα in the sample derived from the subject and the level of FRα in the control sample is an indication that the subject is afflicted with an FRα-expressing cancer. In a particular embodiment, the level of FRα in the sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα not bound to a cell and is selected from the group consisting of (a) an antibody that binds the same epitope as the MORAb-003 antibody; and (b) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT)

as CDRL3. In one embodiment, the sample is selected from the group consisting of urine and serum.

In another aspect, the present invention provides a method of assessing whether a subject is afflicted with an FRα-expressing cancer such as lung cancer or ovarian cancer, the method comprising determining the level of folate receptor alpha (FRα) not bound to a cell in a urine sample derived from the subject; and comparing the level of folate receptor alpha (FRα) in the urine sample derived from the subject with the level of FRα in a control sample, wherein a difference between the level of FRα in the urine sample derived from the subject and the level of FRα in the control sample is an indication that the subject is afflicted with an FRα-expressing cancer.

In another aspect, the invention provides a method of assessing whether a subject is afflicted with an FRα-expressing cancer, by determining the level of folate receptor alpha (FRα) not bound to a cell in a serum sample derived from the subject; and comparing the level of folate receptor alpha (FRα) in the serum sample derived from the subject with the level of FRα in a control sample, wherein a difference between the level of FRα in the serum sample derived from the subject and the level of FRα in the control sample is an indication that the subject is afflicted with an FRα-expressing cancer. In particular embodiments, the subject has not been treated with an agent, such as a steroid, that enhances the levels of FRα in serum. In a specific embodiment, the FRα-expressing cancer is ovarian cancer and the subject has not been treated with an agent, such as a steroid, that enhances the levels of FRα in serum.

In the methods and kits of the present invention, FRα-expressing cancers include cancers characterized in that the cancer cells express FRα. In particular embodiments, the FRα is released from the cancer cells, for example, from the surface of the cancer cell, and into the biological fluids of the subject. FRα-expressing cancers include lung cancer (*e.g.*, bronchioalveolar carcinomas, carcinoid tumors, and non-small cell lung cancers, such as adenocarcinomas); mesothelioma; ovarian cancer; renal cancer; brain cancer (*e.g.*, anaplastic ependymoma and cerebellar juvenile pilocytic astrocytoma); cervical cancer; nasopharyngeal cancer; mesodermally derived tumor; squamous cell carcinoma of the head and neck; endometrial cancer; endometrioid adenocarcinomas of the ovary, serous cystadenocarcinomas, breast cancer; bladder cancer; pancreatic cancer; bone cancer (*e.g.*, high-grade osteosarcoma); and pituitary cancer (*e.g.*, pituitary adenoma). In a particular embodiment, the FRα-expressing cancer is ovarian cancer.

In certain embodiments of the methods and kits of the present invention, the FRα-expressing cancer is lung cancer. In more specific embodiments, the lung cancer is non-small cell lung carcinoma (NSCLC). In one such embodiment, the NSCLC is selected from the group consisting of adenocarcinoma, squamous cell lung carcinoma, large cell lung carcinoma, pleomorphic NSCLC, carcinoid tumor, salivary gland carcinoma, and unclassified carcinoma. In a preferred embodiment, the NSCLC is adenocarcinoma. In alternative embodiments, the lung cancer is small cell lung carcinoma (SCLC). In another embodiment, the lung cancer is bronchioalveolar carcinoma. In yet another embodiment, the lung cancer is a lung carcinoid tumor.

The present invention also provides methods to assess whether a subject is afflicted with ovarian cancer by determining the level of folate receptor alpha (FRα) not bound to a cell in a urine sample derived from the subject, wherein the presence of FRα in the urine sample at a concentration of greater than about 3000 a.u./ml is an indication that the subject is afflicted with ovarian cancer. In particular embodiments, the presence of FRα in the urine sample at a concentration of greater than about 4000 a.u./ml, about 5000 a.u./ml, about 6000 a.u./ml, about 7000 a.u./ml, about 8000 a.u./ml, about 9000 a.u./ml, about 10,000 a.u./ml, about 11,000 a.u./ml, about 12,000 a.u./ml, about 13,000 a.u./ml, about 14,000 a.u./ml, about 15,000 a.u./ml, about 16,000 a.u./ml, about 17,000 a.u./ml, about 18,000 a.u./ml, about 19,000 a.u./ml, about 20,000 a.u./ml, about 21,000 a.u./ml, about 22,000 a.u./ml, about 23,000 a.u./ml, about 24,000 a.u./ml, about 25,000 a.u./ml, about 26,000 a.u./ml, about 27,000 a.u./ml, about 28,000 a.u./ml, about 29,000 a.u./ml or about 30,000 a.u./ml is an indication that the subject is afflicted with ovarian cancer.

In yet another aspect, the present invention provides a method of assessing whether a subject is afflicted with ovarian cancer, by determining the level of folate receptor alpha (FRα) in a urine sample derived from the subject, wherein the presence of FRα in the urine sample at a concentration of greater than about 9100 pg/ml is an indication that the subject is afflicted with ovarian cancer or wherein a concentration of less than about 9100 pg/ml is an indication that the subject is not afflicted with ovarian cancer. For example, the presence of FRα in the urine sample at a concentration of greater than about 9500 pg/mL, about 10,000 pg/mL, about 11,000 pg/mL, about 12,000 pg/mL, about 13,000 pg/mL, about 14,000 pg/mL, about 15,000 pg/mL, about 16,000 pg/mL, about 17,000 pg/mL, about 18,000 pg/mL, about 19,000 pg/mL, about 20,000

pg/mL, about 21,000 pg/mL, about 22,000 pg/mL, about 23,000 pg/mL, about 24,000 pg/mL, about 25,000 pg/mL, about 26,000 pg/mL, about 27,000 pg/mL, about 28,000 pg/mL, about 29,000 pg/mL, about 30,000 pg/mL, about 40,000 pg/mL, about 50,000 pg/mL, about 60,000 pg/mL, about 70,000 pg/mL, about 80,000 pg/ml, about 90,000 pg/ml, about 100,000 pg/ml or about 150,000 pg/ml is an indication that the subject is afflicted with ovarian cancer.

In certain embodiments of the foregoing aspects of the invention, the levels of FRα not bound to a cell in a sample (*e.g.*, a sample such as a urine sample or serum sample) derived from a subject are compared with the levels of FRα in a control sample, wherein a difference between the levels is an indication that the subject is afflicted with an FRα-expressing cancer such as lung or ovarian cancer. In a particular embodiment, the difference constitutes an increase in the level of FRα not bound to a cell in the sample derived from the subject as compared with the level of FRα in the control sample, wherein this increase is indicative of the presence or growth of FRα-expressing cancer. Alternatively, the difference constitutes a decrease in the level of FRα, wherein the decrease is indicative of the absence or regression of FRα-expressing cancer. As used herein, "a difference" between the level of folate receptor alpha not bound to a cell in a sample from a subject (*i.e.*, a test sample) and the level of folate receptor alpha in a control sample refers broadly to any clinically relevant change (including an increase or a decrease) and/or statistically significant difference in the level of folate receptor alpha in the two samples. In an exemplary embodiment, the difference is selected based on a cutoff value determined using a receiver operating characteristic (ROC) analysis, an example of which is presented in Example 6. The optimal cutoff value may vary depending on the assay methods and conditions employed. In other embodiments, the difference must be greater than the limits of detection of the method for determining the level of FRα not bound to a cell. It is preferred that the difference be at least greater than the standard error of the assessment method, and preferably a difference of at least about 2-, about 3-, about 4-, about 5-, about 6-, about 7-, about 8-, about 9-, about 10-, about 15-, about 20-, about 25-, about 100-, about 500-, about 1000-fold or greater than the standard error of the assessment method. The difference may be assessed by any appropriate comparison, including any appropriate parametric or nonparametric descriptive statistic or comparison. For example, "an increase" in the level of FRα may refer to a level that exceeds a cutoff value determined using an ROC analysis. It may

also refer to a level in a test sample that is two, and more preferably about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 150%, about 200%, about 300%, about 400%, about 500%, about 600%, about 700%, about 800%, about 900% or about 1000% more than the level of FRα in the control sample. An increase may also refer to a level in a test sample that is preferably at least about 1.5, and more preferably about two, about three, about four, about five or more standard deviations above the average level of FRα in the control sample. Likewise, "a decrease" in the level of FRα not bound to a cell may refer to a level in a test sample that does not exceed a cutoff value determined using an ROC analysis. It may also refer to a level in a test sample that is about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% less than the level of FRα in the control sample. A decrease may also refer to a level in a test sample that is preferably at least about 1.5, and more preferably about two, about three, about four, about five or more standard deviations below the average level of FRα in the control sample.

Samples useful in the methods and kits of the invention include any tissue, cell, biopsy, or bodily fluid that may contain detectable levels of FRα not bound to a cell. In one embodiment, a sample may be a tissue, a cell, whole blood, plasma, buccal scrape, saliva, cerebrospinal fluid, stool, or bronchoalveolar lavage. In some embodiments, the sample is FRα-expressing tumor sample or a sample of tissues or cells where FRα-expressing cancer may be found. In preferred embodiments, the sample is a urine or serum sample.

Body samples may be obtained from a subject by a variety of techniques known in the art including, for example, by the use of a biopsy or by scraping or swabbing an area or by using a needle to aspirate bodily fluids. Methods for collecting various body samples are well known in the art.

Samples suitable for detecting and quantitating the FRα protein level may be fresh, frozen, or fixed according to methods known to one of skill in the art. Suitable tissue samples are preferably sectioned and placed on a microscope slide for further analyses. Solid samples, *i.e.*, tissue samples, may be solubilized and/or homogenized and subsequently analyzed as soluble extracts. Liquid samples may also be subjected to physical or chemical treatments. In some embodiments, urine samples are treated by

centrifugation, vortexing, dilution and/or treatment with a solubilizing substance (such as, for example, guanidine treatment).

In one embodiment, a freshly obtained biopsy sample is frozen using, for example, liquid nitrogen or difluorodichloromethane. The frozen sample is mounted for sectioning using, for example, OCT, and serially sectioned in a cryostat. The serial sections are collected on a glass microscope slide. For immunohistochemical staining the slides may be coated with, for example, chrome-alum, gelatine or poly-L-lysine to ensure that the sections stick to the slides. In another embodiment, samples are fixed and embedded prior to sectioning. For example, a tissue sample may be fixed in, for example, formalin, serially dehydrated and embedded in, for example, paraffin.

Once the sample is obtained, any method known in the art to be suitable for detecting and quantitating FRα not bound to a cell may be used (either at the nucleic acid or, preferably, at the protein level), as described in section (B) below. Exemplary methods are well known in the art and include but are not limited to western blots, northern blots, southern blots, immunohistochemistry, solution phase assay, ELISA, *e.g.*, amplified ELISA, immunoprecipitation, immunofluorescence, flow cytometry, immunocytochemistry, mass spectrometrometric analyses, *e.g.*, MALDI-TOF and SELDI-TOF, nucleic acid hybridization techniques, nucleic acid reverse transcription methods, and nucleic acid amplification methods.

In many embodiments, the level of FRα not bound to a cell in the sample (such as, for example, urine or serum) is assessed by contacting the sample with an antibody that binds FRα. Antibodies that bind FRα are known in the art and include (i) the murine monoclonal LK26 antibody (the heavy and light chains thereof are presented herein as SEQ ID NOs: 22 and 23), as described in European Patent Application No. 86104170.5 (Rettig) (the entire contents of which are incorporated herein by reference); (ii) the MORAB-003 antibody, as described in International Publication No. WO2004/113388 and U.S. Patent No. 5,646,253, the entire contents of each of which are incorporated herein by reference. The monoclonal antibodies MOV18 and MOv19 also bind different epitopes on the FRα molecule (previously known as gp38/FBP). Miotti, S. *et al. Int J Cancer*, 38: 297-303 (1987). For example, the MOV18 antibody binds the epitope set forth herein as SEQ ID NO:26 (TELLNVXMNAK*XKEKPXPX*KLXXQX) (note that at position 12, a tryptophan or

histidine residue is possible, and at position 21, an aspartic acid or glutamic acid residue is possible), as taught in Coney *et al. Cancer Res*, 51: 6125-6132 (1991).

As used herein, the term "MORAb-003" refers to an antibody that specifically binds FRα and which comprises the mature heavy chain amino acid sequence as set forth in SEQ ID NO:7 and the mature light chain sequence of SEQ ID NO:8. The corresponding pre-protein amino acid sequences for MORAb-003 are set forth in SEQ ID NOs: 9 (heavy chain) and 10 (light chain). The MORAb-003 antibody comprises the following CDRs: SEQ ID NO:1 as CDRH1, SEQ ID NO:2 as CDRH2, SEQ ID NO:3 as CDRH3, SEQ ID NO:4 as CDRL1, SEQ ID NO:5 as CDRL2, and SEQ ID NO:6 as CDRL3. MORAb-003 antibody producing cells have been deposited with the American Type Culture Collection (10801 University Blvd., Manassas, Virginia 20110-2209) on April 24, 2006 and have been assigned Accession No. PTA-7552.

Other antibodies that bind FRα and for use in the methods of the present invention include 9F3.H9.H3.H3.B5.G2 (also referred to as 9F3),19D4.B7 (also referred to as 19D4), 24F12.B1 (also referred to as 24F12), and 26B3.F2 (also referred to as 26B3). The amino acid sequences of these antibodies, their CDRs, and their heavy and light chain variable domains, as well as polynucleotide sequences that may encode them, are provided in Table 33. In some embodiments, these antibodies are murine IgG, or derivatives thereof. In other embodiments, the antibodies are human, humanized, or chimeric.

9F3

In some embodiments, the antibody that binds FRα is an antibody or antigen-binding fragment that includes a light chain CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:27. In some embodiments, the antibody that binds FRα includes a light chain CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:28. In some embodiments, the antibody that binds FRα includes a light chain CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO 29. In some embodiments, the antibody that binds FRα includes a heavy chain CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO 31. In some embodiments, the antibody that binds FRα includes a heavy chain CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:32. In some embodiments, the antibody that binds FRα includes a heavy chain CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:33. The antibody

that binds FRα may include a light chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:27; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:28; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:29. The antibody that binds FRα may include a heavy chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:31; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 32; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:33. The antibody that binds FRα may include a light chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:27; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:28; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:29, and also have a heavy chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:31; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:32; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:33.

The antibody that binds FRα may include a light chain variable domain that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:30. The antibody that binds FRα may include a heavy chain variable domain that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:34. The antibody that binds FRα may include a light and a heavy chain variable domains, wherein the light chain variable domain includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:30, and the heavy chain variable domain includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:34. In some embodiments the antibody that binds FRα is the 9F3.H9.H3.H3.B5.G2 (9F3) antibody or an antigen-binding fragment thereof, capable of binding either a native or nonreduced form of FRα. In some embodiments, the antibody has a murine IgG2a constant region.

In some embodiments, the antibody that binds FRα is an antibody that is produced by antibody-producing cells deposited with the American Type Culture Collection (10801 University Blvd., Manassas, Virginia 20110-2209) on May 19, 2011 and have been assigned Accession No. PTA-11887. In some embodiments, the antibody that binds FRα comprises one or more of the light and heavy chain CDRs of the antibodies produced by the deposited antibody-producing cells. In some embodiments,

antibody that binds FRα comprises the light and heavy chain variable regions of the antibodies produced by the deposited antibody-producing cells.

19D4

In some embodiments, the antibody that binds FRα is an antibody or antigen-binding fragment that includes a light chain CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:35. In some embodiments, the antibody that binds FRα includes a light chain CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:36. In some embodiments, the antibody that binds FRα includes a light chain CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:37. In some embodiments, the antibody that binds FRα includes a heavy chain CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:39. In some embodiments, the antibody that binds FRα includes a heavy chain CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:40. In some embodiments, the antibody that binds FRα includes a heavy chain CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:41. The antibody that binds FRα may include a light chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:35; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:36; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:37. The antibody that binds FRα may include a heavy chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:39; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 40; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:41. The antibody that binds FRα may include a light chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:35; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:36; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:37, and also have a heavy chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:39; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:40; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:41.

The antibody that binds FRα may include a light chain variable domain that includes an amino acid sequence substantially the same as, or identical to, SEQ ID

NO:38. The antibody that binds FRα may include a heavy chain variable domain that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:42. The antibody that binds FRα may include a light and a heavy chain variable domains, wherein the light chain variable domain includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:38, and the heavy chain variable domain includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:42. In some embodiments, the antibody that binds FRα is the 19D4.B7 (19D4) antibody or an antigen-binding fragment thereof, capable of binding either a native or nonreduced form of FRα. In some embodiments, the antibody has a murine IgG2a constant region.

In some embodiments, the antibody that binds FRα is an antibody that is produced by antibody-producing cells deposited with the American Type Culture Collection (10801 University Blvd., Manassas, Virginia 20110-2209) on May 19, 2011 and have been assigned Accession No. PTA-11884. In some embodiments, the antibody that binds FRα comprises one or more of the light and heavy chain CDRs of the antibodies produced by the deposited antibody-producing cells. In some embodiments, antibody that binds FRα comprises the light and heavy chain variable regions of the antibodies produced by the deposited antibody-producing cells.

24F12

In some embodiments, the antibody that binds FRα is an antibody or antigen-binding fragment that includes a light chain CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:43. In some embodiments, the antibody that binds FRα includes a light chain CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:44. In some embodiments, the antibody that binds FRα includes a light chain CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:45. In some embodiments, the antibody that binds FRα includes a heavy chain CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:47. In some embodiments, the antibody that binds FRα includes a heavy chain CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:48. In some embodiments, the antibody that binds FRα includes a heavy chain CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:49. The antibody

that binds FRα may include a light chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:43; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:44; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:45. The antibody that binds FRα may include a heavy chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:47; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 48; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:49. The antibody that binds FRα may include a light chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:43; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:44; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:45, and also have a heavy chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:47; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:48; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:49.

The antibody that binds FRα may include a light chain variable domain that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:46. The antibody that binds FRα may include a heavy chain variable domain that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:50. The antibody that binds FRα may include a light and a heavy chain variable domains, wherein the light chain variable domain includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:46, and the heavy chain variable domain includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:50. In some embodiments the antibody that binds FRα is the 24F12.B1 (24F12) antibody or an antigen-binding fragment thereof, capable of binding either a native or nonreduced form of FRα. In some embodiments, the antibody has a murine IgG1 constant region.

In some embodiments, the antibody that binds FRα is an antibody that is produced by antibody-producing cells deposited with the American Type Culture Collection (10801 University Blvd., Manassas, Virginia 20110-2209) on May 19, 2011 and have been assigned Accession No. PTA-11886. In some embodiments, the antibody that binds FRα comprises one or more of the light and heavy chain CDRs of the antibodies produced by the deposited antibody-producing cells. In some embodiments,

antibody that binds FRα comprises the light and heavy chain variable regions of the antibodies produced by the deposited antibody-producing cells.

26B3

In some embodiments, the antibody that binds FRα is an antibody or antigen-binding fragment that includes a light chain CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:51. In some embodiments, the antibody that binds FRα includes a light chain CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:52. In some embodiments, the antibody that binds FRα includes a light chain CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:53. In some embodiments, the antibody that binds FRα includes a heavy chain CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:55. In some embodiments, the antibody that binds FRα includes a heavy chain CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:56. In some embodiments, the antibody that binds FRα includes a heavy chain CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:57. The antibody that binds FRα may include a light chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:51; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:52; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:53. The antibody that binds FRα may include a heavy chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:55; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:56; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:57. The antibody that binds FRα may include a light chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:51; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:52; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:53, and also have a heavy chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO:55; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO:56; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO:57.

The antibody that binds FRα may include a light chain variable domain that includes an amino acid sequence substantially the same as, or identical to, SEQ ID

NO:54. The antibody that binds FRα may include a heavy chain variable domain that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:58. The antibody that binds FRα may include a light and a heavy chain variable domains, wherein the light chain variable domain includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:54, and the heavy chain variable domain includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO:58. In some embodiments the antibody that binds FRα is the 26B3.F2 (26B3) antibody or an antigen-binding fragment thereof, capable of binding either a native or nonreduced form of FRα. In some embodiments, the antibody has a murine IgG1 constant region.

In some embodiments, the antibody that binds FRα is an antibody that is produced by antibody-producing cells deposited with the American Type Culture Collection (10801 University Blvd., Manassas, Virginia 20110-2209) on May 19, 2011 and have been assigned Accession No. PTA-11885. In some embodiments, the antibody that binds FRα comprises one or more of the light and heavy chain CDRs of the antibodies produced by the deposited antibody-producing cells. In some embodiments, antibody that binds FRα comprises the light and heavy chain variable regions of the antibodies produced by the deposited antibody-producing cells.

Antigen binding arrangements of CDRs may be engineered using antibody-like proteins as CDR scaffolding. Engineered antigen-binding proteins are included within the scope of antibodies that bind FRα.

Other reagent antibodies that bind FRα are known in the art, and presently, multiple such reagent antibodies are commercially available (based on search of anti-FRα antibodies at http://www.biocompare.com), as listed in the table below.

Product	Company	Quantity	Applications	Reactivity
Mouse Anti-Human FRα Purified - MaxPab Polyclonal Antibody, Unconjugated	Abnova Corporation	50 µg	Detection Antibody, Western Blot (Transfected lysate)	Human
Mouse Anti-Human FRα Purified MaxPab Polyclonal Antibody, Unconjugated	Abnova Corporation	50 µg	Detection Antibody, Western Blot (Transfected lysate)	Human
Rabbit Anti-Human FRα Purified MaxPab Polyclonal Antibody, Unconjugated	Abnova Corporation	100 µg	Detection Antibody, Western Blot (Transfected lysate)	Human

Rabbit Anti-FRα Polyclonal Antibody, Unconjugated	Aviva Systems Biology	50 µg	Western Blot	Human, Mouse, Rat
Rabbit Anti-Human FRα Polyclonal Antibody, Unconjugated	GeneTex	100 µL	Western blot. The usefulness of this product in other applications has not been determined.	Human
Goat Anti-Bovine Folate Receptor Alpha (FRα) Polyclonal, Biotin Conjugated	LifeSpan BioSciences	10 mg	ELISA (1:4000 - 1:20000), Immunofluorescence, Immunohistochemistry, Western Blot	Bovine
Goat Anti-Bovine Folate Receptor Alpha (FRα) Polyclonal, Biotin Conjugated	LifeSpan BioSciences	Not provided	ELISA (1:5000 - 1:25000), Western Blot	Bovine
Goat Anti-Bovine Folate Receptor Alpha (FRα) Polyclonal, Hrp Conjugated	LifeSpan BioSciences	20 mg	ELISA (1:2000 - 1:10000), Immunohistochemistry, Western Blot	Bovine
Goat Anti-Bovine Folate Receptor Alpha (FRα) Polyclonal, Hrp Conjugated	LifeSpan BioSciences	1000 µg	ELISA (1:2000 - 1:12000), Gel Shift, Immunohistochemistry (1:100 - 1:200), Immunohistochemistry	Bovine
Goat Anti-Bovine Folate Receptor Alpha (FRα) Polyclonal, Hrp Conjugated	LifeSpan BioSciences	2000 µg (200 µl)	ELISA, Western Blot	Bovine
Goat Anti-Bovine Folate Receptor Alpha (FRα) Polyclonal, Hrp Conjugated	LifeSpan BioSciences	Not provided	ELISA, Immunohistochemistry (Frozen sections), Immunohistochemistry (Parrafin), Western Blot	Bovine
Goat Anti-Bovine Folate Receptor Alpha (FRα) Polyclonal, Unconjugated	LifeSpan BioSciences	50 mg	ELISA (1:10000 - 1:40000), Immunoprecipitation, Western Blot	Bovine
Goat Anti-Bovine Folate Receptor Alpha (FRα) Polyclonal, Unconjugated	LifeSpan BioSciences	10000 µg	ELISA (1:10000 - 1:40000), Immunoprecipitation, Western Blot	Bovine
Goat Anti-Bovine Folate Receptor Alpha (FRα) Polyclonal, Unconjugated	LifeSpan BioSciences	1 ml	ELISA (1:3000 - 1:9000), Immunoprecipitation, Western Blot	Bovine
Goat Anti-Bovine Folate Receptor Alpha (FRα) Polyclonal, Unconjugated Folate Receptor Alpha (FRα)	LifeSpan BioSciences	Not provided	ELISA (1:3000 - 1:9000), Immunoprecipitation, Western Blot	Bovine
Mouse Anti-Bovine Folate Receptor Alpha (FRα) Monoclonal, Unconjugated	LifeSpan BioSciences	200 µg	ELISA	Bovine
Mouse Anti-Bovine Folate Receptor Alpha	LifeSpan BioSciences	200 µg	ELISA	Human

(FRα) Monoclonal, Unconjugated	Folate Receptor Alpha (FRα)			
Mouse Anti-Bovine Folate Receptor Alpha (FRα) Monoclonal, Unconjugated	LifeSpan BioSciences	200 µg	ELISA	Human
Mouse Anti-Bovine Folate Receptor Alpha (FRα) Monoclonal, Unconjugated	LifeSpan BioSciences	200 µg	ELISA	Not provided
Mouse Anti-Human Folate Receptor Alpha (FRα) Monoclonal, Unconjugated, Clone 6d398	LifeSpan BioSciences	100 µl	ELISA (1 - 10 µg/ml), Flow Cytometry, Immunocytochemistry, Immunohistochemistry (Frozen sections)	Monkey
Rabbit Anti-Bovine Folate Receptor Alpha (FRα) Polyclonal, Unconjugated	LifeSpan BioSciences	1 ml	ELISA	Bovine
Rabbit Anti-Bovine Folate Receptor Alpha (FRα) Polyclonal, Unconjugated	LifeSpan BioSciences	Not provided	Not provided	Bovine
Mouse Anti-Human FRα Polyclonal antibody, Unconjugated, Clone folate receptor 1 (adult)	Novus Biologicals	0.05 ml	Western Blot, ELISA	
Mouse Anti-Human FRα Polyclonal, Unconjugated	Novus Biologicals	0.05 mg	ELISA, Western Blot	Human
Goat Anti-Human FRα Affinity purified Polyclonal antibody, Biotin Conjugated	R&D Systems	50 µg	Western Blot	Human
Goat Anti-Human FRα Affinity purified Polyclonal antibody, Unconjugated	R&D Systems	100 µg	Flow Cytometry, Western Blot	Human
Mouse Anti-Human FRα Monoclonal Antibody, Allophycocyanin Conjugated, Clone 548908	R&D Systems	100 Tests	Flow Cytometry	Human
Mouse Anti-Human FRα Monoclonal Antibody, Phycoerythrin Conjugated, Clone 548908	R&D Systems	100 Tests	Flow Cytometry	Human
Mouse Anti-Human FRα Monoclonal antibody, Unconjugated, Clone 548908	R&D Systems	100 µg	Flow Cytometry, Immunocytochemistry, Western Blot	Human
Mouse Anti- Human FRα Monoclonal Antibody, Unconjugated	United States Biological	100 µg	ELISA, Flow Cytometry, Immunocytochemistry, Western Blot	Human

In a preferred embodiment, the antibody that binds FRα comprises at least one of the following CDRs, as derived from the murine LK26 heavy and light chains: SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3. See US Patent No. 5,646,253, the contents of which, as they relate to the anti-FRα antibodies that may be used in the present invention, are incorporated herein by reference. Further mutations may be made in the framework regions as taught in US Patent No. 5,646,253, the contents of which are hereby incorporated by reference.

In another preferred embodiment, the antibody includes a variable region light chain selected from the group consisting of LK26HuVK (SEQ ID NO: 13) LK26HuVKY (SEQ ID NO: 14), LK26HuVKPW (SEQ ID NO: 15), and LK26HuVKPW,Y (SEQ ID NO: 16); and a variable region heavy chain selected from the group consisting of LK26HuVH (SEQ ID NO: 17); LK26HuVH FAIS,N (SEQ ID NO: 18); LK26HuVH SLF (SEQ ID NO: 19); LK26HuVH I,I (SEQ ID NO: 20); and LK26KOLHuVH (SEQ ID NO: 21). See US Patent No. 5,646,253 and US Patent No. 6,124,106. In another embodiment, the antibody comprises the heavy chain variable region LK26KOLHuVH (SEQ ID NO: 21) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16). In another embodiment, the antibody comprises the heavy chain variable region LK26HuVH SLF (SEQ ID NO: 19) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16). In a further embodiment, the antibody comprises the heavy chain variable region LK26HuVH FAIS,N (SEQ ID NO: 18) and the light chain variable region LK26HuVKPW,Y (SEQ ID NO: 16).

In some embodiments, samples may need to be modified in order to make FRα accessible to antibody binding. In a particular aspect of the immunocytochemistry or immunohistochemistry methods, slides may be transferred to a pretreatment buffer and optionally heated to increase antigen accessibility. Heating of the sample in the pretreatment buffer rapidly disrupts the lipid bi-layer of the cells and makes the antigens (may be the case in fresh specimens, but not typically what occurs in fixed specimens) (*i.e.*, the FRα protein) more accessible for antibody binding. The term "pretreatment buffer" are used interchangeably herein to refer to a buffer that is used to prepare cytology or histology samples for immunostaining, particularly by increasing FRα

protein accessibility for antibody binding. The pretreatment buffer may comprise a pH-specific salt solution, a polymer, a detergent, or a nonionic or anionic surfactant such as, for example, an ethyloxylated anionic or nonionic surfactant, an alkanoate or an alkoxylate or even blends of these surfactants or even the use of a bile salt. The pretreatment buffer may, for example, be a solution of 0.1% to 1% of deoxycholic acid, sodium salt, or a solution of sodium laureth-13-carboxylate (*e.g.*, Sandopan LS) or and ethoxylated anionic complex. In some embodiments, the pretreatment buffer may also be used as a slide storage buffer. In a particular embodiment, the sample, for example, the urine sample, is centrifuged, vortexed, diluted and/or subjected to guanidine treatment.

Any method for making FRα protein more accessible for antibody binding may be used in the practice of the invention, including the antigen retrieval methods known in the art. See, for example, Bibbo, *et al.* (2002) *Acta. Cytol.* 46:25-29; Saqi, *et al.* (2003) *Diagn. Cytopathol.* 27:365-370; Bibbo, *et al.* (2003) *Anal. Quant. Cytol. Histol.* 25:8-11, the entire contents of each of which are incorporated herein by reference.

Following pretreatment to increase FRα protein accessibility, samples may be blocked using an appropriate blocking agent, *e.g.*, a peroxidase blocking reagent such as hydrogen peroxide. In some embodiments, the samples may be blocked using a protein blocking reagent to prevent non-specific binding of the antibody. The protein blocking reagent may comprise, for example, purified casein. An antibody, particularly a monoclonal or polyclonal antibody, that specifically binds to FRα is then incubated with the sample.

Techniques for detecting antibody binding are well known in the art. Antibody binding to FRα may be detected through the use of chemical reagents that generate a detectable signal that corresponds to the level of antibody binding and, accordingly, to the level of FRα protein expression. In one of the immunohistochemistry or immunocytochemistry methods of the invention, antibody binding is detected through the use of a secondary antibody that is conjugated to a labeled polymer. Examples of labeled polymers include but are not limited to polymer-enzyme conjugates. The enzymes in these complexes are typically used to catalyze the deposition of a chromagen at the antigen-antibody binding site, thereby resulting in cell staining that corresponds to expression level of the biomarker of interest. Enzymes include, but are not limited to, horseradish peroxidase (HRP) and alkaline phosphatase (AP).

In one immunohistochemistry or immunocytochemistry method of the invention, antibody binding to the FRα protein is detected through the use of an HRP-labeled polymer that is conjugated to a secondary antibody. Antibody binding can also be detected through the use of a species-specific probe reagent, which binds to monoclonal or polyclonal antibodies, and a polymer conjugated to HRP, which binds to the species specific probe reagent. Slides are stained for antibody binding using any chromagen, *e.g.*, the chromagen 3,3-diaminobenzidine (DAB), and then counterstained with hematoxylin and, optionally, a bluing agent such as ammonium hydroxide or TBS/Tween-20. Other suitable chromagens include, for example, 3-amino-9-ethylcarbazole (AEC). In some aspects of the invention, slides are reviewed microscopically by a cytotechnologist and/or a pathologist to assess cell staining, *e.g.*, fluorescent staining (*i.e.*, FRα expression). Alternatively, samples may be reviewed *via* automated microscopy or by personnel with the assistance of computer software that facilitates the identification of positive staining cells.

In a preferred embodiment of the invention, the antibody is labeled. For example, detection of antibody binding can be facilitated by coupling the anti-FRα antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S, ^{14}C, or ^{3}H. In a particular embodiment, the antibody is labeled with a radio-label, chromophore-label, fluorophore-label, or enzyme-label.

In one embodiment of the invention frozen samples are prepared as described above and subsequently stained with antibodies against FRα diluted to an appropriate concentration using, for example, Tris-buffered saline (TBS). Primary antibodies can be detected by incubating the slides in biotinylated anti-immunoglobulin. This signal can optionally be amplified and visualized using diaminobenzidine precipitation of the

antigen. Furthermore, slides can be optionally counterstained with, for example, hematoxylin, to visualize the cells.

In another embodiment, fixed and embedded samples are stained with antibodies against FRα and counterstained as described above for frozen sections. In addition, samples may be optionally treated with agents to amplify the signal in order to visualize antibody staining. For example, a peroxidase-catalyzed deposition of biotinyl-tyramide, which in turn is reacted with peroxidase-conjugated streptavidin (Catalyzed Signal Amplification (CSA) System, DAKO, Carpinteria, CA) may be used.

One of skill in the art will recognize that the concentration of a particular antibody used to practice the methods of the invention will vary depending on such factors as time for binding, level of specificity of the antibody for FRα, and method of sample preparation. Moreover, when multiple antibodies are used, the required concentration may be affected by the order in which the antibodies are applied to the sample, *e.g.*, simultaneously as a cocktail or sequentially as individual antibody reagents. Furthermore, the detection chemistry used to visualize antibody binding to FRα must be optimized to produce the desired signal to noise ratio.

In one embodiment of the invention, proteomic methods, *e.g.*, mass spectrometry, are used for detecting and quantitating the FRα protein. For example, matrix-associated laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) or surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) which involves the application of a sample, such as serum, to a protein-binding chip (Wright, G.L., Jr., *et al.* (2002) *Expert Rev Mol Diagn* 2:549; Li, J., *et al.* (2002) *Clin Chem* 48:1296; Laronga, C., *et al.* (2003) *Dis Markers* 19:229; Petricoin, E.F., *et al.* (2002) 359:572; Adam, B.L., *et al.* (2002) *Cancer Res* 62:3609; Tolson, J., *et al.* (2004) *Lab Invest* 84:845; Xiao, Z., *et al.* (2001) *Cancer Res* 61:6029) can be used to detect and quantitate the FRα protein. Mass spectrometric methods are described in, for example, U.S. Patent Nos. 5,622,824, 5,605,798 and 5,547,835, the entire contents of each of which are incorporated herein by reference.

The present invention is further predicated, at least in part, on the identification of FRα as a prognostic biomarker, *i.e.*, as a biomarker of the progression and/ or severity, of an FRα-expressing cancer such as ovarian cancer or non-small cell lung cancer. Accordingly, the present invention provides methods of assessing the progression of an FRα-expressing cancer in a subject afflicted with ovarian cancer by

comparing the level of FRα in a sample derived from a subject with the level of FRα in a control sample, wherein a difference in the level of FRα in the sample (such as a urine or serum sample) derived from the subject compared with the control sample is an indication that the cancer will progress rapidly. Similarly, methods of assessing the level of risk that a subject will develop an FRα-expressing cancer involve comparing the level of FRα in a sample derived from a subject with the level of FRα in a control sample, wherein a difference in the level of FRα in the sample (such as urine or serum sample) derived from the subject compared with the control sample is an indication that the subject has a higher level of risk of developing an FRα-expressing cancer as compared to normal risk in a healthy individual.

In one embodiment, the difference is an increase. In another embodiment, the difference is a decrease. In some types of cancers (*e.g.*, squamous cell carcinoma of the head and neck, ovarian cancer), a higher level of FRα expression is associated with a worse prognosis, whereas in other types of cancers (*e.g.*, non-small-cell lung cancers), a higher level of FRα expression is associated with a better prognosis. Thus, in one specific embodiment, the FRα-expressing cancer is ovarian cancer or squamous cell carcinoma of the head and neck and the difference is an increase. In another specific embodiment, the FRα-expressing cancer is a non small-cell lung cancer, and the difference is a decrease.

In certain aspects, the invention provides methods of assessing the progression of an FRα-expressing cancer in a subject afflicted with an FRα-expressing cancer by comparing the level of FRα in a sample derived from a subject with the level of FRα in a control sample, wherein an increase in the level of FRα in the sample (such as a urine or serum sample) derived from the subject compared with the control sample is an indication that the cancer will progress rapidly, or a decrease in the level of FRα in the sample derived from the subject as compared with the level of FRα in the control sample is an indication that the cancer will progress slowly or will regress. Similarly, methods of assessing the level of risk that a subject will develop an FRα-expressing cancer involve comparing the level of FRα in a sample derived from a subject with the level of FRα in a control sample, wherein an increase in the level of FRα in the sample (such as urine or serum sample) derived from the subject compared with the control sample is an indication that the subject has a higher level of risk of developing an FRα-expressing cancer as compared to normal risk in a healthy individual, or a decrease in

the level of FRα in the sample derived from the subject as compared with the level of FRα in the control sample is an indication that the subject has a lower level of risk of developing an FRα-expressing cancer as compared to a normal risk in a healthy individual.

Any clinically relevant or statistically significant increase or decrease, using any analytical method known in the art, may be utilized in the prognostic, risk assessment and other methods of the invention. In one embodiment, an increase in the level of FRα in the level of FRα refers to a level that exceeds a cutoff value determined using an ROC analysis as exemplified in Example 6. In another embodiment, a decrease in the level of FRα refers to a level in a test sample that does not exceed a cutoff value determined using an ROC analysis.

In other embodiments, the increase or decrease must be greater than the limits of detection of the method for determining the level of FRα. In further embodiments, the increase or decrease be at least greater than the standard error of the assessment method, and preferably a difference of at least about 2-, about 3-, about 4-, about 5-, about 6-, about 7-, about 8-, about 9-, about 10-, about 15-, about 20-, about 25-, about 100-, about 500-, about 1000-fold or greater than the standard error of the assessment method. In some embodiments, the increase or decrease is assessed using parametric or nonparametric descriptive statistics, comparisons, regression analyses, and the like.

In other embodiments, the increase or decrease is a level in the sample derived from the subject that is about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 150%, about 200%, about 300%, about 400%, about 500%, about 600%, about 700%, about 800%, about 900% or about 1000% more or less than the level of FRα in the control sample. In alternative embodiments, the increase or decrease is a level in the sample derived from the subject that is at least about 1.5, and more preferably about two, about three, about four, about five or more standard deviations above or below the average level of FRα in the control sample. As used herein, the phrase "progression of an FRα-expressing cancer in a subject afflicted with an FRα-expressing cancer" may refer to the progression of an FRα-expressing cancer from a less severe to a more severe cancer state. This could include an increase in the number or severity of tumors, the degree of metastasis, the speed with which the cancer is growing and spreading, and the like. In certain embodiments, the progression is a progression

from a less severe stage to a more severe stage, wherein the stage is assessed according to a staging scheme known in the art. In one embodiment, wherein the FRα-expressing cancer is ovarian cancer, the progression refers to a progression from Stage I to Stage II, from Stage II to Stage III, etc. In another embodiment, wherein the FRα-expressing cancer is non-small cell lung cancer (NSCLC), the progression refers to a progression from Stage 0 to Stage IA, Stage IA to Stage IB, Stage IB to Stage IIA, Stage IIA to Stage IIB, Stage IIB to Stage IIC, etc. In another embodiment, wherein the FRα-expressing cancer is non-small cell lung cancer (NSCLC), the progression refers to a progression from a less severe to a more severe stage as determined under the TNM classification system. See Spira; Greene; Sobin.

Alternatively, the phrase "progression of an FRα-expressing cancer in a subject afflicted with an FRα-expressing cancer" may refer to a regression of an FRα-expressing cancer from a more severe state to a less severe state, such as a decrease in the number or severity of tumors, the degree of metastasis, the speed with which the cancer is growing and spreading, and the like. In certain embodiments, the progression is a progression from a more severe stage to a less severe stage, wherein the stage is assessed according to a staging scheme known in the art. In one embodiment, wherein the FRα-expressing cancer is ovarian cancer, the progression refers to a regression from Stage IV to Stage III, from Stage III to Stage II, etc. In another embodiment, wherein the FRα-expressing cancer is non-small cell lung cancer (NSCLC), the progression refers to a progression from Stage IV to Stage IIIB, Stage IIIB to Stage IIIA, Stage IIIA to Stage IIB, etc. In another embodiment, wherein the FRα-expressing cancer is non-small cell lung cancer (NSCLC), the progression refers to a progression from a more severe to a less severe stage as determined under the TNM classification system. See Spira; Greene; Sobin.

In further embodiments, the level of FRα may be used to calculate the likelihood that a subject is afflicted with an FRα-expressing cancer, the progression of an FRα-expressing cancer in a subject, the level of risk of developing an FRα-expressing cancer, the risk of cancer recurrence in a subject being treated for an FRα-expressing, the survival of a subject being treated for an FRα-expressing cancer, the efficacy of a treatment regimen for treating an FRα-expressing cancer, and the like, using the methods of the invention, which may include methods of regression analysis known to one of skill in the art. For example, suitable regression models include, but are not

limited to CART (*e.g.*, Hill, T, and Lewicki, P. (2006) "STATISTICS Methods and Applications" StatSoft, Tulsa, OK), Cox (*e.g.*, www.evidence-based-medicine.co.uk), exponential, normal and log normal (*e.g.*, www.obgyn.cam.ac.uk/mrg/statsbook/stsurvan.html), logistic (*e.g.*, www.en.wikipedia.org/wiki/Logistic_regression), parametric, non-parametric, semi-parametric (*e.g.*, www.socserv.mcmaster.ca/jfox/Books/Companion), linear (*e.g.*, www.en.wikipedia.org/wiki/Linear_regression), or additive (*e.g.*, www.en.wikipedia.org/wiki/Generalized_additive_model).

In one embodiment, a regression analysis includes the level of FRα. In further embodiments, a regression analysis may include additional clinical and/or molecular co-variates. Such clinical co-variates include, but are not limited to, age of the subject, tumor stage, tumor grade, tumor size, treatment regime, *e.g.*, chemotherapy and/or radiation therapy, clinical outcome (*e.g.*, relapse, disease-specific survival, therapy failure), and/or clinical outcome as a function of time after diagnosis, time after initiation of therapy, and/or time after completion of treatment. Molecular co-variates can include, but are not limited to additional molecular marker values. For example, in embodiments wherein the FRα-expressing cancer is ovarian cancer, such markers may include, *e.g.*, serum CA125 levels, serum DF3 levels, and/or plasma LPA levels.

In other aspects, the invention provides methods for monitoring the effectiveness of a therapy or treatment regimen. For example, the present invention provides methods for monitoring the efficacy of MORAb-003 treatment of ovarian cancer or lung cancer in a subject suffering from ovarian cancer or lung cancer. Specifically, the methods involve determining the level of folate receptor alpha (FRα) which is not bound to a cell, in a sample derived from said subject, wherein said subject has been previously administered MORAb-003; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell with the level of FRα in a control sample, wherein an increase or no change in the level of FRα in the sample derived from said subject as compared with the level of FRα in the control sample is an indication that the MORAb-003 treatment is not efficacious; and wherein a decrease in the level of FRα in the sample derived from said subject as compared with the level of FRα in the control sample is an indication that the MORAb-003 treatment is efficacious.

For example, the control sample may be derived from a subject not subjected to the treatment regimen and a test sample may be derived from a subject subjected to at least a portion of the treatment regimen. Alternatively, the test sample and the control sample may be derived from the same subject. For example, the test sample may be a sample derived from a subject after administration of a therapeutic, such as MORAb-003. The control sample may be a sample derived from a subject prior to administration of therapeutic or at an earlier stage of therapeutic regimen. Accordingly, a decrease in the level of expression of FRα in the test sample, relative to the control sample, is an indication that therapy has decreased the progression of the FRα-expressing cancer, for example, ovarian cancer. For FRα-expressing cancers wherein a higher level of FRα is associated with a worse prognosis, such as *e.g.*, ovarian cancer or squamous cell carcinoma of the head and neck, a decrease in the level of expression of FRα in the test sample, relative to the control sample, is an indication that therapy is effective in slowing the progression of the FRα-expressing cancer, or in causing a regression of the cancer, in the subject afflicted with the FRα-expressing cancer. In a preferred embodiment, the FRα-expressing cancer is ovarian cancer.

In various embodiments of this aspect of the invention, the sample may be urine, serum, plasma or ascites. In particular embodiments, the sample is urine or serum. Moreover, the FRα may be determined by contacting the sample with an antibody that binds FRα, optionally, using antibodies as described herein and assay methods as described herein.

In various embodiments, the MORAb-003 treatment antibody is (a) an antibody that comprises the heavy chain amino acid sequence as set forth in SEQ ID NO:7 and the light chain amino acid sequence as set forth in SEQ ID NO:8; (b) an antibody that binds the same epitope as the MORAb-003 antibody; or (c) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3..

In a particular embodiment, the FRα-expressing cancer is ovarian cancer. In other embodiments, the FRα-expressing cancer is lung cancer. In more specific embodiments, the lung cancer is non-small cell lung cancer (NSCLC). In one such embodiment, the NSCLC is selected from the group consisting of adenocarcinoma,

squamous cell lung carcinoma, large cell lung carcinoma, pleomorphic NSCLC, carcinoid tumor, salivary gland carcinoma, and unclassified carcinoma. In a preferred embodiment, the NSCLC is adenocarcinoma. In alternative embodiments, the lung cancer is small cell lung carcinoma (SCLC). In another embodiment, the lung cancer is bronchioalveolar carcinoma. In yet another embodiment, the lung cancer is a lung carcinoid tumor.

In another aspect, the invention provides methods of stratifying a subject with an FRα-expressing cancer into cancer therapy groups based on the determined level of FRα in a sample. In a preferred embodiment, the method involves stratifying a subject with an FRα-expressing cancer into one of at least four cancer therapy groups. In other embodiments, the method involves stratifying a subject with an FRα-expressing cancer into one of at least about two, about three, about four, about five, about six, about seven, about eight, about nine, or about ten cancer therapy groups.

According to the present invention, the levels of FRα may be associated with the severity, *i.e.*, the stage, of the FRα expressing cancer. For example, ovarian cancer is stratified into different stages based on the severity of the cancer, as set forth herein. Accordingly, the present invention provides methods for stratifying ovarian cancer into Stage I, for example, Stage IA, Stage 1B or Stage IC; Stage II, for example, Stage IIA, Stage IIB or Stage IIC; Stage III, for example, Stage IIIA, Stage IIIB or Stage IIIC; or Stage IV ovarian cancer.

SCLS or NSCLC may be stratified into different stages based on the severity of the cancer, as set forth herein. Accordingly, the present invention provides methods for stratifying the lung cancer, for example, SCLS or NSCLC, into the occult (hidden) stage; stage 0; Stage I, for example, stages IA and IB; Stage II, for example, stages IIA and IIB; Stage III, for example, stages IIIA and IIIB; or Stage IV lung cancer.

In yet another aspect, the present invention is predicated, at least in part, on the finding that FRα can serve as a predictive biomarker for treatment of FRα expressing cancers. Specifically, the methods of the present invention provide for assessing whether a subject will respond to treatment, for example, with MORAb-003, and whether and when to initiate treatment, for example, with MORAb-003, by assessing the levels of FRα in a subject.

In one aspect, the present invention provides a method for predicting whether a subject suffering from an FRα expressing cancer, for example, ovarian or lung cancer, will respond to treatment with MORAb-003, by determining the level of folate receptor alpha (FRα) which is not bound to a cell in a sample derived from said subject; and comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the sample derived from said subject with the level of FRα in a control sample, wherein a difference between the level of FRα in the sample derived from said subject and the level of FRα in the control sample is an indication that the subject will respond to treatment with MORAb-003.

In certain embodiments, the degree of difference between the levels of FRα not bound to a cancer cell in the test sample as compared to the control sample is indicative that the subject will respond to treatment with MORAb-003. For example, a difference of at least about 2-, about 3-, about 4-, about 5-, about 6-, about 7-, about 8-, about 9-, about 10-, about 15-, about 20-, about 25-, about 100-, about 500-, about 1000-fold or greater than the standard error of the assessment method is indicative that the subject will respond to treatment with MORAb-003. Alternatively or in combination, a difference of at least about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 150%, about 200%, about 300%, about 400%, about 500%, about 600%, about 700%, about 800%, about 900% or about 1000% is indicative that the subject will respond to treatment with MORAb-003. Alternatively or in combination, a difference of at least about 1.5, and more preferably about two, about three, about four, about five or more standard deviations is indicative that the subject will respond to treatment with MORAb-003.

In various embodiments, the MORAb-003 treatment antibody is (a) an antibody that binds the same epitope as the MORAb-003 antibody; or (b) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3.

In various embodiments, the sample is urine, plasma, serum or ascites. In particular embodiments, the sample is urine or serum. In further embodiments, the FRα-expressing cancer is selected from the group consisting of lung cancer,

mesothelioma, ovarian cancer, renal cancer, brain cancer, cervical cancer, nasopharyngeal cancer, squamous cell carcinoma of the head and neck, endometrial cancer, breast cancer, bladder cancer, pancreatic cancer, bone cancer, pituitary cancer, colorectal cancer and medullary thyroid cancer. In a particular embodiment, the FRα-expressing cancer is ovarian cancer. In another embodiment, the FRα-expressing cancer is non-small cell lung cancer, such as adenocarcinoma.

B. Anti-FRα Antibody Based Assays for Detecting FRα-Expressing Cancers

There are a variety of assay formats known to those of ordinary skill in the art for using an antibody to detect a polypeptide in a sample, including but not limited to enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunofluorimetry, immunoprecipitation, solution phase assay, equilibrium dialysis, immunodiffusion and other techniques. See, *e.g.*, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; Weir, D. M., Handbook of Experimental Immunology, 1986, Blackwell Scientific, Boston. For example, the assay may be performed in a Western blot format, wherein a protein preparation from the biological sample is submitted to gel electrophoresis, transferred to a suitable membrane and allowed to react with the antibody. The presence of the antibody on the membrane may then be detected using a suitable detection reagent, as is well known in the art and described below.

In another embodiment, the assay involves the use of an antibody immobilized on a solid support to bind to the target FRα polypeptide and remove it from the remainder of the sample. The bound FRα polypeptide may then be detected using a second antibody reactive with a distinct FRα polypeptide antigenic determinant, for example, a reagent that contains a detectable reporter moiety. As a non-limiting example, according to this embodiment the immobilized antibody and the second antibody which recognize distinct antigenic determinants may be any two of the monoclonal antibodies described herein selected from MORAb-003, MOV18, 548908, 6D398 or variants thereof as described herein. Alternatively, a competitive assay may be utilized, in which FRα is labeled with a detectable reporter moiety and allowed to bind to the immobilized anti-FRα antibody after incubation of the immobilized antibody with the sample. The extent to which components of the sample inhibit the binding of

the labeled polypeptide to the antibody is indicative of the reactivity of the sample with the immobilized antibody, and as a result, indicative of the level of FRα in the sample.

The solid support may be any material known to those of ordinary skill in the art to which the antibody may be attached, such as a test well in a microtiter plate, a nitrocellulose filter or another suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic such as polystyrene or polyvinylchloride. The antibody may be immobilized on the solid support using a variety of techniques known to those in the art, which are amply described in the patent and scientific literature.

In certain preferred embodiments, the assay for detection of FRα in a sample is a two-antibody sandwich assay. This assay may be performed by first contacting a FRα specific antibody (*e.g.*, MORAb-003, MOV18, 548908, 6D398 or variants thereof as described herein) that has been immobilized on a solid support, commonly the well of a microtiter plate, with the biological sample, such that a soluble molecule naturally occurring in the sample and having an antigenic determinant that is reactive with the antibody is allowed to bind to the immobilized antibody (*e.g.*, a 30 minute incubation time at room temperature is generally sufficient) to form an antigen-antibody complex or an immune complex. Unbound constituents of the sample are then removed from the immobilized immune complexes. Next, a second antibody specific for FRα is added, wherein the antigen combining site of the second antibody does not competitively inhibit binding of the antigen combining site of the immobilized first antibody to FRα (*e.g.*, MORAb-003, MOV18, 548908, 6D398 or variants thereof as described herein, that is not the same as the monoclonal antibody immobilized on the solid support). The second antibody may be detectably labeled as provided herein, such that it may be directly detected. Alternatively, the second antibody may be indirectly detected through the use of a detectably labeled secondary (or "second stage") anti-antibody, or by using a specific detection reagent as provided herein. The subject invention method is not limited to any particular detection procedure, as those having familiarity with immunoassays will appreciate that there are numerous reagents and configurations for immunologically detecting a particular antigen (*e.g.*, FRα) in a two-antibody sandwich immunoassay.

In certain preferred embodiments of the invention using the two-antibody sandwich assay described above, the first, immobilized antibody specific for FRα is a

polyclonal antibody and the second antibody specific for FRα is a polyclonal antibody. In certain other embodiments of the invention, the first, immobilized antibody specific for FRα is a monoclonal antibody and the second antibody specific for FRα is a polyclonal antibody. In certain other embodiments of the invention the first, immobilized antibody specific for FRα is a polyclonal antibody and the second antibody specific for FRα is a monoclonal antibody. In certain other embodiments of the invention, the first, immobilized antibody specific for FRα is a monoclonal antibody and the second antibody specific for FRα is a monoclonal antibody. For example, in these embodiments it should be noted that monoclonal antibodies MORAb-003, MOV18, 548908, 6D398 or variants thereof as described herein, as provided herein recognize distinct and noncompetitive antigenic determinants (*e.g.*, epitopes) on FRα polypeptides, such that any pairwise combination of these monoclonal antibodies may be employed. In other preferred embodiments of the invention, the first, immobilized antibody specific for FRα and/or the second antibody specific for FRα may be any of the kinds of antibodies known in the art and referred to herein, for example, by way of illustration and not limitation, Fab fragments, $F(ab')_2$ fragments, immunoglobulin V-region fusion proteins or single chain antibodies. Those familiar with the art will appreciate that the present invention encompasses the use of other antibody forms, fragments, derivatives and the like in the methods disclosed and claimed herein.

In certain particularly preferred embodiments, the second antibody may contain a detectable reporter moiety or label such as an enzyme, dye, radionuclide, luminescent group, fluorescent group or biotin, or the like. The amount of the second antibody that remains bound to the solid support is then determined using a method appropriate for the specific detectable reporter moiety or label. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Antibody-enzyme conjugates may be prepared using a variety of coupling techniques (for review see, *e.g.*, Scouten, W. H., Methods in Enzymology 135:30-65, 1987). Spectroscopic methods may be used to detect dyes (including, for example, colorimetric products of enzyme reactions), luminescent groups and fluorescent groups. Biotin may be detected using avidin or streptavidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic, spectrophotometric or other analysis of the reaction products. Standards

and standard additions may be used to determine the level of mesothelin polypeptide in a sample, using well known techniques.

A method of screening for the presence of an FRα expressing cancer according to the present invention may be further enhanced by the detection of more than one tumor associated marker in a biological sample from a subject. Accordingly, in certain embodiments the present invention provides a method of screening that, in addition to detecting reactivity of FRα not bound to a cell, also includes detection of at least one additional soluble marker of a malignant condition using established methods as known in the art and provided herein. As noted above, there are currently a number of soluble tumor associated antigens that are detectable in samples of readily obtained biological fluids.

C. Kits of the Invention

The invention also provides kits for assessing whether a subject is afflicted with an FRα-expressing cancer, for assessing the progression of an FRα-expressing cancer, for assessing the level of risk that a subject will develop an FRα-expressing cancer, or for monitoring the effectiveness of a therapy or treatment regimen for an FRα-expressing cancer. These kits include means for determining the level of expression of FRα and instructions for use of the kit to assess the progression of an FRα-expressing cancer, to assess the level of risk that a subject will develop an FRα-expressing cancer, or to monitor the effectiveness of a therapy or treatment regimen for an FRα-expressing cancer.

The kits of the invention may optionally comprise additional components useful for performing the methods of the invention. By way of example, the kits may comprise means for obtaining a sample from a subject, a control sample, *e.g.*, a sample from a subject having slowly progressing cancer and/or a subject not having cancer, one or more sample compartments, and instructional material which describes performance of a method of the invention and tissue specific controls/standards.

The means for determining the level of FRα include known methods in the art for assessing protein levels, as discussed above, and specific preferred embodiments, for example, utilizing the MORAb-003 antibody, as discussed herein. Thus, for example, in one embodiment, the level of FRα is assessed by contacting a sample derived from a subject (such as urine or serum) with a folate receptor alpha (FRα) binding agent. In a

preferred embodiment, the binding agent is an antibody. Many of the types of antibodies that bind FRα are discussed above in the methods of the invention and may also be utilized in the kits of the invention.

The means for determining the level of FRα can further include, for example, buffers or other reagents for use in an assay for determining the level of FRα. The instructions can be, for example, printed instructions for performing the assay and/or instructions for evaluating the level of expression of FRα.

The kits of the inventions may also include means for isolating a sample from a subject. These means can comprise one or more items of equipment or reagents that can be used to obtain a fluid or tissue from a subject. The means for obtaining a sample from a subject may also comprise means for isolating blood components, such as serum, from a blood sample. Preferably, the kit is designed for use with a human subject.

III. Screening Assays

In further embodiments, the invention also provides methods (also referred to herein as "screening assays") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs), which modulate the growth, progression and/or aggressiveness of cancer, *e.g.*, an FRα-expressing cancer, or a cancer cell, *e.g.*, an ovarian cancer cell, by monitoring and comparing the levels of FRα in a sample. Such assays typically comprise a test compound, or a combination of test compounds, whose activity against cancer or a cancer cell is to be evaluated. Compounds identified *via* assays such as those described herein may be useful, for example, for modulating, *e.g.*, inhibiting, ameliorating, treating, or preventing aggressiveness of an FRα-expressing cancer or a cancer cell, *e.g.*, an ovarian cancer cell. By monitoring the level of FRα in a sample, one can determine whether the FRα-expressing cancer is progressing or regressing and whether the test compound has the desired effect. For example, in embodiments wherein the FRα-expressing cancer is a cancer for which higher levels of FRα are associated with a worse prognosis, a decrease in the level of FRα after administration of the test compound(s) would be indicative of the efficacy of the test compound. By contrast, an increase in the level of FRα after administration of the test compound(s) would indicate that the test compound is not effective in treating ovarian

cancer. By contrast, in embodiments wherein the FRα-expressing cancer is a cancer for which higher levels of FRα are associated with a better prognosis, an increase in the level of FRα after administration of the test compound(s) would be indicative of the efficacy of the test compound. By contrast, an decrease in the level of FRα after administration of the test compound(s) would indicate that the test compound is not effective in treating ovarian cancer.

The test compounds used in the screening assays of the present invention may be obtained from any available source, including systematic libraries of natural and/or synthetic compounds. Test compounds may also be obtained by any of the numerous approaches in combinatorial library methods known in the art, including biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, *e.g.*, Zuckermann *et al.*, 1994, *J. Med. Chem.* 37:2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, 1997, *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992, *Biotechniques* 13:412-421), or on beads (Lam, 1991, *Nature* 354:82-84), chips (Fodor, 1993, *Nature* 364:555-556), bacteria and/or spores, (Ladner, USP 5,223,409), plasmids (Cull *et al*, 1992, *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith, 1990, *Science* 249:386-390; Devlin, 1990, *Science* 249:404-406; Cwirla *et al*, 1990, *Proc. Natl. Acad. Sci.* 87:6378-6382; Felici, 1991, *J. Mol. Biol.* 222:301-310; Ladner, *supra.*).

The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures, are expressly incorporated herein by reference in their entirety.

EXAMPLES

EXAMPLE 1. DETERMINATION OF FRα LEVELS IN URINE SAMPLES FROM HUMAN SUBJECTS WITH AND WITHOUT OVARIAN CANCER AS MEASURED BY ELECTROCHEMILUMINESCENCE IMMUNOASSAY (ECLIA).

Materials and Methods

Urine samples were obtained from human subjects, including subjects afflicted with ovarian cancer and normal control subjects not afflicted with ovarian cancer. The levels of FRα in urine samples were determined using an electrochemiluminescence immunoassay (ECLIA) according to the following procedure (see Namba *et al.* (1999) *Analytical Science* 15:1087-1093):

i. Antibody coating to micro beads

The monoclonal anti-folate receptor alpha antibody was coated over the surface of micro beads (Dynabeads M-450 Epoxy, Dynal). Thirty six milligrams of micro beads were mixed with 1.2 mL of antibody MOV18 (0.36 mg/mL, Enzo Life Science) in 0.15 mol/L phosphate buffer saline pH 7.8 (PBS), followed by gentle mixing for 16 hours at room temperature. The micro beads were then washed 5 times with 50 mM HEPES buffer containing 0.1% normal rabbit serum (NRS), 150 mmol/L NaCl, 0.01% Tween 20 pH 7.5 (wash buffer). Thereafter, the coated micro beads were suspended in 1.2 mL 50 mM HEPES buffer containing 20% NRS, 150 mmol/L NaCl and 0.01% Tween 20 pH 7.5 (reaction buffer) to block the unbound surface, followed by gentle mixing for 3.5 hours at room temperature. Finally, the micro beads were washed 5 times with wash buffer and re-suspended with 1.2 mL 50 mM HEPES buffer containing 10% NRS, 150 mmol/L NaCl, 10 mmol/L EDTA-2Na and 0.01% Tween 20 pH 7.5 (reaction buffer) so that the concentration of micro beads was 30 mg/mL. The micro beads were stored at 4°C until use.

ii. Antibody labeling with Ruthenium-chelate-NHS (Ru)

One milliliter of MORAB-003 (1 mg/mL) in PBS was mixed with 14 µL of Ru (10 mg/mL), initial molar ratio of antibody to Ru was 1:20, followed by shaking for 30 minutes at room temperature in the dark. The reaction was terminated by adding 25 µL

of 2 mol/L glycine solution followed by incubation for 20 minutes. The labeled antibody was purified by gel filtration using Sephadex G-25 (GE Healthcare) eluted with PBS. The first eluted yellow fraction was collected and the concentration of antibody and Ru were determined by means of the Pierce BCA protein assay kit (Thermo Scientific) and absorption at 455 nm respectively. The final molar ratio was calculated by the formula: Final molar ratio = [(absorption at 455)/13700]/[Ab(mg/mL/150000)]. The labeled antibody was stored at 4°C until use.

iii. One Step Immunoassay

The antibody coated micro beads were set on the reagent table of the Picolumi 8220 (Sanko, Tokyo, Japan) after adjusting the concentration of the beads to 1.5 mg/ml (working solution) in reaction buffer. The Ru labeled antibody was set on the reagent table of the Picolumi 8220 after adjusting the concentration of antibody to 2 µg/ml (working solution) in reaction buffer.

Ten microliters of urine (diluted 1:51 in reaction buffer) or standard FRα (prepared in reaction buffer) and 100 µL of reaction buffer were dispensed into a reaction tube (Sanko, Tokyo, Japan) and set on Picolumi 8220.

The following steps were automatically run by Picolumi 8220. Twenty five microliters of beads (working solution) and 180 µL of Ru labeled antibody (working solution) were dispensed. After 26 minutes of incubation at 30 +/-2°C, the beads were washed and suspended with 300 µL of electrolyte solution (Sanko, Tokyo, Japan). The washed beads were subsequently transferred to the electrode and electrochemiluminescence (ECL) emission was measured.

All ECL measurements were carried out in duplicate.

Results

Table 1 depicts the urine levels of FRα in individual subjects with ovarian cancer and non-afflicted female control subjects.

Table 1: FRα levels in urine of subjects with ovarian cancer and normal female control subjects

Group	Sample #	FRα (pg/mL)

ovarian cancer	1	27800
	2	40242
	3	85580
	4	4994
	5	2017
	6	3781
	7	29469
	8	47456
	9	4479
	10	11920
	11	18352
	12	162017
	13	30630
	14	14431
	15	11801
	16	13470
	17	11563
	18	22185
	19	52106
normal control	20	8491
	21	4885
	22	3595
	23	21301
	24	22757
	25	16578
	26	6081
	27	4195
	28	12169
	29	20639

Figure 2 depicts the distribution of FRα levels in urine in subjects with ovarian cancer and in normal female control subjects, as set forth in Table 1.

Table 2 summarizes the number of subjects (n), mean, standard deviation (SD), maximum (Max.) and minimum (Min.) values for the levels of FRα in the ovarian cancer group and the normal female control group.

Table 2: Summary of urine FRα measurement

	FRα (pg/mL)	
	ovarian cancer	**normal female**
N	19	10
Mean	31279	12069
SD	37895	7654
Max.	162017	22757

Min.	2017	3595

Discussion

A high level of FRα was detected in the urine of subjects with ovarian cancer. Moreover, the levels of FRα differed significantly between ovarian cancer and normal female control groups (p=0.03, one-sided).

EXAMPLE 2. DILUTION LINEARITY - DETERMINATION OF FRα LEVELS IN SERIALLY DILUTED URINE SAMPLES MEASURED BY ELECTROCHEMILUMINESCENCE IMMUNOASSAY (ECLIA)

Dilution Linearity is a measure of accuracy of an assay. Two urine samples were serially diluted by a factor of 10 and 100. The FRα levels of each sample were measured as set forth in Example 1 and compared to assess the percent error. Percent error was calculated as follows:

$$\frac{[[(\text{FR}\alpha \text{ in diluted sample}) * (\text{dilution factor})] - (\text{FR}\alpha \text{ in undiluted sample})] * 100}{(\text{FR}\alpha \text{ in undiluted sample})}$$

The results are set forth in Table 3:

Table 3: Dilution Linearity for Urine

Sample	Dilution factor	FRα (pg/mL)	Error (%)
1	1	25037	-
	10	2601	4
	100	279	11
2	1	16649	-
	10	1696	2
	100	173	4

The foregoing results demonstrate dilution linearity in assessing the levels of FRα in human urine samples and that, within acceptable errors, urine can be diluted up to a

factor of at least 100 while retaining accurate levels of FRα. Accordingly, dilution of the urine samples may be considered prior to determining levels of FRα.

EXAMPLE 3: CENTRIFUGATION OF URINE SAMPLES - ADDRESSING REPRODUCIBILITY

The reproducibility of the ECLIA assay for a particular urine sample was also tested. For example, as reflected in Table 4, ECLIA assays of the same sample resulted in varying results.

Table 4. Reproducibility without sample centrifugation

	ECL Counts	
Sample	**Test 1**	**Test 2**
1	29380	15046
2	20912	17227

The presence of insoluble material (precipitates) in urine samples was hypothesized to be responsible for the variability seen in measuring the levels of FRα. As a result, centrifugation of samples in order to remove urine sediment, prior to measurement of FRα levels, was considered as an option to enhance the accuracy and reproducibility of the assay.

Table 5 depicts the results obtained when three samples were centrifuged prior to performance of the ECLIA assay.

Table 5. Reproducibility with sample centrifugation

		FRα concentration (ng/mL)	
Test	**Sample 1**	**Sample 2**	**Sample 3**
1	10.4	9.2	13.3
2	10.5	8.9	14.0
3	10.3	9.2	13.4
Mean	10.4	9.1	13.6
SD	0.1	0.1	0.3
CV(%)	1.0	1.1	2.2

As set forth above, the results indicate that centrifugation provided more consistent measurements of FRα concentration.

Additionally, two samples were subjected to (i) centrifugation (at 2000 x g for 2 min) and the supernatant removed for measurement of FRα (depicted as sample "A" below in Table 6) and (ii) centrifugation followed by vortexing (depicted as sample "B" below in Table 6), prior to measurement of FRα levels by the ECLIA assay set forth in Example 1. The results are reflected in Table 6 below.

Table 6: Effect of centrifugation on FRα levels in urine

Sample	Sediment after centrifugation	A/B	FRα (pg/mL)	Difference (%)
1	Yes (++)	A	13678	-
		B	16559	21
2	Yes (+)	A	12271	-
		B	13206	8

Difference (%) was determined as follows:

$$\frac{[(\text{Level of FR}\alpha \text{ in "B"}) - (\text{Level of FR}\alpha \text{ in "A"})] * 100}{(\text{Level of FR}\alpha \text{ in "A"})}$$

As shown in Table 6, the levels of FRα as determined by the ECLIA assay vary depending on whether urine was clarified by centrifugation to remove precipitates or whether urine was vortexed to suspend or disperse sediments. Accordingly, in certain embodiments centrifuging or vortexing of urine samples may be performed prior to determining levels of FRα.

EXAMPLE 4. DETERMINATION OF FRα LEVELS IN CENTRIFUGED URINE SAMPLES FROM HUMAN SUBJECTS WITH AND WITHOUT OVARIAN CANCER MEASURED BY ELECTROCHEMILUMINESCENCE IMMUNOASSAY (ECLIA).

Based on the results of Example 3, the assay for assessing FRα levels in subjects was modified to introduce a centrifugation step. FRα levels were determined on the same samples utilized in Example 1, including the group of subjects with ovarian cancer and the group of normal female control subjects.

Materials and Methods

The methodology utilized was as described in Example 1, except that the urine samples were centrifuged for 10000 x g for 1 minute and the resulting supernatant subsequently diluted by 1:51 in reaction buffer.

Results

Table 7 depicts the levels of FRα in centrifuged and non-centrifuged urine samples from subjects afflicted with ovarian cancer and healthy female control subjects.

Table 7: Urine FRα1 level in ovarian cancer and normal control group

		FRα (pg/mL)		
Group	**Sample #**	**Without centrifugation**	**With centrifugation**	**sediment after centrifugation**
ovarian cancer	1	27800	23960	+
	2	40242	37852	+
	3	85580	78976	+
	4	4994	3766	+
	5	2017	1512	-
	6	3781	3443	-
	7	29469	25728	+
	8	47456	16556	+
	9	4479	3357	-
	10	11920	5020	+
	11	18352	16695	-
	12	162017	82705	+
	13	30630	4496	+
	14	14431	8786	+
	15	11801	10582	-
	16	13470	5611	+
	17	11563	5463	+
	18	22185	14443	+
	19	52106	38327	-
normal control	20	8491	6867	+
	21	4885	3754	-
	22	3595	3529	-

	23	21301	15047	+
	24	22757	4850	+
	25	16578	14366	+
	26	6081	5201	-
	27	4195	3135	-
	28	12169	499	+
	29	20639	2439	+

According to the results set forth in Table 7, centrifugation resulted in a decrease in the measurement of FRα levels in some samples, as previously demonstrated in Table 6.

EXAMPLE 5: DETECTION OF FRα IN URINE SEDIMENT BY IMMUNOBLOTTING

Based on the results shown in Examples 3 and 4, the presence or absence of FRα in urine sediment/precipitate was assessed using western blotting.

Materials and Methods

Urine samples from 2 ovarian cancer patients for whom FRα concentrations were measured at 18,747 pg/mL and 145,564 pg/mL, respectively (See Table 10 *supra*), were subjected to the following procedures. Control samples consisted of HeLa cell lysate 10 µg, liver tissue lysate 20 µg, and ovarian cancer tissue lysate 20 µg.

- 900 µL urine was centrifuged for 2 minutes at 10000g
- supernatant was removed
- the remaining pellet was dissolved in 15 µL of PAGE sample buffer (containing 292 mM LDS) and subsequently boiled at 70°C for 10 min
- The entire sample (approx. 20 µL) was loaded onto the NuPAGE bis-tris gel (Invitrogen)
- After electrophoresis, proteins were transferred to PVDF membrane
- 1% skim milk / 0.05% Tween 20 / PBS was added for blocking
- The membrane was washed with 0.05% Tween 20 / PBS

- 0.5 mL of monoclonal antibody 548908 (R&D Systems) at 2 μg/ml was added and incubated for 60 minutes at room temperature
- The membrane was washed with 0.05% Tween 20 / PBS
- 10 mL of anti-mouse IgG-HRP (DAKO p0447, 1:2000) was added and allowed to incubate for 60 minutes
- The membrane was washed with 0.05% Tween 20 / PBS
- Pierce ECL Substrate was added to the membrane
- The membrane was removed from the substrates and then imaged using the LAS-3000 (FUJIFILM) system

Results

The resulting immunoblot is shown in Figure 3. In this figure, lanes 1-5 correspond to FRα detected from the following sources:

(1) urine from ovarian cancer patient with a measured FRα level of 18,747 pg/mL

(2) urine from ovarian cancer patient with a measured FRα level of 145,564 pg/mL

(3) HeLa cell lysate: 10 μg

(4) Liver tissue lysate: 20 μg

(5) ovarian cancer tissue lysate: 20 μg

Lane 6 in the western blot represents molecular weight markers and demonstrates that the observed band in lanes 1, 2, 3 and 5 runs at the expected molecular weight for FRα.

Lanes 3 and 5 are positive control samples and lane 4 is a negative control sample. The faint band on lane 1 and the clear band on lane 2 demonstrate that FRα can be detected in the urine sediment of ovarian cancer patients by western blotting.

EXAMPLE 6. DETERMINATION OF FRα LEVELS IN GUANIDINE-TREATED NORMAL HUMAN URINE SAMPLES BY ELECTROCHEMILUMINESCENCE IMMUNOASSAY (ECLIA).

Based on the results of Example 4 in which centrifugation resulted in decreased FRα levels, and the results of Example 5 where the urine sediment obtained from centrifugation was shown to contain immunoreactive FRα, methods were sought to

solubilize the sediments of urine to obtain more quantitative and accurate measurements of FRα.

In this regard, treatment of normal female urine samples with guanidine prior to assessing FRα levels was attempted.

The methodology utilized was as described in Example 1, except that the urine samples were mixed in a 1:1 ratio with either 6 M guanidine in buffer (PBS) or buffer alone. Subsequently, the urine samples were diluted by 1:51 in reaction buffer.

The results of this assay are shown in Table 8.

Table 8: Normal urine FRα level with or without guanidine treatment

Sample		Guanidine treatment	FRα (pg/mL)
	Std Ag	Yes	83964
		No	82512
Normal Urine	1	Yes	9431
		No	7796
	2	Yes	5713
		No	4066
	3	Yes	9687
		No	9428

The results of this experiment indicate that guanidine does not interfere with FRα measurements. As can be seen for the pure antigen control (Std Ag), this methodology of guanidine treatment and subsequent dilution has no effect on the measurement of FRα. Further, it will be noted that in all three (3) urine samples assessed, the levels of FRα were higher in the samples treated (solubilized) with guanidine relative to the samples not treated with guanidine.

The reliability of guanidine pre-treatment of urine samples was further assessed by exposing three samples to guanidine and measuring the FRα concentration of each guanidine treated sample 3 times using the ECLIA assay. The results are reflected in Table 9 below:

Table 9: Intra-assay reproducibility of guanidine treated urine

	FRα (pg/mL)		
Test	**Sample 1**	**Sample 2**	**Sample 3**

1	9210	5477	9889
2	9638	5405	10047
3	10192	5812	10944
Mean	9680	5565	10293
SD	492	217	569
CV(%)	5.1	3.9	5.5

As set forth above, the results indicate that guanidine treatment of urine prior to FRα assay provided consistent measurements of FRα concentration with very low CV's.

EXAMPLE 7. DETERMINATION OF FRα LEVELS IN GUANIDINE-TREATED URINE SAMPLES FROM HUMAN SUBJECTS WITH AND WITHOUT OVARIAN CANCER MEASURED BY ELECTROCHEMILUMINESCENCE IMMUNOASSAY (ECLIA).

Based on the results of Example 6 in which guanidine treatment was shown not to interfere with FRα assays, a modified assay protocol was employed to measure FRα in the urine samples from the subjects with and without ovarian cancer in Example 1.

The following assay protocol was employed:

Materials and Methods

The methodology utilized was as described in Example 1, except that the urine samples were mixed in a 1:1 ratio with a 6 M guanidine buffer and subsequently diluted by 1:26 in reaction buffer.

Results

Table 10 depicts the levels of FRα in guanidine treated urine samples from subjects afflicted with ovarian cancer and healthy female control subjects.

Table 10: Urine FRα level in ovarian cancer and normal control group

Group	Sample #	FRα (pg/mL)
ovarian cancer	1	27015
	2	37315

	3	79579
	4	285
	5	1864
	6	2902
	7	27914
	8	51864
	9	2699
	10	9455
	11	18396
	12	145564
	13	19046
	14	10440
	15	10977
	16	9199
	17	18223
	18	18747
	19	51098
normal control	20	8012
	21	3797
	22	3323
	23	20976
	24	6941
	25	14512
	26	7286
	27	2789
	28	2617
	29	7233

Figure 4 shows the distribution of FRα levels in the urine of ovarian cancer afflicted subjects and normal female control subjects using the modified protocol with guanidine treatment. A statistically significant difference between groups was observed. Table 11 summarizes these results.

Table 11: Summary of urine FRα measurement

	FRα (pg/mL)	
	ovarian cancer	normal control
n	19	10
Mean	28557	7749
SD	34990	5850
Max.	145564	20976
Min.	285	2617

Using the data from this experiment, a receiver operating characteristic (ROC) analysis was performed. Figure 5 shows an ROC curve of the sensitivity and specificity of the ECLIA measurement of FRα levels in urine after the urine was treated with guanidine. AUC is the area under the curve, which measures the accuracy of the test in separating ovarian cancer from control subjects.

Using an arbitrary cutoff value of 9100 pg FRα/mL, the AUC was 0.70 with a positive predictive value of 70% and a negative predictive value of 80%, as shown in Table 12. Using this cutoff value, 15/19 ovarian cancer patients had a concentration of FRα above 9100 pg/mL and 8/10 normal subjects had a concentration of FRα less than 9100 pg/mL.

Table 12: Guanidine treatment for urine measurement

	ovarian cancer	control
Number of Samples	19	10
Positive	15	2
Predictive value (%)	78.9	80.0

EXAMPLE 8: CREATININE CORRECTION OF FRα CONCENTRATIONS DETERMINED IN GUANIDINE-TREATED URINE SAMPLES BY ELECTROCHEMILUMINESCENCE IMMUNOASSAY (ECLIA)

Concentrations of FRα were previously determined using ECLIA of guanidine-treated urine samples from ovarian cancer patients and normal female controls (See Example 7, Table 10). Here, these FRα concentrations were corrected for urine creatinine levels in order to normalize for the glomerular filtration rate. The resulting values were subjected to an ROC analysis.

Methods

The urine creatinine level was determined by the Ministry of Health, Labour and Welfare approved test kit, determiner L CRE (Kyowa Medex, Japan). The corrected value for urine FRα concentration was calculated as follows:

Urine FRα Creatinine correction (ng/g)

= (Urine FRα (ng/L) x 1000) / (Urine Creatinine (mg/dL) x10)

= (Urine FRα (ng/L) x 1000) / Urine Creatinine (mg/L)

= Urine FRα (ng/L) / Urine Creatinine (g/L)

= Urine FRα (ng) / Urine Creatinine (g)

or

= 1/1000 x Urine FRα (μg) / Urine Creatinine (g)

Results

Table 13 presents the resulting creatinine-corrected FRα levels.

Table 13: Creatinine-corrected FRα levels determined using ECLIA of guanidine-treated urine samples

Group	Sample #	FRα (pg/mL)	Corrected FRα (μg FRα/ g creatinine)
ovarian cancer	1	27015	11.6
	2	37315	37.8
	3	79579	33.9
	4	285	0.6
	5	1864	6.7
	6	2902	7.1
	7	27914	54.9
	8	51864	17.1
	9	2699	13.5
	9	9455	14.5
	10	18396	23.1
	11	145564	66.0
	12	19046	9.1
	13	10440	8.5
	14	10977	7.4
	15	9199	5.9
	16	18223	13.0
	17	18747	9.6
normal control	18	3797	7.7
	19	3323	7.9
	20	20976	10.7
	21	6941	4.3
	22	14512	8.6
	23	7286	13.8
	24	2789	4.3
	25	2617	1.9

	26	7233	3.1

Figure 6 shows the distribution of FRα levels in ovarian cancer (OC) and normal female control subjects after correction for urine creatinine levels. There is a statistically significant difference between ovarian cancer patients and controls in creatinine-corrected levels of FRα (p=0.007).

The summary data for ovarian cancer and normal control subjects are provided in Table 14.

Table 14: Summary statistics for creatinine-corrected FRα levels

	FRα (μg/g-creatinine)	
	ovarian cancer	**normal control**
n	18	9
Mean	18.9	6.9
SD	17.9	3.9
Max.	66.0	13.8
Min.	0.6	1.9

The creatinine-corrected FRα levels were further subjected to an ROC analysis. The ROC curve is shown in Figure 7. Table 15 presents the sensitivity, specificity, and area under the curve (AUC) for various cutoff values of the creatinine-corrected test.

Table 15: Sensitivity, specificity, and AUC for various cutoff values of the creatinine-corrected FRα test

Cut-off	Sensitivity	Specificity	AUC
3.0	94.4%	11.1%	0.67
4.0	94.4%	22.2%	0.70
5.0	94.4%	44.4%	0.78
6.0	88.9%	44.4%	0.74
9.0	66.7%	77.8%	0.70

As previously noted, there is a clear discrimination between urines of ovarian cancer patients and those from healthy female control subjects.

EXAMPLE 9: ENZYME IMMUNOASSAY (EIA) AND OPTIMIZATION THEREOF

1. Enzyme Immunoassay (EIA)

Antibody coating to microtiter plates

The monoclonal anti-folate receptor alpha antibody was coated on the surface of microtiter plates (Nunc-immunoplate, Thermo Scientific) as follows. One hundred microliter of antibody (absorbance 0.02 at 280 nm) in 50 mmol/L carbonate buffer pH 9.4 was dispensed into wells, followed by coating for 16 hours at 4°C. The microplates were then washed 2 times with PBS containing 0.05% Tween20 (PBS-T). Thereafter 0.15 mL of PBS containing 20% normal rabbit serum pH 7.8 was dispensed into wells to block the unbounded surface, followed by blocking for 1 hour at room temperature. Finally, the microplates were washed 2 times with PBS-T. The antibody coated plates were dried and kept at 4°C in aluminum bags until use.

Biotin labeling

Biotin labeling was conducted according to the manufacturers recommendations for the EZ-Link Sulfo-NHS-LC-LC-Biotin (Product No. 21338, Thermo Scientific). Briefly, 1 mg of antibody in 0.4 mL of PBS was mixed with 0.013 mL of 10 mM Sulfo-NHS-LC-LC-Biotin, with an initial molar ratio of antibody to biotin of 1:20, followed by incubation for 30 min at room temperature. The biotin coupled antibody was purified by gel filtration using a PD-10 column (GE Healthcare) eluted with PBS to remove non-reacted biotin. In order to determine the level of biotin incorporation, the EZ Biotin quantitation kit (Product No. 28005, Thermo Scientific) was used. The biotin labeled antibody was stored at -80°C until use.

Two step immunoassay

For the first reaction, 40 µL of plasma or standard antigen and 60 µL of 50 mM HEPES buffer containing 10% NRS, 150 mmol/L NaCl, 10 mmol/L EDTA-2Na, 0.01% Tween 20 pH 7.5 (reaction buffer) was dispensed into antibody coated wells. The plate

was incubated for 18 hours at 4°C, and subsequently washed 5 times with PBS-T. For the second reaction, 100 μL of 10 μg/mL biotin labeled antibody in reaction buffer was dispensed. The plate was incubated for 1 hour at room temperature, and subsequently washed 5 times with PBS-T. 100 μL of horse radish peroxidase labeled streptavidin (Pierce) was dispensed. After 30 minutes incubation at room temperature, the plates were washed 5 times with PBS-T. Finally, for the color development, 100 μL of TMB solution (KPL) was dispensed and left for 15 minutes in dark. After stopping color development by adding 100 μL of 1N HCl, the absorption at 450 nm was read using a plate reader. All washing steps were automatically done by auto-plate washer (AMW-8, BioTec, Japan), and all EIA measurements were carried out in duplicate.

Figure 8 depicts the EIA assay using MOV18 as the capture antibody and biotinylated MORAb-003 as the detector antibody.

2. Optimization of EIA procedures

The above EIA procedures were arrived at in part based on the following experiments designed to optimize the procedure.

First, avidin-HRP, biotin labeled antibody and HRP labeled antibody were compared. Compared with HRP labeled antibody, biotin labeled antibody and avidin-HRP provided a higher signal; therefore, biotin labeled antibody and avidin-HRP were employed.

Second, one and two-step incubation procedures were compared. As depicted in Figure 9, a two-step incubation procedure yielded a higher signal and was thus employed.

Third, to optimize the second incubation time, incubation times of one to four hours were compared. The results indicated that one hour incubation times provided the highest signal to noise ratio and therefore an incubation time of one hour was subsequently employed.

Fourth, in order to optimize the working concentration of biotin labeled antibody, HRP labeled antibody and sample volume, various concentrations were employed as set forth in the above description of the EIA assay. The optimal values concentrations are described above.

EXAMPLE 10: COMPARISON OF FRα IN HUMAN PLASMA USING ELECTROCHEMILUMINESCENCE IMMUNOASSAY (ECLIA) AND ENZYME IMMUNOASSAY (EIA)

The levels of FRα were measured in human plasma samples taken from ovarian cancer patients and healthy female controls using the electrochemiluminescence assay (ECLIA) described in Example 1 and Figure 1 (using MORAb-003 as the capture antibody and ruthenium (Ru)-labeled MOV-18 as the labeled detector antibody) and the enzyme immunoassay (EIA) described in Example 9 and Figure 8. In both assays, 40 µL of plasma was assayed.

Table 16 shows the plasma levels of FRα in ovarian cancer and normal control subjects, as determined using the EIA and ECLIA.

Table 16: Plasma concentrations of FRα determined using EIA and ECLIA methods

Group	Sample #	FRα concentration (pg/mL)	
		EIA	ECLIA
Ovarian cancer	1	10	73
	2	<10	200
	3	56	286
	4	44	286
	5	353	1606
	6	83	494
Healthy control	7	110	127
	8	162	112
	9	88	252
	10	180	254
	11	262	471
	12	206	396

With only one exception, the results for all of the subjects indicated that the concentrations of FRα detected in serum using EIA are lower than the levels detected using ECLIA, demonstrating that the EIA assay, as formatted, is not as sensitive as the ECLIA assay when this particular combination of capture (MOV-18) and detector

antibodies (MORAb-003) is used. Therefore, further experiments with other types of antibodies were conducted to develop a more sensitive EIA procedure.

EXAMPLE 11: FEASIBILITY OF DIFFERENT TYPES OF ANTIBODIES FOR EIA MEASUREMENT OF FRα IN HUMAN PLASMA

1. Preliminary experimentation of Antibody Combinations

Various combinations of capture/detector antibodies were considered. Preliminary experimentation rendered the results set forth in Table 17.

Table 17

		Capture antibody							
		MORAB-003		MOV18		548908(R&D)		6D398	
Biotin-labeled detection antibody	003	Blank Std. 1-S	Low - -	Blank Std. 1-S	Low +++ ++	Blank Std. 1-S	Low ++ ++	Blank Std. 1-S	Low ++ +
	MOV18	Blank Std. 1-S	Low + ++	Blank Std. 1-S	High - +++	Blank Std. 1-S	High - ++	Blank Std. 1-S	High - +++
	548908							Blank Std. 1-S	Low - ++

2. Comparison of EIA assays using various antibody combinations and comparison to the ECLIA assay

The levels of FRα in plasma from ovarian cancer patients and normal healthy female controls were measured using an enzyme-linked immunosorbent assay (EIA) with different combinations of capture and biotin-labeled antibodies and compared with the levels of FRα measured using the ECLIA assay.

Materials and Methods

The ECLIA method was as described in Example 1 and depicted in Figure 1 (using the MORAb-003 antibody as the capture antibody and the Mov-18 antibody as

the labeled detector antibody). The EIA method was as described in Example 9, except that three different combinations of capture/detector antibodies were employed, as depicted in Figure 10: MOV18/MORAb-003, 548908/MORAb-003 and 6D398/MORAb-003. The antibodies 548908 and 6D398 are commercially available. The 548908 antibody was obtained from R&D Systems (North Las Vegas, NV) and the 6D398 antibody was obtained from US Biological (Swampscott, MA 01907).

Results

The concentrations of FRα (pg/mL) determined using the EIA and ECLIA methods are shown in Table 18. In addition, the concentrations of FRα (pg/ml) determined by EIA using various combinations of capture/detector antibodies are depicted graphically in Figure 11.

Table 18: Plasma concentrations of FRα (pg/mL) determined using the EIA and ECLIA methods with various combinations of capture and detector antibodies.

Group	Sample #	EIA 548908-MORAb-003	EIA 6D398-MORAb-003	EIA MOV18-MORAb-003	ECLIA MOV18-MORAb-003
Ovarian cancer	1	176	2	10	217
	2	85	<0	-	165
	3	257	35	-	296
	4	117	42	44	322
	5	2048	370	353	1335
	6	447	63	83	390
Normal control	7	247	66	110	137
	8	213	110	162	185
	9	367	78	88	219
	10	364	152	180	228
	11	804	224	262	388
	12	473	194	206	318

The data in Table 18 indicate that the measurements of FRα levels with EIA using the 54908-MORAb-003 combination yields results that are most similar to the

results obtained using the ECLIA assay. Quantitive analyses were performed, confirming this observation. Further, these data demonstrate that the detection of FRα is highly dependent on the antibodies and antibody combination employed. Accordingly, different antibody combinations can be employed for the determination of FRα in biological fluids. In addition, since the data obtained from the EIA and the ECLIA assay formats are similar, various assay formats can be used for the determination of FRα.

For each of the three combinations of capture and detector antibodies used for the EIA method, a regression analysis was performed, and the concentrations of FRα (pg/mL) determined with EIA were correlated with the concentrations determined with the ECLIA assay. The results of this analysis are shown in Table 19 and in Figure 12.

Table 19: Correlations of plasma concentrations of FRα measured by ECLIA with concentrations measured by EIA using three combinations of capture and detector antibodies

Capture antibody-detector antibody	548098-MORAb-003	6D398-MORAb-003	MOV18-MORAB-003
r	0.960	0.781	0.715
Slope	1.595	0.285	0.223
Intercept	-87.06	-0.64	58.62

The results for EIA using the 548098-MORAb-003 capture-detector combination correlated highly (r=0.96) with the results for ECLIA.

EXAMPLE 12: PLASMA LEVELS OF FRα DETERMINED BY EIA AND ECLIA IN SAMPLES FROM OVARIAN CANCER PATIENTS

Measurements of serum FRα levels were determined in a group of ovarian cancer patients (n=17) and normal controls (n=35) using ECLIA and EIA. For the EIA measurements, the 548908 capture/ MORAb-003 detector antibody combination was employed. The EIA procedure was otherwise as described in Example 9. The ECLIA procedure was as described in Example 1. The results are shown in Table 20.

Table 20: Plasma FRα concentrations in ovarian cancer patients and normal controls, as determined using EIA and ECLIA

Group	Sample #	EIA	ECLIA
		pg/mL	pg/mL
Ovarian cancer	1	245	217
	2	247	223
	3	194	229
	4	2613	1335
	5	154	153
	6	319	215
	7	516	390
	8	370	271
	9	933	449
	10	4768	4502
	11	385	266
	12	251	322
	13	404	349
	14	338	371
	15	4147	2344
	16	179	165
	17	380	296
Control	18	232	181
	19	372	173
	20	332	189
	21	380	203
	22	376	290
	23	406	217
	24	281	182
	25	348	191
	26	490	247
	27	253	137
	28	368	185
	29	338	195
	30	289	219
	31	338	206
	32	406	226
	33	365	228
	34	501	280
	35	806	388
	36	613	286
	37	380	250
	38	420	281
	39	393	280
	40	552	284

	41	664	318
	42	429	261
	43	499	286
	44	310	218
	45	281	217
	46	215	202
	47	293	217
	48	380	256
	49	270	195
	50	393	234
	51	425	308
	52	226	199

Figure 13 shows the distribution of plasma FRα concentrations in subjects with ovarian cancer and normal female control subjects as determined using EIA.

Table 21 shows summary descriptive statistics for the plasma FRα concentrations in ovarian cancer and normal female control subjects as determined using EIA.

Table 21: Summary of FRα plasma concentrations in ovarian cancer and normal female control subjects as determined using EIA.

	FRα (pg/mL)	
	Ovarian cancer	**Normal control**
n	**17**	**35**
Mean	**967**	**389**
SD	**1438**	**126**
Max.	**4768**	**806**
Min.	**154**	**215**

Figure 14 further depicts the correlation between FRα plasma concentrations determined using EIA and ECLIA. The correlation is high (r=0.95).

EXAMPLE 13. DETERMINATION OF FRα LEVELS IN MATCHED URINE AND SERUM SAMPLES FROM LUNG CANCER PATIENTS AND OVARIAN CANCER PATIENTS AS MEASURED BY ELECTROCHEMILUMINESCENCE IMMUNOASSAY (ECLIA)

FRα levels were determined in matched urine and serum samples from lung cancer and ovarian cancer patients using ECLIA where the samples were taken from the same patient. The correlation between serum and urine FRα levels was also determined.

Materials and Methods

The ECLIA methodology utilized is as described in Example 1. Guanidine was used to solubilize urine sediments as described in Example 6.

Results

The results of the ECLIA assays of serum and urine from lung cancer and ovarian cancer patients are presented in Table 22.

Table 22: FRα concentrations in matched urine and serum samples of lung cancer patients and ovarian cancer patients, as determined by ECLIA

		Serum	Urine
Group	set ID	pg/mL	pg/mL
Lung cancer	1	146	2009
	2	153	4496
	3	206	-
	4	70	3562
	5	195	12381
	6	352	21873
	7	198	11296
	8	120	18570
	9	275	4455
	10	163	8662
	11	145	5294
	12	178	-
	13	165	1106
	14	187	7446
	15	168	11167
	16	217	24448
	17	142	6724
	18	177	14514
	19	236	822
	20	101	4826
	21	145	7723
	22	213	9887
	23	143	7422
	24	253	3376
	25	421	8045
ovarian cancer	26	282	9414
	27	1605	7651
	28	240	13059
	29	695	10549

Summary data for serum and urine FRα levels of lung cancer patients is presented in Table 23.

Table 23: Summary statistics for FRα concentrations in matched serum and urine samples of lung cancer patients, as determined by ECLIA

Lung cancer	FRα (pg/mL)	
	Serum	Urine
n	25	23
Mean	191	8700
SD	75	6291
Max.	421	24448
Min.	70	822

Summary data for serum and urine FRα levels of ovarian cancer patients is presented in Table 24.

Table 24: Summary statistics for FRα concentrations in matched serum and urine samples of ovarian cancer patients, as determined by ECLIA

Ovarian cancer	FRα (pg/mL)	
	Serum	Urine
n	4	4
Mean	705	10168
SD	634	2266
Max.	1605	13059
Min.	240	7651

Figure 15 shows correlations between ECLIA measures of FRα levels in matched serum and urine samples taken from the same patient. The correlation for lung cancer patients was r=0.24 (upper panel) and the correlation for ovarian cancer patients was r=-0.76 (lower panel).

These data demonstrate the relative lack of correlation between FRα concentrations measured in urine versus serum, especially as shown for lung cancer patients. Further, these data demonstrate that FRα is basically non-detectable above

background levels in the serum of lung cancer patients versus normal controls whereas FRα is detectable in the urine of these patients.

EXAMPLE 14. ASSESSMENT OF LEVELS OF FRα IN SERUM SAMPLES FROM PATIENTS WITH OVARIAN CANCER, PATIENTS WITH LUNG CANCER, AND NORMAL CONTROLS

FRα levels in the serum of patients with ovarian cancer, patients with lung cancer, and normal controls were assessed. Serum FRα levels were assessed using ECLIA with two different pairs of capture-detector antibodies: **Pair 1**, in which 9F3 was the capture antibody and 24F12 was the detector antibody, and **Pair 2**, in which 26B3 was the capture antibody and 19D4 was the detector antibody.

The FRα pairs were tested with full calibrator curves and 196 individual serums diluted 1:4. In one experiment, 26B3 was used as the capture antibody after CR processing on a plate lot (75 µg/mL, +B, +T) and 19D4 was used as the detector antibody at 1.0µg/mL. In another experiment, 9F3 was used as the capture antibody and 24F12 was used as the detector antibody at 1.0µg/mL. Each were CR processed (lot 10070) with an label to protein ratio (L/P) of 13.3. Diluent 100 (Meso Scale Discovery, Gaithersburg, Maryland) + human anti-mouse antibody (HAMA) +mIgG was used for samples and calibrator. Diluent 3 (Meso Scale Discovery, Gaithersburg, Maryland) was used for detections.

The following protocol was employed for the ECLIA. Samples were added at 50µL/well. The samples were shaken for 2 hours and subsequently washed with Phosphate Buffered Saline (PBS) solution with the detergent Tween 20 (PBST). The detector antibody was added at 25µL/well. The samples were shaken for 2 hours and then were washed with PBST. Finally, the electrochemiluminescence (ECL) emission of the samples was read with 2X MSD® Buffer T.

The results are shown in Table 25 below.

Table 25: FRα levels in serum of patients with ovarian cancer, patients with lung cancer, and normal controls

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	Sample Type	Adjusted backfit conc[2] (pg/mL)	%CV	Adjusted backfit conc[2] (pg/mL)	%CV	stage	grade	gender	comments
1	Ovarian Serum	3760	4%	3585	4%	III	2	F	Adenocarcinoma-Ovary
2	Ovarian Serum	223	3%	273	2%	III	3	F	Adenocarcinoma-Ovary
3	Ovarian Serum	950	1%	3346	8%	IIIC		F	Papillary Serous Carcinoma
4	Ovarian Serum	3827	4%	968	0%	III	2	F	Adenocarcinoma-Ovary
5	Ovarian Serum	251	6%	468	2%	IV	2	F	Adenocarcinoma-Ovary
6	Ovarian Serum	199	6%	328	1%	IIIC	2	F	Cystadenocarcinoma
7	Ovarian Serum	166	1%	257	5%	IC	2	F	Cystadenocarcinoma
8	Ovarian Serum	182	4%	248	2%	IIIC	2	F	Cystadenocarcinoma
9	Ovarian Serum	155	6%	265	2%	IIIC	2	F	Cystadenocarcinoma
10	Ovarian Serum	145	9%	253	5%	IIIC	2	F	Cystadenocarcinoma
11	Ovarian Serum	142	5%	186	1%	IIB	1	F	Serous adenocarcinoma
12	Ovarian Serum	299	5%	456	2%	IB	3	F	Serous adenocarcinoma
13	Ovarian Serum	315	0%	768	8%	IIIB	high grade	F	Serous cystadenocarcinoma of the ovary

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	**Sample Type**	**Adjusted backfit conc[2] (pg/mL)**	**%CV**	**Adjusted backfit conc[2] (pg/mL)**	**%CV**	**stage**	**grade**	**gender**	**comments**
14	**Ovarian Serum**	168	6%	351	1%	I	high grade	F	Serous cystadenocarcinoma of the ovary
15	**Ovarian Serum**	187	8%	263	1%	I	Well to moderately differentiated	F	Papillary serous cystadenocarcinoma of the ovary
16	**Ovarian Serum**	423	3%	229	5%	IA	poorly differentiated	F	Mucinous cystadenocarcinoma of the ovary
17	**Ovarian Serum**	86	7%	289	1%	I	moderately differentiated	F	Papillary cystadenocarcinoma of the ovary
18	**Ovarian Serum**	57	3%	255	1%	I	well differentiated	F	Mucinous cystadenocarcinoma of the ovary
19	**Ovarian Serum**	108	1%	393	1%	III	moderately differentiated	F	Papillary mucinous cystadenocarcinoma of the ovary
20	**Ovarian Serum**	207	3%	328	1%	missing	poorly differentiated	F	Papillary cystadenocarcinoma of the ovary
21	**Ovarian Serum**	66	0%	254	1%	missing	high grade	F	Adenocarcinoma of the ovary
22	**Ovarian Serum**	108	2%	197	2%	III	low grade	F	Papillary cystadenocarcinoma of the ovary
23	**Ovarian Serum**	201	2%	457	1%	II	high grade	F	Papillary serous cystadenocarcinoma of the ovary
24	**Ovarian Serum**	477	1%	808	11%	III	high grade	F	Serous cystadenocarcinoma

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	Sample Type	Adjusted backfit conc[2] (pg/mL)	%CV	Adjusted backfit conc[2] (pg/mL)	%CV	stage	grade	gender	comments
									of the ovary
25	Ovarian Serum	423	3%	613	3%	n/a	n/a	F	transitional cell carcinoma
26	Ovarian Serum	249	8%	432	1%	n/a	2	F	ovarian carcinoma - endometrioid type
27	Ovarian Serum	152	7%	235	2%	IV	2	F	ovarian carcinoma - serous papillary type
28	Ovarian Serum	1409	5%	1287	3%	III C	3	F	ovarian carcinoma - serous type
29	Ovarian Serum	443	2%	547	2%	III C	2	F	ovarian carcinoma - serous papillary type
30	Ovarian Serum	208	1%	298	13%	IA	1	F	ovarian carcinoma - serous type
31	Ovarian Serum	114	9%	344	1%	III B	2	F	ovarian carcinoma - serous papillary type
32	Ovarian Serum	223	4%	157	133%	III C	1	F	ovarian carcinoma - serous papillary type
33	Ovarian Serum	5034	1%	4405	1%	III C	3	F	transitional cell carcinoma
34	Ovarian Serum	32966	6%	23228	4%	IV	n/a	F	ovarian carcinoma - serous papillary type
35	Ovarian Serum	94	6%	188	3%	n/a	3	F	clear cell adenocarcinoma
36	Ovarian Serum	866	6%	1317	1%	III C	3	F	poorly differentiated adenocarcinoma
37	Ovarian Serum	2916	8%	3121	0%	IV	3	F	ovarian carcinoma - serous papillary type
38	Ovarian Serum	679	4%	1037	17%	IV	3	F	ovarian carcinoma - serous type
39	Ovarian Serum	294	8%	478	3%	III C	3	F	ovarian carcinoma - serous papillary type

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	**Sample Type**	**Adjusted backfit conc[2] (pg/mL)**	**%CV**	**Adjusted backfit conc[2] (pg/mL)**	**%CV**	**stage**	**grade**	**gender**	**comments**
40	**Ovarian Serum**	2037	4%	12	74%	III C	3	F	ovarian carcinoma - serous type
41	**Ovarian Serum**	16289	6%	10431	3%	III C	2	F	ovarian carcinoma - serous papillary type
42	**Ovarian Serum**	386	6%	736	6%	n/a	3	F	ovarian carcinoma - serous papillary type
43	**Ovarian Serum**	1474	6%	2382	3%	IIIC		F	Papillary serous cystadenocarcinoma of the pelvic
44	**Ovarian Serum**	169	2%	438	1%	I	3	F	Adenocarcinoma of the ovary
45	**Ovarian Serum**	257	4%	635	10%	I	high grade	F	Adenocarcinoma of the ovary
46	**Ovarian Serum**	184	6%	393	0%	IIIC	poorly differentiated	F	Adenocarcinoma of the ovary
47	**Ovarian Serum**	251	6%	659	1%	IIA	2	F	Adenocarcinoma of the ovary
48	**Ovarian Serum**	165	2%	394	7%	I	2	F	Adenocarcinoma of the ovary
49	**Ovarian Serum**	64	7%	245	6%	IIIB	undifferentiated	F	Serous cystadenocarcinoma of the ovary
50	**Ovarian Serum**	90	3%	203	3%	IIIC		F	Serous adenocarcinoma nos
51	**Ovarian Serum**	167	3%	376	11%	IIIC	high grade	F	Serous papillary adenocarcinoma nos
52	**Ovarian Serum**	112	4%	260	5%	IIIC	high grade	F	Serous adenocarcinoma nos
53	**Ovarian Serum**	198	2%	340	6%	IV	high grade	F	Serous cystadenocarcinoma, nos

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		*FRα Pair 1*					
MSD Sample Testing Number	**Sample Type**	**Adjusted backfit conc[2] (pg/mL)**	**%CV**	**Adjusted backfit conc[2] (pg/mL)**	**%CV**	**stage**	**grade**	**gender**	**comments**
54	**Ovarian Serum**	134	3%	363	6%	I	moderately differentiated	F	Papillary mucinous cystadenocarcinoma of the ovary
55	**Ovarian Serum**	118	5%	304	10%	IB	high grade	F	Papillary serous cystadenocarcinoma of the ovary
56	**Ovarian Serum**	107	1%	326	4%	IIIB	high grade	F	Papillary serous cystadenocarcinoma of the ovary
57	**Ovarian Serum**	728	1%	1670	2%	IIIB		F	Papillary serous cystadenocarcinoma of the ovary
58	**Ovarian Serum**	2138	1%	3257	2%	IIIC		F	Papillary adenocarcinoma of the ovary
59	**Ovarian Serum**	167	4%	410	1%	III		F	Papillary serous cystadenocarcinoma of the ovary
60	**Ovarian Serum**	3054	4%	3285	14%	IIIC	high grade	F	Serous carcinoma of the ovary
61	**Ovarian Serum**	97	3%	215	0%	IIIB	high grade	F	Papillary serous cystadenocarcinoma of the ovary
63	**Lung Serum**	61	3%	366	10%	IA	moderate to poorly differentiated	M	Adenocarcinoma of the lung
64	**Lung Serum**	106	1%	319	4%	IB		M	Adenocarcinoma of the lung
65	**Lung Serum**	49	2%	257	1%	IB	moderately differentiated	M	Adenocarcinoma of the lung
66	**Lung**	75	2%	301	2%	II	3	M	Adenocarcinoma

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	Sample Type	Adjusted backfit conc[2] (pg/mL)	%CV	Adjusted backfit conc[2] (pg/mL)	%CV	stage	grade	gender	comments
	Serum								
67	Lung Serum	79	3%	283	8%	II	2	M	Adenocarcinoma
68	Lung Serum	145	2%	492	0%	II	2	F	Adenocarcinoma
69	Lung Serum	142	2%	367	6%	IV	n/a	F	adenocarcinoma
70	Lung Serum	103	2%	231	7%	IV	n/a	F	adenocarcinoma
71	Lung Serum	153	1%	311	3%	III B	n/a	F	adenocarcinoma
72	Lung Serum	54	3%	124	6%	III A	missing	M	large and solid cell carcinoma
73	Lung Serum	183	3%	391	10%	III B	missing	F	adenocarcinoma
74	Lung Serum	91	2%	199	7%	missing	3	M	poorly differentiated non-keratinizing squamous cell carcinoma
75	Lung Serum	89	7%	221	2%	III B	3	F	poorly differentiated adenocarcinoma
76	Lung Serum	139	1%	330	1%	I A	2	F	moderately differentiated adenocarcinoma
77	Lung Serum	197	3%	452	7%	III A	2	F	moderately differentiated adenocarcinoma
78	Lung Serum	52	3%	183	5%	III A	n/a	F	pleomorphic carcinoma
79	Lung Serum	80	3%	249	8%	III A	n/a	M	pleomorphic carcinoma

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	Sample Type	Adjusted backfit conc[2] (pg/mL)	%CV	Adjusted backfit conc[2] (pg/mL)	%CV	stage	grade	gender	comments
80	Lung Serum	72	1%	158	7%	IIIA	2	F	moderately differentiated adenocarcinoma
81	Lung Serum	130	12%	221	1%	IA	3	M	large and solid cell carcinoma
82	Lung Serum	81	6%	155	1%	IB	missing	M	large and solid cell carcinoma
83	Lung Serum	127	4%	278	3%	IIIB	missing	F	large and solid cell carcinoma
84	Lung Serum	129	3%	240	2%	missing	missing	F	large and solid cell carcinoma
85	Lung Serum	135	2%	231	7%	III B	2	M	moderately differentiated adenocarcinoma
86	Lung Serum	235	1%	330	0%	IV	2	M	adenocarcinoma
87	Lung Serum	243	5%	396	2%	IV	3	F	poorly differentiated adenocarcinoma
88	Lung Serum	204	3%	572	6%	IA	moderately differentiated	F	Squamous cell carcinoma of the lung
89	Lung Serum	54	9%	214	4%	IB	moderately differentiated	M	Adenocarcinoma of the lung
90	Lung Serum	116	7%	270	0%	IB	moderately differentiated	F	Mucinous adenocarcinoma of the lung
91	Lung Serum	117	3%	292	5%	IIA	moderately differentiated	M	Adenocarcinoma of the lung
92	Lung Serum	248	1%	578	2%	missing	moderately differentiated	M	Adenocarcinoma of the lung
93	Lung	86	2%	300	9%	IB	poorly	M	Adenocarcinoma of

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	**Sample Type**	**Adjusted backfit conc[2] (pg/mL)**	**%CV**	**Adjusted backfit conc[2] (pg/mL)**	**%CV**	**stage**	**grade**	**gender**	**comments**
	Serum						differentiated		the lung
94	**Lung Serum**	33	6%	117	5%	IIIA	moderate to poorly differentiated	M	Adenocarcinoma of the lung
95	**Lung Serum**	36	3%	196	3%	IIIA	poorly differentiated	M	Adenocarcinoma of the lung
96	**Lung Serum**	237	3%	722	4%	IB	well differentiated	M	Adenocarcinoma of the lung
97	**Lung Serum**	82	9%	286	2%	IB	poorly differentiated	M	Adenocarcinoma of the lung
98	**Lung Serum**	112	1%	431	0%	IB	Well to moderately differentiated	F	Adenocarcinoma of the lung
99	**Lung Serum**	137	5%	379	0%	IA	moderately differentiated	F	Alveolar adenocarcinoma of the lung
100	**Lung Serum**	65	2%	181	9%	IA	moderate to poorly differentiated	M	Adenocarcinoma of the lung
101	**Lung Serum**	119	6%	280	4%	IB		M	Adenocarcinoma of the lung
102	**Normal Serum**	187	6%	391	2%			F	
103	**Normal Serum**	337	4%	560	6%			F	
104	**Normal Serum**	203	4%	405	3%			F	
105	**Normal Serum**	97	3%	311	2%			F	
106	**Normal Serum**	210	11%	393	6%			F	

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	Sample Type	Adjusted backfit conc[2] (pg/mL)	%CV	Adjusted backfit conc[2] (pg/mL)	%CV	stage	grade	gender	comments
107	Normal Serum	135	9%	271	6%			M	
108	Normal Serum	145	2%	225	3%			F	
109	Normal Serum	182	4%	257	5%			M	
110	Normal Serum	186	5%	297	2%			M	
111	Normal Serum	129	8%	197	4%			M	
112	Normal Serum	133	4%	254	4%			M	
113	Normal Serum	136	1%	298	9%			M	
114	Normal Serum	189	3%	321	1%			M	
115	Normal Serum	167	1%	257	6%			M	
116	Normal Serum	159	1%	315	1%			M	
117	Normal Serum	166	3%	270	1%			M	
118	Normal Serum	197	2%	339	0%			M	
119	Normal Serum	148	1%	389	4%			M	
120	Normal Serum	198	4%	833	9%			M	
121	Normal Serum	101	3%	137	0%			M	
122	Normal	111	8%	266	1%			M	

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	Sample Type	Adjusted backfit conc[2] (pg/mL)	%CV	Adjusted backfit conc[2] (pg/mL)	%CV	stage	grade	gender	comments
	Serum								
123	Normal Serum	96	0%	172	2%			M	
124	Normal Serum	224	1%	249	7%			M	
125	Normal Serum	203	8%	312	1%			M	
126	Normal Serum	277	10%	388	5%			M	
127	Normal Serum	191	4%	272	4%			F	
128	Normal Serum	206	4%	297	3%			F	
129	Normal Serum	204	15%	194	3%			F	
130	Normal Serum	156	1%	106	2%			F	
131	Normal Serum	177	3%	195	3%			F	
132	Normal Serum	109	0%	148	5%			M	
134	Normal Serum	116	1%	281	1%			F	
135	Normal Serum	182	7%	250	6%			M	
136	Normal Serum	324	2%	475	7%			F	
137	Normal Serum	122	18%	191	1%			F	
138	Normal Serum	135	7%	185	1%			F	

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	Sample Type	Adjusted backfit conc[2] (pg/mL)	%CV	Adjusted backfit conc[2] (pg/mL)	%CV	stage	grade	gender	comments
139	Normal Serum	264	4%	372	2%			F	
140	Normal Serum	105	4%	188	5%			M	
141	Normal Serum	374	0%	649	0%			M	
142	Normal Serum	93	7%	162	7%			M	
143	Normal Serum	143	7%	326	4%			F	
144	Normal Serum	108	3%	202	2%			F	
145	Normal Serum	153	8%	341	3%			F	
146	Normal Serum	448	8%	400	5%			F	
147	Normal Serum	109	4%	196	3%			F	
148	Normal Serum	142	6%	218	1%			F	
149	Normal Serum	174	4%	309	9%			F	
150	Normal Serum	185	5%	270	2%			F	
151	Normal Serum	180	3%	241	0%			M	
152	Normal Serum	125	5%	314	4%			F	
153	Normal Serum	270	0%	449	2%			F	
154	Normal	127	9%	232	1%			M	

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	Sample Type	Adjusted backfit conc[2] (pg/mL)	%CV	Adjusted backfit conc[2] (pg/mL)	%CV	stage	grade	gender	comments
	Serum								
155	Normal Serum	251	3%	415	6%			F	
156	Normal Serum	121	1%	349	0%			M	
157	Normal Serum	137	8%	223	0%			M	
158	Normal Serum	77	3%	173	6%			M	
159	Normal Serum	143	4%	223	7%			F	
160	Normal Serum	121	5%	411	8%			M	
161	Normal Serum	99	8%	199	3%			F	
162	Normal Serum	158	2%	236	0%			F	
163	Normal Serum	138	7%	235	3%			F	
164	Normal Serum	175	18%	290	2%			F	
165	Normal Serum	339	4%	589	8%			M	
166	Normal Serum	155	4%	372	1%			F	
167	Normal Serum	166	0%	278	1%			M	
168	Normal Serum	231	7%	377	3%			M	
169	Normal Serum	148	10%	255	3%			F	

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	Sample Type	Adjusted backfit conc[2] (pg/mL)	%CV	Adjusted backfit conc[2] (pg/mL)	%CV	stage	grade	gender	comments
170	Normal Serum	172	2%	312	4%			M	
171	Normal Serum	146	6%	344	1%			M	
172	Normal Serum	158	3%	306	4%			M	
173	Normal Serum	145	2%	274	6%			F	
174	Normal Serum	163	12%	279	1%			M	
175	Normal Serum	83	5%	196	0%			M	
176	Normal Serum	102	7%	282	5%			M	
177	Normal Serum	140	3%	330	6%			M	
178	Normal Serum	174	9%	277	15%			F	
179	Normal Serum	295	3%	281	8%			M	
180	Normal Serum	67	4%	308	13%			F	
181	Normal Serum	115	3%	324	0%			F	
182	Normal Serum	128	5%	287	0%			F	
183	Normal Serum	128	1%	112	79%			M	
184	Normal Serum	76	3%	147	53%			F	
185	Normal	264	7%	377	4%			F	

		LLOQ[1] = 1pg/mL		LLOQ[1] = 5 pg/mL					
		FRα- Pair 2		FRα Pair 1					
MSD Sample Testing Number	Sample Type	Adjusted backfit conc[2] (pg/mL)	%CV	Adjusted backfit conc[2] (pg/mL)	%CV	stage	grade	gender	comments
	Serum								
186	Normal Serum	146	13%	258	3%			M	
187	Normal Serum	132	0%	264	1%			F	
188	Normal Serum	92	4%	249	1%			M	
189	Normal Serum	89	11%	251	4%			F	
190	Normal Serum	135	5%	268	4%			M	
191	Normal Serum	177	4%	394	3%			F	
192	Normal Serum	184	8%	367	2%			F	
193	Normal Serum	156	3%	387	5%			F	
194	Normal Serum	118	8%	275	4%			M	
195	Normal Serum	74	7%	217	6%			M	
196	Normal Serum	185	8%	373	5%			F	
197	Normal Serum	159	3%	378	2%			F	
198	Normal Serum	94	2%	245	1%			F	

[1] LLOQ is the lower limit of quantilation

[2] The adjusted backfit concentration is adjusted to take into account the sample dilution.

Based on the foregoing data, it was apparent that the 9F3, 2412, 26B3 and 19D4 antibodies were useful in detecting levels of FRα in biological samples, for example serum, derived from a subject. Moreover, the particular combinations of (i) 9F3 as a capture antibody and 24F12 as a detector antibody and (ii) 26B3 as a capture antibody and 19D4 as a detector antibody were capable and particularly effective of assessing levels of FRα in biological samples.

EXAMPLE 15. ASSESSMENT OF LEVELS OF FRα IN URINE USING THREE DIFFERENT DETECTOR AND CAPTURE ANTIBODY PAIRS

The ability of three anti-FRα antibody pairs in detecting the levels of FRα in urine samples was assessed. The antibody pairs utilized were as follows: (1) 26B3 as detector antibody and 9F3 as capture antibody, and (2) 24F12 as detector antibody and 9F3 as capture antibody.

Method

Two antibody pairs were tested with full calibrator curves and urine pretreated with a 1:1 dilution for 2 minutes in either 6M guanidine, 3M guanidine or PBS control. The following urine samples were tested: three human urine pools diluted 1:80, and five human individual urines diluted 1:80 (one male, four female).

Plates were Biodotted at 150µg/mL, +B, +T, on 4spot STD ((Meso Scale Discovery, Gaithersburg, Maryland)), one capture per well. Detects were run at 1 µg/mL. Diluent 100 + HAMA + mIgG was used for samples and calibrator. Diluent 3 was used for detections. Diluents were commercially available diluents obtained from Meso Scale Discovery.

The following protocol was employed for the ECLIA. Samples were added at 50µL/well. The samples were shaken for 2 hours. The samples were washed with Phosphate Buffered Saline (PBS) solution with the detergent Tween 20 (PBST). The detector antibody was added at 25µL/well. The samples were shaken for 2 hours and subsequently washed with PBST. The electrochemiluminescence (ECL) emission of the samples was read with 2X MSD Buffer T.

The results of these experiments are shown in Tables 26-27.

Table 26: Detection of FRα levels in urine using 26B3 as detector antibody and 9F3 as capture antibody

Detect	26B3							
Capture	9F3							
	6M Guanidine			3M Guanidine			PBS Control	
Sample ID	Adjusted Backfit conc pg/mL	%CV	% of control	Adjusted Backfit conc pg/mL	%CV	% of control	Adjusted Backfit conc pg/mL	%CV
Urine pool 1	13,252	2%	114%	14,505	10%	124%	11,655	17%
Urine pool 2	14,827	5%	133%	17,039	4%	152%	11,187	5%
Urine pool 3	11,280	5%	119%	9,065	10%	96%	9,479	9%
Urine Ind 1	1,747	3%	99%	1,754	6%	100%	1,760	12%
Urine Ind 2	40,505	7%	145%	46,622	5%	167%	27,920	13%
Urine Ind 3	1,623	1%	117%	1,496	5%	108%	1,381	4%
Urine Ind 4	12,091	2%	86%	14,941	5%	107%	13,996	13%
Urine Ind 5	22,829	2%	128%	24,607	8%	137%	17,899	5%
	Average	3%	118%	Average	7%	**124%**	Average	10%
					difference from 6M condition	6%		

Table 27: Detection of FRα levels in urine using 24F12 as detector antibody and 9F3 as capture antibody

Detect	24F12							
Capture	9F3							
	6M Guanidine			3M Guanidine			PBS Control	
Sample ID	Adjusted Backfit conc pg/mL	%CV	% of control	Adjusted Backfit conc pg/mL	%CV	% of control	Adjusted Backfit conc pg/mL	%CV
Urine pool 1	10,883	2%	53%	14,689	9%	72%	20,504	10%
Urine pool 2	11,763	8%	60%	16,487	7%	85%	19,468	1%
Urine pool 3	7,456	9%	40%	9,362	17%	50%	18,677	7%
Urine Ind 1	1,376	1%	39%	1,894	7%	54%	3,501	3%
Urine Ind 2	29,567	0%	61%	37,843	13%	78%	48,607	5%
Urine Ind 3	1,621	4%	61%	2,153	2%	81%	2,667	1%
Urine Ind 4	10,470	6%	58%	14,116	5%	78%	18,175	6%
Urine Ind 5	19,390	6%	68%	22,076	23%	78%	28,421	4%
	Average	5%	55%	Average	10%	**72%**	Average	5%
					difference from 6M condition	17%		

Based on the foregoing data, it was apparent that the 9F3, 24F12, 26B3 and 19D4 antibodies were useful in detecting levels of FRα in biological samples derived from a subject. Moreover, the combinations of (1) 26B3 as detector antibody and 9F3 as capture antibody and (2) 24F12 as detector antibody and 9F3 as capture antibody were capable and particularly effective of assessing levels of FRα in biological samples.

A second set of experiments, following the protocol described above and using the same two pairs of antibodies, were conducted utilizing four female human individual urines diluted 1:80. The urine was pretreated with a 1:1 dilution for 2 minutes in either 3M guanidine or PBS control. The results are shown in Tables 28-29.

Table 28: Detection of FRα levels in urine using 26B3 as detector antibody and 9F3 as capture antibody

Detect	26B3				
Capture	9F3				
	3M Guanidine			PBS Control	
Sample ID	Adjusted Backfit conc pg/mL	%CV	% of control	Adjusted Backfit conc pg/mL	%CV
Urine Ind 2	33,824	4%	98%	34,569	2%
Urine Ind 3	2,086	4%	99%	2,107	3%
Urine Ind 4	15,283	5%	97%	15,696	2%
Urine Ind 5	24,955	4%	92%	26,991	3%
	Average	4%	97%	Average	3%

Table 29: Detection of FRα levels in urine using 24F12 as detector antibody and 9F3 as capture antibody

Detect	24F12				
Capture	9F3				
	3M Guanidine			PBS Control	
Sample ID	Adjusted Backfit conc pg/mL	%CV	% of control	Adjusted Backfit conc pg/mL	%CV
Urine Ind 2	38,455	4%	106%	36,414	6%
Urine Ind 3	2,447	2%	109%	2,250	2%
Urine Ind 4	15,303	3%	81%	18,964	8%
Urine Ind 5	27,216	0%	95%	28,651	5%

	Average	2%	98%	Average	5%

The results of this second set of experiments further confirm the results of the first set of experiments and demonstrate that the level of FRα which is not bound to a cell can be reliably assessed, for example, in urine, using assays such as the ECLIA assay and using the 26B3, 9F3, 24F12 antibodies. Further, the results demonstrate that such assays can effectively detect FRα using pairs of detector and capture antibodies that bind FRα (such as, *e.g.*, 26B3 as detector antibody and 9F3 as capture antibody, and 24F12 as detector antibody and 9F3 as capture antibody).

EXAMPLE 16. ASSESSMENT OF LEVELS OF FRα IN SERUM AND PLASMA

The levels of FRα were assessed in samples of serum and plasma on two separate days. The subjects from whom the samples were derived were either normal subjects or patients with ovarian or lung cancer.

The protocol for assessing FRα levels was the same as set forth in Example 14 above. The pairs of antibodies used for assessing FRα levels were also the same as in Example 14, *i.e.*, **Pair 1**, in which 9F3 was the capture antibody and 24F12 was the detector antibody, and **Pair 2**, in which 26B3 was the capture antibody and 19D4 was the detector antibody.

The results are provided in Table 30.

Table 30: Levels of FRα as assessed in serum and plasma samples on different days

				Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
				FRα/pair1	**FRα/pair1**	**FRα/pair1**	**FRα/pair1**	**FRα/pair2**	**FRα/pair2**	**FRα/pair2**	**FRα/pair2**
Donor ID	**Disease**	**Biosample Confirmed Diagnosis**	**Stage**	**Serum (pg/ml)**	**Serum (pg/ml)**	**Plasma (pg/ml)**	**Plasma (pg/ml)**	**Serum (pg/ml)**	**Serum (pg/ml)**	**Plasma (pg/mL)**	**Plasma (pg/mL)**
17168	ovary	Serum FRA - Pair 1 versus Pair 2	I	1236	1282	1466	1183	1298	1384	1410	1336
46464	ovary	Serous carcinoma	IIIC	1589	1848	2027	2147	1966	2018	2066	2210
47219	ovary	Adenocarcinoma	missing	447	432	1208	748	435	446	695	577
47721	ovary	Papillary serous cystadenocarcinoma	IIIB	1307	1400	2291	2100	1642	1479	1940	1807
48185	ovary	Adenocarcinoma	missing	1058	1038	883	652	918	872	811	781
48254	ovary	Adenocarcinoma	IIIC	511	495	3030	2569	506	445	1332	1370
48258	ovary	Adenocarcinoma	missing	471	552	1978	1070	629	547	978	798
48282	ovary	Adenocarcinoma	missing	375	388	688	536	446	407	540	428
48698	ovary	Carcinoma, undifferentiated	IIIB	279	231	308	225	340	255	328	228
49028	ovary	Serous carcinoma	IIIC	727	590	158	169	468	526	192	192
49030	ovary	Serous carcinoma	IIIC	215	205	695	485	362	291	734	590
49033	ovary	Serous cystadenocarcinoma	IIIC	579	457	805	717	511	451	629	668
49071	ovary	Serous cystadenocarcinoma	IV	335	338	1884	1462	559	457	1224	1028
49092	ovary	Papillary cystadenocarcinoma	missing	272	228	1235	615	399	334	678	535
49258	ovary	Papillary serous cystadenocarcinoma	IIIB	201	190	343	142	328	253	368	244

				Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
				FRα/pair1	**FRα/pair1**	**FRα/pair1**	**FRα/pair1**	**FRα/pair2**	**FRα/pair2**	**FRα/pair2**	**FRα/pair2**
Donor ID	**Disease**	**Biosample Confirmed Diagnosis**	**Stage**	**Serum (pg/ml)**	**Serum (pg/ml)**	**Plasma (pg/ml)**	**Plasma (pg/ml)**	**Serum (pg/ml)**	**Serum (pg/ml)**	**Plasma (pg/mL)**	**Plasma (pg/mL)**
49335	ovary	Serous cystadenocarcinoma	IIIB	1904	1698	1560	1485	1879	1503	1868	1627
49369	ovary	Serous carcinoma	IIIC	2451	2805	2589	2641	3402	2659	2898	2605
49551	ovary	Serous carcinoma	III	408	385	364	342	505	432	436	418
50009	ovary	Serous carcinoma	IIIC	2887	4127	2641	3811	4466	3914	3533	3519
50370	ovary	Serous cystadenocarcinoma	IIIB	619	570	788	611	813	762	708	758
50378	ovary	Papillary serous cystadenocarcinoma	I	410	387	330	256	388	464	381	387
50460	ovary	Papillary serous cystadenocarcinoma	IIIB	254	274	688	414	284	272	489	420
50467	ovary	Clear cell adenocarcinoma	I	427	314	414	300	272	313	292	309
50635	ovary	Serous cystadenocarcinoma	IA	247	227	462	367	355	345	392	392
51503	ovary	Papillary cystadenocarcinoma	I	291	250	925	651	315	326	818	498
51504	ovary	Mucinous cystadenoma, borderline malignancy	I	224	184	2438	1395	225	240	916	856
51506	ovary	Papillary mucinous cystadenocarcinoma	III	270	258	713	324	335	384	457	424
52949	ovary	Adenocarcinoma	missing	446	436	288	257	435	483	395	390

				Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
				FRα/pair1	**FRα/pair1**	**FRα/pair1**	**FRα/pair1**	**FRα/pair2**	**FRα/pair2**	**FRα/pair2**	**FRα/pair2**
Donor ID	**Disease**	**Biosample Confirmed Diagnosis**	**Stage**	**Serum (pg/ml)**	**Serum (pg/ml)**	**Plasma (pg/ml)**	**Plasma (pg/ml)**	**Serum (pg/ml)**	**Serum (pg/ml)**	**Plasma (pg/mL)**	**Plasma (pg/mL)**
52952	ovary	Papillary serous cystadenocarcinoma	missing	480	415	451	376	310	351	299	321
52957	ovary	Papillary serous cystadenocarcinoma	III	373	334	274	221	318	374	303	330
52978	ovary	Papillary mucinous cystadenocarcinoma	II	348	318	533	429	525	559	596	624
52980	ovary	Serous cystadenocarcinoma	III	627	630	1447	1019	834	905	964	996
DLSN-057	normal			309	402	413	394	515	527	509	531
DLSN-056	normal			273	254	483	498	480	458	522	524
DLSN-052	normal			282	289	293	298	446	483	438	401
DLSN-049	normal			282	256	351	320	363	394	430	359
DLSN-048	normal			362	399	454	434	746	722	824	639
DLSN-047	normal			188	176	208	167	275	263	251	212
DLSN-046	normal			295	321	276	240	354	310	315	257
DLSN-045	normal			259	259	259	189	292	279	273	235
DLSN-044	normal			244	236	246	254	284	273	284	271
DLSN-042	normal			245	199	210	185	328	301	285	258
DLSN-040	normal			392	376	408	406	481	499	502	413
DLSN-039	normal			463	470	469	446	617	599	567	528
DLSN-037	normal			256	231	256	223	367	338	351	289
DLSN-029	normal			285	270	348	337	453	417	418	351
DLSN-023	normal			265	254	286	298	458	402	370	386
DLSN-020	normal			237	238	311	287	530	496	504	446

				Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
				FRα/pair1	**FRα/pair1**	**FRα/pair1**	**FRα/pair1**	**FRα/pair2**	**FRα/pair2**	**FRα/pair2**	**FRα/pair2**
Donor ID	**Disease**	**Biosample Confirmed Diagnosis**	**Stage**	**Serum (pg/ml)**	**Serum (pg/ml)**	**Plasma (pg/ml)**	**Plasma (pg/ml)**	**Serum (pg/ml)**	**Serum (pg/ml)**	**Plasma (pg/mL)**	**Plasma (pg/mL)**
DLSN-011	normal			344	328	210	207	688	603	297	267
DLSN-039	normal			196	178	204	183	259	219	242	248
DLSN-047	normal				336		385		489		429
DLSN-052	normal				226		208		346		315
DLSL-012	lung				340		220		529		375
DLSL-015	lung				199		180		236		292
DLSL-023	lung				581		347		568		523
DLSL-031	lung				400		251		415		343
DLSL-034	lung				133		138		299		331
1	lung				238		339		462		460
3	lung				120		185		203		334
6	lung				295		1646		431		868
18	lung				328		288		466		418
18639	lung				124		333		128		216
18640	lung				246		275		221		221
50666	lung				372		357		405		338
2	lung				214		327		274		269
4	lung				347		625		526		575
7	lung				224		308		421		452
12	lung				409		424		778		725
13	lung				509		590		1251		1360
14	lung				250		250		409		357
15	lung				502		466		606		594

				Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
				FRα/pair1	**FRα/pair1**	**FRα/pair1**	**FRα/pair1**	**FRα/pair2**	**FRα/pair2**	**FRα/pair2**	**FRα/pair2**
Donor ID	**Disease**	**Biosample Confirmed Diagnosis**	**Stage**	**Serum (pg/ml)**	**Serum (pg/ml)**	**Plasma (pg/ml)**	**Plasma (pg/ml)**	**Serum (pg/ml)**	**Serum (pg/ml)**	**Plasma (pg/mL)**	**Plasma (pg/mL)**
16	lung				153		274		246		275
19	lung				357		905		428		576
DLS4-0002	lung				234		245		410		347
DLS4-0004	lung				472		520		507		538
DLSO-007	ovary				389		593		574		544
DLSO-008	ovary				175		295		386		364
DLSO-009	ovary				232		331		299		349
DLSO-014	ovary				265		300		430		467
DLSO-020	ovary				536		261		453		389
DLSO-026	ovary										
DLSO-027	ovary				290		140		269		218
DLSO-028	ovary				357		207		397		18
DLSO-029	ovary				412		236		369		361
DLSO-030	ovary				387		498		595		616
DLSO-031	ovary				348		496		353		413
DLSO-034	ovary				197		222		308		346
DLSO-035	ovary				144		540		298		587
DLSO-023	ovary				404		216		423		337
DLSO-025	ovary				306		156		301		272
DLSO-026	ovary				231		293		406		378
DLSO-032	ovary				151		190		240		255
DLSO-018	ovary				155		340		303		343
DLSO-019	ovary				350		330				

				Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
				FRα/pair1	**FRα/pair1**	**FRα/pair1**	**FRα/pair1**	**FRα/pair2**	**FRα/pair2**	**FRα/pair2**	**FRα/pair2**
Donor ID	**Disease**	**Biosample Confirmed Diagnosis**	**Stage**	**Serum (pg/ml)**	**Serum (pg/ml)**	**Plasma (pg/ml)**	**Plasma (pg/ml)**	**Serum (pg/ml)**	**Serum (pg/ml)**	**Plasma (pg/mL)**	**Plasma (pg/mL)**
DLSO-021	ovary				359		365		346		434
DLSO-024	ovary				260		140		338		318
10001627	ovary				1481		1396		1583		1655
11025393	ovary				5241		4069		4998		5486
11025394	ovary				473		371		488		518
110025395	ovary				3215		2920		3466		4043
110025397	ovary				109		145		245		232
110025398	ovary				476		497		613		543
110025399	ovary				166		145		234		229
110025402	ovary				219		221		389		357
110025403	ovary				7529		6990		13033		11888
110025405	ovary				510		508		836		835
5	ovary				164		328		252		375
8	ovary				251		709		473		573
9	ovary				249		743		482		561
10	ovary				288		569		412		446
11	ovary				180		417		310		397
17	ovary				296		760		342		628
110025392	ovary				1094		2210		907		1294

Based on the foregoing data, it was apparent that the 9F3, 2412, 26B3 and 19D4 antibodies were useful in detecting levels of FRα in biological samples, for example, serum or plasma, derived from a subject. Moreover, the particular combinations of (i) 9F3 as a capture antibody and 24F12 as a detector antibody and (ii) 26B3 as a capture antibody and 19D4 as a detector antibody were capable and particularly effective in assessing levels of FRα in biological samples.

For assays conducted using both Pair 1 and Pair 2, there was a high correlation between serum and plasma FRα levels . Figure 16 shows the correlation in serum versus plasma FRα levels for assays conducted using Pair 1 (see Example 16). The R^2 value was 0.8604. Figure 17 shows the correlation in serum versus plasma FRα levels for assays conducted using Pair 2 (see Example 16). The R^2 value was 0.9766.

For both serum and plasma samples, there was a high correlation between FRα levels measured using Pair 1 and Pair 2. Figure 18 shows the correlation in serum FRα levels for assays conducted using Pair 1 versus Pair 2 (see Example 16). The R^2 value was 0.9028. Figure 19 shows the correlation in plasma FRα levels for assays conducted using pair 1 versus pair 2 (see Example 16). The R^2 value was 0.8773.

The results also showed that there was a high correlation between FRα levels measured on different days. Figure 20 shows the interday correlation in serum FRα levels for assays conducted using pair 2. The R^2 value was 0.9839.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims. Any combination of the embodiments disclosed in the dependent claims are contemplated to be within the scope of the invention.

Table 33: **SEQUENCES**

SEQ ID NO:	DESCRIPTION	SEQUENCE
1	MORAB-003 CDRH1	GFTFSGYGLS
2	MORAB-003 CDRH2	MISSGGSYTYYADSVKG
3	MORAB-003 CDRH3	HGDDPAWFAY
4	MORAB-003 CDRL1	SVSSSISSNNLH
5	MORAB-003 CDRL2	GTSNLAS
6	MORAB-003 CDRL3	QQWSSYPYMYT
7	MORAb-003 Heavy Chain Mature Polypeptide Amino Acid Sequence	1 EVQLVESGGG VVQPGRSLRL SCSASGFTFS GYGLSWVRQA PGKGLEWVAM 51 ISSGGSYTYY ADSVKGRFAI SRDNAKNTLF LQMDSLRPED TGVYFCARHG 101 DDPAWFAYWG QGTPVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD 151 YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY 201 ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP SVFLFPPKPK 251 DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS 301 TYRVVSVLIV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV 351 YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL 401 DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK
8	MORAb-003 Light Chain Mature Polypeptide Amino Acid Sequence	1 DIQLTQSPSS LSASVGDRVI ITCSVSSSIS SNNLHWYQQK PGKAPKPWIY 51 GTSNLASGVP SRFSGSGSGT DYTFTISSLQ PEDIATYYCQ QWSSYPYMYT 101 FGQGTKVEIK RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ 151 WKVDNALQSG NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT 201 HQGLSSPVTK SFNRGEC
9	MORAb-003 Heavy Chain full length pre-protein amino acid sequence	1 MGWSCIILFL VATATGVHSE VQLVESGGGV VQPGRSLRLS CSASGFTFSG 51 YGLSWVRQAP GKGLEWVAMI SSGGSYTYYA DSVKGRFAIS RDNAKNTLFL 101 QMDSLRPEDT GVYFCARHGD DPAWFAYWGQ GTPVTVSSAS TKGPSVFPLA 151 PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL 201 YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC 251 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV 301 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP 351 APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV 401 EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH 451 EALHNHYTQK SLSLSPGK
10	MORAb-003 Light Chain full length pre-protein amino acid sequence	1 MGWSCIILFL VATATGVHSD IQLTQSPSSL SASVGDRVTI TCSVSSSISS 51 NNLHWYQQKP GKAPKPWIYG TSNLASGVPS RFSGSGSGTD YTFTISSLQP 101 EDIATYYCQQ WSSYPYMYTF GQGTKVEIKR TVAAPSVFIF PPSDEQLKSG 151 TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYSLSST 201 LTLSKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC
11	MORAb-003 Heavy Chain Nucleotide	1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT 51 CCACTCCGAG GTCCAACTGG TGGAGAGCGG TGGAGGTGTT GTGCAACCTG 101 GCCGGTCCCT GCGCCTGTCC TGCTCCGCAT CTGGCTTCAC CTTCAGCGGC 151 TATGGGTTGT CTTGGGTGAG ACAGGCACCT GGAAAAGGTC TTGAGTGGGT 201 TGCAATGATT AGTAGTGGTG GTAGTTATAC CTACTATGCA GACAGTGTGA 251 AGGGTAGATT TGCAATATCG CGAGACAACG CCAAGAACAC ATTGTTCCTG 301 CAAATGGACA GCCTGAGACC CGAAGACACC GGGGTCTATT TTTGTGCAAG 351 ACATGGGGAC GATCCCGCCT GGTTCGCTTA TTGGGGCCAA GGGACCCCGG 401 TCACCGTCTC CTCAGCCTCC ACCAAGGGCC CATCGGTCTT CCCCCTGGCA 451 CCCTCCTCCA AGAGCACCTC TGGGGGCACA GCGGCCCTGG GCTGCCTGGT 501 CAAGGACTAC TTCCCCGAAC CGGTGACGGT GTCGTGGAAC TCAGGCGCCC 551 TGACCAGCGG CGTGCACACC TTCCCGGCTG TCCTACAGTC CTCAGGACTC 601 TACTCCCTCA GCAGCGTGGT GACCGTGCCC TCCAGCAGCT TGGGCACCCA

		651 GACCTACATC TGCAACGTGA ATCACAAGCC CAGCAACACC AAGGTGGACA 701 AGAAAGTTGA GCCCAAATCT TGTGACAAAA CTCACACATG CCCACCGTGC 751 CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCAAA 801 ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG 851 TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG 901 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA 951 CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT 1001 GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA 1051 GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC 1101 ACAGGTGTAC ACCCTGCCCC CATCCCGGGA TGAGCTGACC AAGAACCAGG 1151 TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG 1201 GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC 1251 CGTGCTGGAC TCCGACGGCT CCTTCTTCTT ATATTCAAAG CTCACCGTGG 1301 ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT 1351 GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCCGG 1401 GAAATGA
12	MORAb-003 Light Chain Nucleotide	1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT 51 CCACTCCGAC ATCCAGCTGA CCCAGAGCCC AAGCAGCCTG AGCGCCAGCG 101 TGGGTGACAG AGTGACCATC ACCTGTAGTG TCAGCTCAAG TATAAGTTCC 151 AACAACTTGC ACTGGTACCA GCAGAAGCCA GGTAAGGCTC CAAAGCCATG 201 GATCTACGGC ACATCCAACC TGGCTTCTGG TGTGCCAAGC AGATTCAGCG 251 GTAGCGGTAG CGGTACCGAC TACACCTTCA CCATCAGCAG CCTCCAGCCA 301 GAGGACATCG CCACCTACTA CTGCCAACAG TGGAGTAGTT ACCCGTACAT 351 GTACACGTTC GGCCAAGGGA CCAAGGTGGA AATCAAACGA ACTGTGGCTG 401 CACCATCTGT CTTCATCTTC CCGCCATCTG ATGAGCAGTT GAAATCTGGA 451 ACTGCCTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA GAGAGGCCAA 501 AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCCCAGGAGA 551 GTGTCACAGA GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGCAGCACC 601 CTGACGCTGA GCAAAGCAGA CTACGAGAAA CACAAAGTCT ACGCCTGCGA 651 AGTCACCCAT CAGGGCCTGA GCTCGCCCGT CACAAAGAGC TTCAACAGGG 701 GAGAGTGTTA A
13	LK26HuVK	DIQLTQSPSSLSASVGDRVT ITCSVSSSISSNNLHWYQQK PGKAPKLLIYGTSNLASGVP SRFSGSGSGTDFTFTISSLQ PEDIATYYCQQWSSYPYMYT FGQGTKVEIK*
14	LK26HuVKY	DIQLTQSPSSLSASVGDRVT ITCSVSSSISSNNLHWYQQK PGKAPKLLIYGTSNLASGVP SRFSGSGSGTDYTFTISSLQ PEDIATYYCQQWSSYPYMYT FGQGTKVEIK*
15	LK26HuVKPW	DIQLTQSPSSLSASVGDRVT ITCSVSSSISSNNLHWYQQK PGKAPKPWIYGTSNLASGVP SRFSGSGSGTDFTFTISSLQ PEDIATYYCQQWSSYPYMYT FGQGTKVEIK
16	LK26HuVKPW, Y	DIQLTQSPSSLSASVGDRVT ITCSVSSSISSNNLHWYQQK PGKAPKPWIYGTSNLASGVP SRFSGSGSGTDYTFTISSLQ PEDIATYYCQQWSSYPYMYT FGQGTKVEIK
17	LK26HuVH	QVQLQESGPGLVRPSQTLSL TCTASGFTFSGYGLSWVRQP PGRGLEWVAMISSGGSYTYY ADSVKGRVTMLRDTSKNQFS

		LRLSSVTAADTAVYYCARHG DDPAWFAYWGQGSLVTVSS
18	LK26HuVHFAIS, N	QVQLQESGPGLVRPSQTLSL TCTASGFTFSGYGLSWVRQP PGRGLEWVAMISSGGSYTYY ADSVKGRFAISRDNSKNQFS LRLSSVTAADTAVYYCARHG DDPAWFAYWGQGSLVTVSS*
19	LK26HuVHSLF	QVQLQESGPGLVRPSQTLSL TCTASGFTFSGYGLSWVRQP PGRGLEWVAMISSGGSYTYY ADSVKGRVTMLRDTSKNSLF LRLSSVTAADTAVYYCARHG DDPAWFAYWGQGTTVTVSS*
20	LK26HuVHI, I	QVQLQESGPGLVRPSQTLSL TCTASGFTFSGYGLSWVRQP PGRGLEWVAMISSGGSYTYY ADSVKGRVTMLRDTSKNQFS LRLSSVTAADTAIYICARHG DDPAWFAYWGQGSLVTVSS*
21	LK26KOLHuVH	EVQLVESGGGVVQPGRSLRL SCSASGFTFSGYGLSWVRQA PGKGLEWVAMISSGGSYTYY ADSVKGRFAISRDNAKNTLF LQMDSLRPEDTGVYFCARHG DDPAWFAYWGQGTPVTVSS*
22	Murine LK26 Ab Heavy Chain Sequence	QVXLQXSGGDLVKPGGSLKL SCAASGFTFSGYGLSWVRQT PDKRLEWVAMISSGGSYTYY ADSVKGRFAISRDNAKNSLF LQMSSLKSDDTAIYICARHG DDPAWFAYWGQGTLVTVSA*
23	Murine LK26 Ab Light Chain Sequence	DIELTQSPALMAASPGEKVT ITCSVSSSISSNNLHWYQQK SETSPKPWIYGTSNLASGVP LRFRGFGSGTSYSLTISSME AEDAATYYCQQWSSYPYMYT FGGGTKLEIK*
24	Consensus Human FRα Nucleotide Sequence	tcaaggttaa acgacaagga cagacatggc tcagcggatg acaacacagc tgctgctcct tctagtgtgg gtggctgtag taggggaggc tcagacaagg attgcatggg ccaggactga gcttctcaat gtctgcatga acgccaagca ccacaaggaa aagccaggcc ccgaggacaa gttgcatgag cagtgtcgac cctggaggaa gaatgcctgc tgttctacca acaccagcca ggaagcccat aaggatgttt cctacctata tagattcaac tggaaccact gtggagagat ggcacctgcc tgcaaacggc atttcatcca ggacacctgc ctctacgagt gctcccccaa cttggggccc tggatccagc aggtggatca gagctggcgc aaagagcggg tactgaacgt gcccctgtgc aaagaggact gtgagcaatg gtgggaagat tgtcgcacct cctacacctg caagagcaac tggcacaagg gctggaactg gacttcaggg tttaacaagt gcgcagtggg agctgcctgc caacctttcc atttctactt ccccacaccc actgttctgt gcaatgaaat ctggactcac tcctacaagg tcagcaacta cagccgaggg agtggccgct gcatccagat gtggttcgac ccagcccagg gcaaccccaa tgaggaggtg gcgaggttct atgctgcagc catgagtggg gctgggccct gggcagcctg gcctttcctg cttagcctgg ccctaatgct gctgtggctg ctcagctgac ctcctttac cttctgatac ctggaaatcc ctgccctgtt cagccccaca gctcccaact atttggttcc tgctccatgg tcgggcctct gacagccact ttgaataaac cagacaccgc acatgtgtct tgagaattat ttggaaaaaa aaaaaaaaaa aa
25	Consensus Human FRα Amino Acid Sequence	maqrmttqll lllvwvavvg eaqtriawar tellnvcmna khhkekpgpe dklheqcrpw rknaccstnt sqeahkdvsy lyrfnwnhcg emapackrhf iqdtclyecs pnlgpwiqqv dqswrkervl nvplckedce qwwedcrtsy tcksnwhkgw nwtsgfnkca vgaacqpfhf yfptptvlcn eiwthsykvs nysrgsgrci qmwfdpaqgn pneevarfya aamsgagpwa awpfllslal mllwlls
26	Mov-18	TELLNVXMNAK*XKEKPXPX

	epitope sequence	*KLXXQX
27	9F3 Light Chain CDR1	RASSTVSYSYLH
28	9F3 Light Chain CDR2	GTSNLAS
29	9F3 Light Chain CDR3	QQYSGYPLT
30	9F3 Light Chain Variable Domain	PAIMSASPGEKVTMTCRASSTVSYSYLHWYQQKSGASPQLWIYGTSNLAS GVPARFSGSGSGTSYSLTISSVEAEDAATYYCQQYSGYPLTFGAGTKLELK RADAAP
31	9F3 Heavy Chain CDR1	SGYYWN
32	9F3 Heavy Chain CDR2	YIKSDGSNNYNPSLKN
33	9F3 Heavy Chain CDR3	EWKAMDY
34	9F3 Heavy Chain Variable Domain	ESGPGLVRPSQSLSLTCSVTGYSITSGYYWNWIRQFPGSRLEWMGYIKSDG SNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYFCTREWKAMDYW GQGTSVTVSSAKTTPPSVYPLAPGCGDT
35	19D4 Light Chain CDR1	RASESVDTYGNNFIH
36	19D4 Light Chain CDR2	LASNLES
37	19D4 Light Chain CDR3	QQNNGDPWT
38	19D4 Light Chain Variable Domain	PASLAVSLGQRATISCRASESVDTYGNNFIHWYQQKPGQPPKLLIYLASNL ESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNNGDPWTFGGGTKL EIKRADAAP
39	19D4 Heavy Chain CDR1	HPYMH
40	19D4 Heavy Chain CDR2	RIDPANGNTKYDPKFQG
41	19D4 Heavy Chain CDR3	EEVADYTMDY
42	19D4 Heavy Chain Variable Domain	GAELVKPGASVKLSCTASGFNIKHPYMHWVKQRPDQGLEWIGRIDPANG NTKYDPKFQGKATITADTSSNTAYLQLSSLTSEDTAVYYCGREEVADYTM DYWGQGTSVTVSSAKTTAPSVYPLAPV
43	24F12 Light Chain CDR1	SASQGINNFLN
44	24F12 Light Chain CDR2	YTSSLHS
45	24F12 Light Chain CDR3	QHFSKLPWT
46	24F12 Light Chain Variable Domain	TSSLSASLGDRVTISCSASQGINNFLNWYQQKPDGTVKLLIYYTSSLHSGVP SRFSGSGSGTDYSLTISNLEPEDIATYYCQHFSKLPWTFGGGTKLEIKRADA AP
47	24F12 Heavy Chain CDR1	SYAMS
48	24F12 Heavy Chain CDR2	EIGSGGSYTYYPDTVTG
49	24F12 Heavy Chain CDR3	ETTAGYFDY
50	24F12 Heavy Chain	SGGGLVRPGGSLKLSCAASGFTFSSYAMSWVRQSPEKRLEWVAEIGSGGS

	Variable Domain	YTYYPDTVTGRFTISRDNAKSTLYLEMSSLRSEDTAIYYCARETTAGYFDY WGQGTTLTVSS
51	26B3 Light Chain CDR1	RTSENIFSYLA
52	26B3 Light Chain CDR2	NAKTLAE
53	26B3 Light Chain CDR3	QHHYAFPWT
54	26B3 Light Chain Variable Domain	PASLSASVGETVTITCRTSENIFSYLAWYQQKQGISPQLLVYNAKTLAEGV PSRFSGSGSGTQFSLKINSLQPEDFGSYYCQHHYAFPWTFGGGSKLEIKRA DAAP
55	26B3 Heavy Chain CDR1	GYFMN
56	26B3 Heavy Chain CDR2	RIFPYNGDTFYNQKFKG
57	26B3 Heavy Chain CDR3	GTHYFDY
58	26B3 Heavy Chain Variable Domain	GPELVKPGASVKISCKASDYSFTGYFMNWVMQSHGKSLEWIGRIFPYNGD TFYNQKFKGRATLTVDKSSSTAHMELRSLASEDSAVYFCARGTHYFDYW GQGTTLTVSSAKTTPPSVYPLAPGSAAQT
59	9F3 Light Chain CDR1	AGGGCCAGCTCAACTGTAAGTTACAGTTACTTGCAC
60	9F3 Light Chain CDR2	GGCACATCCAACTTGGCTTCT
61	9F3 Light Chain CDR3	CAGCAGTACAGTGGTTACCCACTCACG
62	9F3 Light Chain Variable Domain	CCAGCAATCATGTCTGCATCTCCAGGGGAAAAGGTCACCATGACCTGC AGGGCCAGCTCAACTGTAAGTTACAGTTACTTGCACTGGTACCAGCAG AAGTCAGGTGCCTCCCCCCAACTCTGGATTTATGGCACATCCAACTTGG CTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTA CTCTCTCACAATCAGCAGTGTGGAGGCTGAAGATGCTGCCACTTATTAC TGCCAGCAGTACAGTGGTTACCCACTCACGTTCGGTGCTGGGACCAAG CTGGAGCTGAAACGGGCTGATGCTGCACCAAC
63	9F3 Heavy Chain CDR1	AGTGGTTATTACTGGAAC
64	9F3 Heavy Chain CDR2	TACATAAAGTCCGACGGTAGCAATAATTACAACCCATCTCTCAAAAAT
65	9F3 Heavy Chain CDR3	GAGTGGAAGGCTATGGACTAC
66	9F3 Heavy Chain Variable Domain	GAGTCAGGACCTGGCCTCGTGAGACCTTCTCAGTCTCTGTCTCTCACCT GCTCTGTCACTGGCTACTCCATCACCAGTGGTTATTACTGGAACTGGAT CCGGCAGTTTCCAGGAAGCAGACTGGAATGGATGGGCTACATAAAGTC CGACGGTAGCAATAATTACAACCCATCTCTCAAAAATCGAATCTCCAT CACTCGTGACACATCTAAGAACCAGTTTTTCCTGAAGTTGAATTCTGTG ACTACTGAGGACACAGCTACATATTTCTGTACAAGGGAGTGGAAGGCT ATGGACTACTGGGGTCAGGGAACCTCAGTCACCGTCTCCTCAGCCAAA ACAACACCCCCATCAGTCTATCCACTGGCCCCTGGGTGTGGAGATACA AC
67	19D4 Light Chain CDR1	AGAGCCAGTGAAAGTGTTGATACTTATGGCAATAATTTTATACAC
68	19D4 Light Chain CDR2	CTTGCATCCAACCTAGAATCT
69	19D4 Light Chain CDR3	CAGCAAAATAATGGGGATCCGTGGACG

70	19D4 Light Chain Variable Domain	CCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGGGCCACCATATCCTGCA GAGCCAGTGAAAGTGTTGATACTTATGGCAATAATTTTATACACTGGT ACCAGCAGAAACCAGGACAGCCACCCAAACTCCTCATTTATCTTGCAT CCAACCTAGAATCTGGGGTCCCTGCCAGGTTCAGTGGCAGTGGGTCTA GGACAGACTTCACCCTCACCATTGATCCTGTGGAGGCTGATGATGCTG CAACCTATTACTGTCAGCAAAATAATGGGGATCCGTGGACGTTCGGTG GAGGCACCAAGCTGGAGATCAAACGGGCTGATGCTGCACCAA
71	19D4 Heavy Chain CDR1	CACCCCTATATGCAC
72	19D4 Heavy Chain CDR2	AGGATTGATCCTGCGAATGGTAATACTAAATATGACCCGAAGTTCCAG GGC
73	19D4 Heavy Chain CDR3	GAGGAGGTGGCGGACTATACTATGGACTAC
74	19D4 Heavy Chain Variable Domain	GGGGCAGAGCTTGTGAAGCCAGGGGCCTCAGTCAAGTTGTCCTGCACA GCTTCTGGCTTCAACATTAAACACCCCTATATGCACTGGGTGAAGCAG AGGCCTGACCAGGGCCTGGAGTGGATTGGAAGGATTGATCCTGCGAAT GGTAATACTAAATATGACCCGAAGTTCCAGGGCAAGGCCACTATAACA GCAGACACATCCTCCAACACAGCCTACCTACAGCTCAGCAGCCTGACA TCTGAGGACACTGCCGTCTATTACTGTGGTAGAGAGGAGGTGGCGGAC TATACTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA GCCAAAACAACAGCCCCATCGGTCTATCCACTGGCCCCTGTGTG
75	24F12 Light Chain CDR1	AGTGCAAGTCAGGGCATTAACAATTTTTTAAAC
76	24F12 Light Chain CDR2	TACACATCAAGTTTACACTCA
77	24F12 Light Chain CDR3	CAGCACTTTAGTAAGCTTCCGTGGACG
78	24F12 Light Chain Variable Domain	ACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCACCATCAGTTGCA GTGCAAGTCAGGGCATTAACAATTTTTTAAACTGGTATCAGCAGAAAC CAGATGGCACTGTTAAACTCCTGATCTATTACACATCAAGTTTACACTC AGGAGTCCCATCAAGGTTCAGTGGCAGTGGGTCTGGGACAGATTATTC TCTCACCATCAGCAACCTGGAACCTGAAGATATTGCCATATACTATTGT CAGCACTTTAGTAAGCTTCCGTGGACGTTCGGTGGAGGCACCAAGCTG GAAATCAAACGGGCTGATGCTGCACCAAC
79	24F12 Heavy Chain CDR1	AGCTATGCCATGTCT
80	24F12 Heavy Chain CDR2	GAAATTGGTAGTGGTGGTAGTTACACCTACTATCCAGACACTGTGACG GGC
81	24F12 Heavy Chain CDR3	GAAACTACGGCGGGCTACTTTGACTAC
82	24F12 Heavy Chain Variable Domain	TCTGGGGGAGGCTTAGTGAGGCCTGGAGGGTCCCTGAAACTCTCCTGT GCAGCCTCTGGATTCACTTTCAGTAGCTATGCCATGTCTTGGGTTCGCC AGTCTCCAGAGAAGAGGCTGGAGTGGGTCGCAGAAATTGGTAGTGGTG GTAGTTACACCTACTATCCAGACACTGTGACGGGCCGATTCACCATCTC CAGAGACAATGCCAAGAGCACCCTGTACCTGGAAATGAGCAGTCTGAG GTCTGAGGACACGGCCATCTATTACTGTGCAAGGGAAACTACGGCGGG CTACTTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
83	26B3 Light Chain CDR1	CGAACAAGTGAGAATATTTTCAGTTATTTAGCA
84	26B3 Light Chain CDR2	AATGCAAAAACCTTAGCAGAG
85	26B3 Light Chain CDR3	CAACATCATTATGCTTTTCCGTGGACG

86	26B3 Light Chain Variable Domain	CCAGCCTCCCTATCTGCATCTGTGGGAGAAACTGTCACCATCACATGTC GAACAAGTGAGAATATTTTCAGTTATTTAGCATGGTATCAGCAGAAAC AGGGAATATCTCCTCAGCTCCTGGTCTATAATGCAAAAACCTTAGCAG AGGGTGTGCCATCAAGGTTCAGTGGCAGTGGATCAGGCACACAGTTTT CTCTGAAGATCAACAGCCTGCAGCCTGAAGATTTTGGGAGTTATTACT GTCAACATCATTATGCTTTTCCGTGGACGTTCGGTGGAGGCTCCAAGCT GGAAATCAAACGGGCTGATGCTGCACCAAC
87	26B3 Heavy Chain CDR1	GGCTACTTTATGAAC
88	26B3 Heavy Chain CDR2	CGTATTTTTCCTTACAATGGTGATACTTTCTACAACCAGAAGTTCAAGG GC
89	26B3 Heavy Chain CDR3	GGGACTCATTACTTTGACTAC
90	26B3 Heavy Chain Variable Domain	GGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAGATATCCTGCAAG GCTTCTGATTACTCTTTTACTGGCTACTTTATGAACTGGGTGATGCAGA GCCATGGAAAGAGCCTTGAGTGGATTGGACGTATTTTTCCTTACAATG GTGATACTTTCTACAACCAGAAGTTCAAGGGCAGGGCCACATTGACTG TAGACAAATCCTCTAGCACAGCCCACATGGAGCTCCGGAGCCTGGCAT CTGAGGACTCTGCAGTCTATTTTTGTGCAAGAGGGACTCATTACTTTGA CTACTGGGGCCAAGGCACCACTCTCACTGTCTCCTCAGCCAAAACGAC ACCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAA

CLAIMS

What is claimed:

1. A method of assessing whether a subject is afflicted with ovarian or lung cancer, the method comprising

contacting a urine sample derived from said subject with an antibody that binds FRα, thereby determining the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample;

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample derived from said subject with the level of FRα in a control sample;

identifying an increase in the level of FRα in the urine sample derived from said subject as compared to the level of FRα in the control sample; and

determining that the subject is afflicted with ovarian or lung cancer based on said increase.

2. The method of claim 1, wherein the subject is identified as being afflicted with ovarian cancer.

3. The method of claim 1, wherein the subject is identified as being afflicted with lung cancer.

4. The method of claim 3, wherein the lung cancer is non-small cell lung cancer.

5. The method of claim 4, wherein the non-small cell lung cancer is adenocarcinoma.

6. The method of claim 1, wherein the presence of FRα in said urine sample derived from said subject at a concentration of greater than 9500 pg/mL, 10,000 pg/mL, 11,000 pg/mL, 12,000 pg/mL, 13,000 pg/mL, 14,000 pg/mL, 15,000 pg/mL, 16,000 pg/mL, 17,000 pg/mL, 18,000 pg/mL, 19,000 pg/mL, or 20,000 pg/mL is an indication that the subject is afflicted with ovarian cancer.

7. The method of claim 1, wherein the antibody is:

(a) an antibody that specifically binds the same epitope as the MORAb-003 antibody;

(b) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3;

(c) the MOV18 antibody;

(d) an antibody that specifically binds the same epitope as the MOV18 antibody;

(e) the 548908 antibody;

(f) an antibody that specifically binds the same epitope as the 548908 antibody;

(g) the 6D398 antibody;

(h) an antibody that specifically binds the same epitope as the 6D398 antibody;

(i) an antibody that specifically binds the same epitope as an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 58 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:54;

(j) an antibody comprising SEQ ID NO:55 (GYFMN) as CDRH1, SEQ ID NO:56 (RIFPYNGDTFYNQKFKG) as CDRH2, SEQ ID NO:57 (GTHYFDY) as CDRH3, SEQ ID NO:51 (RTSENIFSYLA) as CDRL1 , SEQ ID NO:52 (NAKTLAE) as CDRL2 and SEQ ID NO:53 (QHHYAFPWT) as CDRL3;

(k) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 58 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:54;

(l) an antibody that specifically binds the same epitope as an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 42 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:38;

(m) an antibody comprising SEQ ID NO:39 (HPYMH) as CDRH1, SEQ ID NO:40 (RIDPANGNTKYDPKFQG) as CDRH2, SEQ ID NO:41 (EEVADYTMDY) as CDRH3, SEQ

ID NO:35 (RASESVDTYGNNFIH) as CDRL1, SEQ ID NO:36 (LASNLES) as CDRL2 and SEQ ID NO:37 (QQNNGDPWT) as CDRL3;

(n) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 42 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:38;

(o) an antibody that specifically binds the same epitope as an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:30;

(p) an antibody comprising SEQ ID NO:31 (SGYYWN) as CDRH1, SEQ ID NO:32 (YIKSDGSNNYNPSLKN) as CDRH2, SEQ ID NO:33 (EWKAMDY) as CDRH3, SEQ ID NO:27 (RASSTVSYSYLH) as CDRL1, SEQ ID NO:28 (GTSNLAS) as CDRL2 and SEQ ID NO:29 (QQYSGYPLT) as CDRL3;

(q) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:30;

(r) an antibody that specifically binds the same epitope as an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:46;

(s) an antibody comprising SEQ ID NO:47 (SYAMS) as CDRH1, SEQ ID NO:48 (EIGSGGSYTYYPDTVTG) as CDRH2, SEQ ID NO:49 (ETTAGYFDY) as CDRH3, SEQ ID NO:43 (SASQGINNFLN) as CDRL1, SEQ ID NO:44 (YTSSLHS) as CDRL2 and SEQ ID NO:45 (QHFSKLPWT) as CDRL3;

(t) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:46;

(u) an antibody that comprises a variable region light chain of SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; or SEQ ID NO: 16;

(v) an antibody that comprises a variable region heavy chain of SEQ ID NO: 17; SEQ ID NO: 18; SEQ ID NO: 19; SEQ ID NO: 20; or SEQ ID NO: 21;

(w) an antibody that comprises the heavy chain variable region SEQ ID NO: 21 and the light chain variable region SEQ ID NO: 16;

(x) an antibody that comprises the heavy chain variable region SEQ ID NO: 19 and the light chain variable region SEQ ID NO: 16; or

(y) an antibody that comprises the heavy chain variable region SEQ ID NO: 18 and the light chain variable region SEQ ID NO: 16.

8. The method of claim 1, wherein the antibody is a murine antibody, a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, a Fab, Fab'2, ScFv, nanobody™, or a domain antibody.

9. The method of claim 1, wherein the antibody is labeled.

10. The method of claim 9, wherein the antibody is labeled with a label that is a radio-label, a biotin-label, a chromophore-label, a fluorophore-label, an ECL label or an enzyme-label.

11. The method of claim 1, wherein the level of FRα is determined by using a technique that is western blot analysis, radioimmunoassay, immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, solution phase assay, electrochemiluminescence immunoassay (ECLIA) or ELISA assay.

12. The method of claim 1, wherein the control sample comprises a standardized control level of FRα in a healthy subject.

13. The method of claim 1, wherein the urine sample derived from said subject is treated with guanidine prior to determining the level of FRα.

14. The method of claim 1, wherein the urine sample derived from said subject is diluted prior to determining the level of FRα.

15. The method of claim 1, wherein the urine sample derived from said subject is centrifuged, vortexed, or both, prior to determining the level of FRα.

16. The method of claim 1, wherein the level of FRα in the urine sample derived from said subject is assessed by contacting the urine sample derived from said subject with a pair of antibodies that are

(a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody,

(b) an antibody comprising SEQ ID NO:31 (SGYYWN) as CDRH1, SEQ ID NO:32 (YIKSDGSNNYNPSLKN) as CDRH2, SEQ ID NO:33 (EWKAMDY) as CDRH3, SEQ ID NO:27 (RASSTVSYSYLH) as CDRL1, SEQ ID NO:28 (GTSNLAS) as CDRL2 and SEQ ID NO:29 (QQYSGYPLT) as CDRL3 immobilized to a solid support and a labeled antibody comprising SEQ ID NO:47 (SYAMS) as CDRH1, SEQ ID NO:48 (EIGSGGSYTYYPDTVTG) as CDRH2, SEQ ID NO:49 (ETTAGYFDY) as CDRH3, SEQ ID NO:43 (SASQGINNFLN) as CDRL1, SEQ ID NO:44 (YTSSLHS) as CDRL2 and SEQ ID NO:45 (QHFSKLPWT) as CDRL3,

(c) an antibody comprising SEQ ID NO:55 (GYFMN) as CDRH1, SEQ ID NO:56 (RIFPYNGDTFYNQKFKG) as CDRH2, SEQ ID NO:57 (GTHYFDY) as CDRH3, SEQ ID NO:51 (RTSENIFSYLA) as CDRL1, SEQ ID NO:52 (NAKTLAE) as CDRL2 and SEQ ID NO:53 (QHHYAFPWT) as CDRL3 immobilized to a solid support and a labeled antibody comprising SEQ ID NO:39 (HPYMH) as CDRH1, SEQ ID NO:40 (RIDPANGNTKYDPKFQG) as CDRH2, SEQ ID NO:41 (EEVADYTMDY) as CDRH3, SEQ ID NO:35 (RASESVDTYGNNFIH) as CDRL1, SEQ ID NO:36 (LASNLES) as CDRL2 and SEQ ID NO:37 (QQNNGDPWT) as CDRL3, or

(d) an antibody comprising SEQ ID NO:31 (SGYYWN) as CDRH1, SEQ ID NO:32 (YIKSDGSNNYNPSLKN) as CDRH2, SEQ ID NO:33 (EWKAMDY) as CDRH3, SEQ ID NO:27 (RASSTVSYSYLH) as CDRL1, SEQ ID NO:28 (GTSNLAS) as CDRL2 and SEQ ID NO:29 (QQYSGYPLT) as CDRL3 immobilized to a solid support and a labeled antibody comprising SEQ ID NO:55 (GYFMN) as CDRH1, SEQ ID NO:56 (RIFPYNGDTFYNQKFKG) as CDRH2, SEQ ID NO:57 (GTHYFDY) as CDRH3, SEQ ID NO:51 (RTSENIFSYLA) as CDRL1, SEQ ID NO:52 (NAKTLAE) as CDRL2 and SEQ ID NO:53 (QHHYAFPWT) as CDRL3.

17. A method of assessing the progression of ovarian cancer in a subject afflicted with ovarian cancer, the method comprising

contacting a urine sample derived from said subject with an antibody that binds FRα, thereby determining the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample;

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample derived from said subject with the level of FRα in a control sample; and

(i) identifying an increase in the level of FRα in the urine sample derived from said subject as compared to the level of FRα in the control sample and determining that the ovarian cancer will progress rapidly based on said increase; or

(ii) identifying a decrease in the level of FRα in the urine sample derived from said subject as compared to the level of FRα in the control sample and determining that the ovarian cancer will progress slowly based on said decrease.

18. A method of stratifying a subject afflicted with ovarian cancer into one of at least four cancer therapy groups comprising:

contacting a urine sample derived from said subject with an antibody that binds FRα, thereby determining the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample;

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample derived from said subject with the level of FRα in a control sample; and

stratifying the subject into one of at least four cancer therapy groups based on the level of folate receptor alpha (FRα) which is not bound to a cell.

19. The method of claim 18, wherein the subject is stratified in Stage I, Stage II, Stage III or Stage IV ovarian cancer.

20. A method of monitoring the efficacy of MORAb-003 treatment of ovarian cancer in a subject suffering from ovarian cancer, the method comprising

contacting a urine sample derived from said subject with an antibody that binds FRα, thereby determining the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample, wherein said subject has been previously administered MORAb-003;

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample derived from said subject with the level of FRα in a control sample; and

(i) identifying an increase in the level of FRα in the urine sample derived from said subject as compared to the level of FRα in the control sample and determining that the MORAb-003 treatment is not efficacious; or

(ii) identifying a decrease in the level of FRα in the urine sample derived from said subject as compared to the level of FRα in the control sample and determining that the MORAb-003 treatment is efficacious.

21. A method for predicting whether a subject suffering from ovarian cancer will respond to treatment with MORAb-003, the method comprising

contacting a urine sample derived from said subject with an antibody that binds FRα, thereby determining the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample;

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in said urine sample derived from said subject with the level of FRα in a control sample; and

identifying an increase in the level of FRα in the urine sample derived from said subject as compared to the level of FRα in the control sample and determining that the subject will respond to treatment with MORAb-003.

22. A method of assessing whether a subject is afflicted with folate receptor alpha (FRα)-expressing ovarian cancer or lung cancer, the method comprising

determining the level of FRα which is not bound to a cell in a serum or plasma sample derived from said subject; and

comparing the level of FRα which is not bound to a cell with the level of FRα in a control sample,

identifying an increase in the level of FRα in the serum or plasma sample derived from said subject as compared to the level of FRα in the control sample; and

determining that the subject is afflicted with ovarian cancer or lung cancer based upon said increase,

wherein the level of FRα which is not bound to a cell in the serum or plasma sample derived from said subject is assessed by contacting the serum or plasma sample with a pair of antibodies that bind FRα in a two-antibody sandwich assay, wherein the two antibody-sandwich assay is an electrochemiluminescence assay (ECLIA) or enzyme immunoassay (EIA).

23. The method of claim 22, wherein the subject is identified as being afflicted with ovarian cancer.

24. The method of claim 22, wherein the subject is identified as being afflicted with lung cancer.

25. The method of claim 24, wherein the lung cancer is non-small cell lung cancer.

26. The method of claim 25, wherein the non-small cell lung cancer is adenocarcinoma.

27. The method of claim 22, wherein the antibodies that bind FRα are selected from the group consisting of:

(a) an antibody that specifically binds the same epitope as the MORAb-003 antibody;

(b) an antibody comprising SEQ ID NO:1 (GFTFSGYGLS) as CDRH1, SEQ ID NO:2 (MISSGGSYTYYADSVKG) as CDRH2, SEQ ID NO:3 (HGDDPAWFAY) as CDRH3, SEQ ID NO:4 (SVSSSISSNNLH) as CDRL1, SEQ ID NO:5 (GTSNLAS) as CDRL2 and SEQ ID NO:6 (QQWSSYPYMYT) as CDRL3;

(c) the MOV18 antibody;

(d) an antibody that specifically binds the same epitope as the MOV18 antibody;

(e) the 548908 antibody;

(f) an antibody that specifically binds the same epitope as the 548908 antibody;

(g) the 6D398 antibody;

(h) an antibody that specifically binds the same epitope as the 6D398 antibody;

(i) an antibody that specifically binds the same epitope as an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 58 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:54;

(j) an antibody comprising SEQ ID NO:55 (GYFMN) as CDRH1, SEQ ID NO:56 (RIFPYNGDTFYNQKFKG) as CDRH2, SEQ ID NO:57 (GTHYFDY) as CDRH3, SEQ ID NO:51 (RTSENIFSYLA) as CDRL1 , SEQ ID NO:52 (NAKTLAE) as CDRL2 and SEQ ID NO:53 (QHHYAFPWT) as CDRL3;

(k) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 58 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:54;

(l) an antibody that specifically binds the same epitope as an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 42 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:38;

(m) an antibody comprising SEQ ID NO:39 (HPYMH) as CDRH1, SEQ ID NO:40 (RIDPANGNTKYDPKFQG) as CDRH2, SEQ ID NO:41 (EEVADYTMDY) as CDRH3, SEQ ID NO:35 (RASESVDTYGNNFIH) as CDRL1, SEQ ID NO:36 (LASNLES) as CDRL2 and SEQ ID NO:37 (QQNNGDPWT) as CDRL3;

(n) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 42 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:38;

(o) an antibody that specifically binds the same epitope as an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:30;

(p) an antibody comprising SEQ ID NO:31 (SGYYWN) as CDRH1, SEQ ID NO:32 (YIKSDGSNNYNPSLKN) as CDRH2, SEQ ID NO:33 (EWKAMDY) as CDRH3, SEQ ID NO:27 (RASSTVSYSYLH) as CDRL1, SEQ ID NO:28 (GTSNLAS) as CDRL2 and SEQ ID NO:29 (QQYSGYPLT) as CDRL3;

(q) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:30;

(r) an antibody that specifically binds the same epitope as an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:46;

(s) an antibody comprising SEQ ID NO:47 (SYAMS) as CDRH1, SEQ ID NO:48 (EIGSGGSYTYYPDTVTG) as CDRH2, SEQ ID NO:49 (ETTAGYFDY) as CDRH3, SEQ ID NO:43 (SASQGINNFLN) as CDRL1, SEQ ID NO:44 (YTSSLHS) as CDRL2 and SEQ ID NO:45 (QHFSKLPWT) as CDRL3;

(t) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:46;

(u) an antibody that comprises a variable region light chain of SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; or SEQ ID NO: 16;

(v) an antibody that comprises a variable region heavy chain of SEQ ID NO: 17; SEQ ID NO: 18; SEQ ID NO: 19; SEQ ID NO: 20; or SEQ ID NO: 21;

(w) an antibody that comprises the heavy chain variable region SEQ ID NO: 21 and the light chain variable region SEQ ID NO: 16;

(x) an antibody that comprises the heavy chain variable region SEQ ID NO: 19 and the light chain variable region SEQ ID NO: 16; and

(y) an antibody that comprises the heavy chain variable region SEQ ID NO: 18 and the light chain variable region SEQ ID NO: 16.

28. The method of claim 22, wherein the antibodies that bind FRα are selected from the group consisting of a murine antibody, a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, a Fab, Fab'2, ScFv, nanobody™, and a domain antibody.

29. The method of claim 22, wherein the level of FRα is determined by electrochemiluminescence immunoassay (ECLIA).

30. The method of claim 22, wherein the control sample comprises a standardized control level of FRα in a healthy subject.

31. The method of claim 22, wherein (i) the serum or plasma sample derived from the subject is treated with guanidine prior to determining the level of FRα, (ii) the serum or plasma sample derived from the subject is diluted prior to determining the level of FRα, and/or (iii) the serum or plasma sample derived from the subject is centrifuged, vortexed, or both, prior to determining the level of FRα.

32. The method of claim 22, wherein the pair of antibodies is selected from the group consisting of:

(a) MOV18 antibody immobilized to a solid support and labeled MORAB-003 antibody,

(b) an antibody comprising SEQ ID NO:31 (SGYYWN) as CDRH1, SEQ ID NO:32 (YIKSDGSNNYNPSLKN) as CDRH2, SEQ ID NO:33 (EWKAMDY) as CDRH3, SEQ ID NO:27 (RASSTVSYSYLH) as CDRL1, SEQ ID NO:28 (GTSNLAS) as CDRL2 and SEQ ID NO:29 (QQYSGYPLT) as CDRL3 immobilized to a solid support and a labeled antibody comprising SEQ ID NO:47 (SYAMS) as CDRH1, SEQ ID NO:48 (EIGSGGSYTYYPDTVTG) as CDRH2, SEQ ID NO:49 (ETTAGYFDY) as CDRH3, SEQ ID NO:43 (SASQGINNFLN) as CDRL1, SEQ ID NO:44 (YTSSLHS) as CDRL2 and SEQ ID NO:45 (QHFSKLPWT) as CDRL3, and

(c) an antibody comprising SEQ ID NO:55 (GYFMN) as CDRH1, SEQ ID NO:56 (RIFPYNGDTFYNQKFKG) as CDRH2, SEQ ID NO:57 (GTHYFDY) as CDRH3, SEQ ID NO:51 (RTSENIFSYLA) as CDRL1 , SEQ ID NO:52 (NAKTLAE) as CDRL2 and SEQ ID NO:53 (QHHYAFPWT) as CDRL3 immobilized to a solid support and a labeled antibody comprising SEQ ID NO:39 (HPYMH) as CDRH1, SEQ ID NO:40 (RIDPANGNTKYDPKFQG) as CDRH2, SEQ ID NO:41 (EEVADYTMDY) as CDRH3, SEQ ID NO:35 (RASESVDTYGNNFIH) as CDRL1, SEQ ID NO:36 (LASNLES) as CDRL2 and SEQ ID NO:37 (QQNNGDPWT) as CDRL3.

33. A method of assessing the progression of ovarian cancer in a subject afflicted with ovarian cancer, the method comprising

determining the level of folate receptor alpha (FRα) which is not bound to a cell in a serum or plasma sample derived from said subject by performing a two antibody sandwich assay,

wherein the two antibody-sandwich assay is an electrochemiluminescence assay (ECLIA) or enzyme immunoassay (EIA);

comparing the level of folate receptor alpha (FRα) which is not bound to a cell with the level of FRα in a control sample; and

(i) identifying an increase in the level of FRα in the serum or plasma sample derived from said subject as compared to the level of FRα in the control sample and determining that the ovarian cancer will progress rapidly based on said increase; or

(ii) identifying a decrease in the level of FRα in the serum or plasma sample derived from said subject as compared to the level of FRα in the control sample and determining that the ovarian cancer will progress slowly based on said decrease.

34. A method of stratifying a subject afflicted with ovarian cancer into one of at least four cancer therapy groups comprising:

determining the level of folate receptor alpha (FRα) which is not bound to a cell, in a serum or plasma sample derived from said subject by performing a two antibody sandwich assay, wherein the two antibody-sandwich assay is an electrochemiluminescence assay (ECLIA) or enzyme immunoassay (EIA); and

stratifying the subject into one of at least four cancer therapy groups based on the level of folate receptor alpha (FRα) which is not bound to a cell.

35. The method of claim 34, wherein the subject is stratified into Stage I, Stage II, Stage III or Stage IV ovarian cancer.

36. A method of monitoring the efficacy of MORAb-003 treatment of ovarian cancer in a subject suffering from ovarian cancer, the method comprising

determining the level of folate receptor alpha (FRα) which is not bound to a cell in a serum or plasma sample derived from said subject by performing a two antibody sandwich assay, wherein the two antibody-sandwich assay is an electrochemiluminescence assay (ECLIA) or enzyme immunoassay (EIA), wherein said subject has been previously administered MORAb-003;

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in said serum or plasma sample derived from said subject with the level of FRα in a control sample; and

(i) identifying an increase in the level of FRα in the serum or plasma sample derived from said subject as compared to the level of FRα in the control sample and determining that the MORAb-003 treatment is not efficacious; or

(ii) identifying a decrease in the level of FRα in the serum or plasma sample derived from said subject as compared to the level of FRα in the control sample and determining that the MORAb-003 treatment is efficacious.

37. A method for predicting whether a subject suffering from ovarian cancer will respond to treatment with MORAb-003, the method comprising

determining the level of folate receptor alpha (FRα) which is not bound to a cell in a serum or plasma sample derived from said subject,

comparing the level of folate receptor alpha (FRα) which is not bound to a cell in the serum or plasma sample derived from said subject with the level of FRα in a control sample,

identifying an increase in the level of FRα in the serum or plasma sample derived from said subject as compared to the level of FRα in the control sample and determining that the subject will respond to treatment with MORAb-003,

wherein the level of FRα which is not bound to a cell in the serum or plasma sample derived from said subject is assessed by contacting the serum or plasma sample with a pair of antibodies that bind FRα in a two-antibody sandwich assay, wherein the two antibody-sandwich assay is an electrochemiluminescence assay (ECLIA) or enzyme immunoassay (EIA).

38. The method of claim 22, wherein at least one of the antibodies that bind FRα is labeled.

39. The method of claim 38, wherein the label is a radio-label, a biotin-label, a chromophore-label, a fluorophore-label, an ECL label or an enzyme-label.