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DESCRIPTION

TECHNICAL FIELD

[0001] The subject matter provided herein relates to folate receptor alpha (FRα)-specific antibodies

BACKGROUND

[0002] In humans, the high affinity receptor for folate comes in four isoforms: alpha, beta, gamma, and delta. The alpha, beta and delta 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 folate receptor may be derived by the action of proteases or phospholipase on membrane anchored folate receptors.

[0003] 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 (1992). Overexpression of FRα has been observed in various cancers, including lung cancer (*e.g.,* 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; papillary serous and endometrioid adenocarcinomas of the ovary, serous cystadenocarcinomas of the ovary, 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 Publication No. 2005/0232919; Int1. Pub1. No. 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); Basal et al., PLoS ONE, 4(7):6292 (2009); Fisher RE J Nucl Med, 49: 899-906 (2008); Franklin, WA et al., Int J Cancer, Suppl 8: 89-95 (1994); Hartmann LC et al., Int J Cancer 121: 938-942 (2007); Iwakiri S et al., Annals of Surgical Oncology, 15(3): 889-899 (2008); European patent publication EP 2199796, Parker N. et al., Analytical Biochemistry, 338: 284-293 (2005); Weitman, SD et al., Cancer Res 52: 3396-3401 (1992); Saba NF 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 high level of FRα expression is associated with a poor prognosis, whereas in other types of cancers (e.g., non-small-cell lung cancers), a higher level of FRα expression is associated with a more favorable prognosis. See, *e.g.,* Iwakiri S et al., Annals of Surgical Oncology, 15(3): 889-

899; Saba NF et al., Head Neck, 31(4): 475-481 (2009).
Smith et al, Hybridoma, vol 26, no. 5, 2007-10 pg 281-288 discloses a monoclonal antibody that may be used to detect folate receptor alpha.

[0004] 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 FRα-expressing cancers and for monitoring existing FRα-expressing cancers.

SUMMARY

[0005] Provided herein are antibodies that specifically bind to FRα. Also described are related polynucleotides capable of encoding the provided antibodies, cells expressing the provided antibodies, as well as associated vectors and detectable antibody labels. In addition, methods of using the provided antibodies are described. For example, the provided antibodies may be used to diagnose cancer; monitor cancer progression, regression, or stable disease; develop a prognosis for cancer in a subject; to determine whether or not a patient should be treated for cancer, or to determine whether or not a subject is afflicted with FRα-expressing cancer and thus may be amenable to treatment with a FRα-specific anti-cancer therapeutic.

[0006] In a first aspect of the present invention provides an isolated antibody, or antigen-binding fragment thereof, specific for folate receptor alpha (FRα) comprising a light chain CDR1 having the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 having the amino acid sequence of SEQ ID NO: 27, a light chain CDR3 having the amino acid sequence of SEQ ID NO: 28, a heavy chain CDR1 having the amino acid sequence of SEQ ID NO: 30, a heavy chain CDR2 having the amino acid sequence of SEQ ID NO: 31, and a heavy chain CDR3 having the amino acid sequence of SEQ ID NO: 32.

[0007] In a second aspect of the present invention provides an isolated polynucleotide encoding an antibody, or antigen-binding fragment thereof, specific for folate receptor alpha (FRα), wherein the light chain CDR1 of the encoded antibody comprises the amino acid sequence of SEQ ID NO: 26, the light chain CDR2 of the encoded antibody comprises the amino acid sequence of SEQ ID NO: 27, the light chain CDR3 of the encoded antibody comprises the amino acid sequence of SEQ ID NO: 28, the heavy chain CDR1 of the encoded antibody comprises the amino acid sequence of SEQ ID NO: 30, the heavy chain CDR2 of the encoded antibody comprises the amino acid sequence of SEQ ID NO: 31, and the heavy chain CDR3 of the encoded antibody comprises the amino acid sequence of SEQ ID NO: 32.

[0008] In a third aspect of the present invention provides a vector comprising the isolated polynucleotide of any one of claims 5 to 6.

[0009] In a fourth aspect of the present invention provides a recombinant cell comprising the vector of claim 7.

[0010] In a fifth aspect of the present invention provides an isolated antibody specific for folate receptor alpha (FRα) produced by the cell line deposited with the ATCC having accession number PTA-11885.

[0011] In a sixth aspect of the present invention provides a method of detecting folate receptor alpha (FRα) or FRα-expressing cancer in a biological sample, comprising exposing the sample to the antibody of claim 1 or 10, or antigen-binding fragment thereof, and detecting folate receptor alpha (FRα).

[0012] In a seventh aspect of the present invention provides A method of diagnosing a folate receptor alpha-expressing cancer in a subject, comprising:

1. a. exposing a biological sample of the subject to the antibody of claim 1 or claim 10, or an antigen-binding fragment thereof;
2. b. quantifying the amount of folate receptor alpha (FRα) present in the sample;
3. c. comparing the amount of folate receptor alpha (FRα) present in the sample to a known standard; and
4. d. determining whether the subject's folate receptor alpha (FRα) levels fall within the levels of folate receptor alpha (FRα) associated with cancer.

[0013] In a eighth aspect of the present invention provides a method of monitoring a folate receptor alpha-expressing cancer in a subject, comprising:

1. a. exposing a biological sample of the subject to the antibody of claim 1 or claim 10, or an antigen-binding fragment thereof;
2. b. quantifying the amount of folate receptor alpha (FRα) present in the sample that is bound by the antibody or antigen-binding fragment thereof;
3. c. comparing the amount of folate receptor alpha (FRα) present in the sample to either
    1. i. a known standard, or
    2. ii. a biological sample obtained from the subject at an earlier point in time; and
4. d. determining whether the subject's folate receptor alpha (FRα) levels are indicative of cancer progression, regression or stable disease.

[0014] In a ninth aspect of the present invention provides a kit for detecting the presence of folate receptor alpha (FRα) in a biological sample, comprising at least one antibody of claim 1 or claim 10, or an antigen-binding fragment thereof.

[0015] In a tenth aspect of the present invention provides a kit for detecting the presence of folate receptor alpha (FRα) in a biological sample, comprising:

at least one antibody of claim 1 or claim 10, or an antigen-binding fragment thereof;

wherein the included antibody, or antigen-binding fragment thereof, is affixed to a solid support.

[0016] In a eleventh aspect of the present invention provides a kit for detecting the presence of folate receptor alpha (FRα) in a biological sample, comprising:

at least one antibody of claim 1 or claim 10, or an antigen-binding fragment thereof;

wherein the included antibody, or antigen-binding fragment thereof, is detectably labeled.

Folate Receptor Alpha (FRα)-Specific Antibodies

[0017] Described herein are isolated antibodies and antigen-binding fragments that specifically bind to FRα. In some embodiments, the antibodies or antigen-binding fragments are murine IgG, or derivatives thereof.

[0018] Described herein are isolated antibodies and antigen-binding fragments that specifically bind to FRα in either a native, nonreduced, or chemically preserved form. In some embodiments, the antibodies or antigen-binding fragments are murine IgG, or derivatives thereof. While the antibodies or antigen-binding fragments may be human, humanized, or chimeric, the antibodies or antigen-binding fragments exemplified herein are murine.

[0019] The described antibodies or antigen-binding fragments may include a light chain variable domain that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 29. In some embodiments, an isolated polynucleotide that includes a sequence substantially the same as, or identical to, SEQ ID NO: 61 may encode this light chain variable domain amino acid sequence. The described antibodies or antigen-binding fragments may include a heavy chain variable domain that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 33. In some embodiments, an isolated polynucleotide that includes a sequence substantially the same as, or identical to, SEQ ID NO: 65 may encode this heavy chain variable domain amino acid sequence. The described antibodies or antigen-binding fragments 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: 29, and the heavy chain variable domain includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 33. In some embodiments are provided the 26B3.F2 (26B3) antibody or antigen-binding fragments thereof, which is capable of binding to the native, nonreduced, or chemically preserved forms of FRα.

[0020] In some embodiments, the 26B3 antibody 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 antibodies, or antigen-binding fragments thereof, have the binding affinity for FRα of the antibodies produced by the deposited antibody-producing cells. In some embodiments, the disclosed antibodies, or antigen-binding fragments thereof, comprise the light and heavy chain CDRs of the antibodies produced by the deposited antibody-producing cells. In some embodiments, the antibodies, or antigen-binding fragments thereof, comprise the light and heavy chain variable regions of the antibodies produced by the deposited antibody-producing cells.

[0021] Also disclosed are polynucleotides that encode antibodies or antigen-binding fragments that specifically bind to the native, nonreduced, or chemically preserved forms of FRα. In some embodiments, the isolated polynucleotides encode an antibody or antigen-binding fragment thereof having a light chain CDR1 sequence substantially the same as, or identical to, SEQ ID NO: 26, for example SEQ ID NO: 58. In some embodiments, the isolated polynucleotides encode an antibody or antigen-binding fragment thereof having a light chain CDR2 substantially the same as, or identical to, SEQ ID NO: 27, for example SEQ ID NO: 59. In some embodiments, the isolated polynucleotides encode an antibody or antigen-binding fragment thereof having a light chain CDR3 substantially the same as, or identical to, SEQ ID NO: 28, for example SEQ ID NO: 60. In some embodiments, the isolated polynucleotides encode an antibody or antigen-binding fragment thereof having a heavy chain CDR1 substantially the same as, or identical to, SEQ ID NO: 30, for example SEQ ID NO: 62. In some embodiments, the isolated polynucleotides encode an antibody or antigen-binding fragment thereof having a heavy chain CDR2 substantially the same as, or identical to, SEQ ID NO: 31, for example SEQ ID NO: 63. In some embodiments, the isolated polynucleotides encode an antibody or antigen-binding fragment thereof having a heavy chain CDR3 substantially the same as, or identical to, SEQ ID NO: 32, for example SEQ ID NO: 64. The polynucleotides may encode an antibody or antigen-binding fragment thereof having a light chain with a CDR1 substantially the same as, or identical to, SEQ ID NO: 26, for example SEQ ID NO: 58; a CDR2 substantially the same as, or identical to, SEQ ID NO: 27, for example SEQ ID NO: 59; and a CDR3 substantially the same as, or identical to, SEQ ID NO: 28, for example SEQ ID NO: 60. The polynucleotides may encode an antibody or antigen-binding fragment thereof having a heavy chain CDR1 substantially the same as, or identical to, SEQ ID NO: 30, for example SEQ ID NO: 62; a CDR2 substantially the same as, or identical to, SEQ ID NO: 31, for example SEQ ID NO: 63; and a CDR3 substantially the same as, or identical to, SEQ ID NO: 32, for example SEQ ID NO: 64. The polynucleotides may encode an antibody or antigen-binding fragment thereof having a light chain CDR1 substantially the same as, or identical to, SEQ ID NO: 26, for example SEQ ID NO: 58; a CDR2 encoded by a nucleotide sequence substantially the same as, or identical to, SEQ ID NO: 27, for example SEQ ID NO: 59; and a CDR3 encoded by a nucleotide sequence substantially the same as, or identical to, SEQ ID NO: 28, for example SEQ ID NO: 60; and a heavy chain CDR1 substantially the same as, or identical to, SEQ ID NO: 30, for example SEQ ID NO: 62; a CDR2 substantially the same as, or identical to, SEQ ID NO: 31, for example SEQ ID NO: 63; and a CDR3 substantially the same as, or identical to, SEQ ID NO: 32, for example SEQ ID NO: 64. Antigen-binding arrangements of CDRs may also be engineered using antibody-like proteins as CDR scaffolding. Such engineered antigen-

binding proteins are within the scope of the disclosure.

[0022] Polynucleotides described herein may encode antibodies or antigen-binding fragments that have a light chain variable domain segment that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 29, for example SEQ ID NO: 61. In some embodiments the described polynucleotides may encode antibodies or antigen-binding fragments that have a heavy chain variable domain segment that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 33, for example SEQ ID NO: 65. In some embodiments the described polynucleotides may encode antibodies or antigen-binding fragments that have a light chain variable domain segment that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 29, for example SEQ ID NO: 61; and a heavy chain variable domain segment that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 33, for example SEQ ID NO: 65. The polynucleotides capable of encoding the variable domain segments provided herein may be included on the same, or different, vectors to produce an antibodies or antigen-binding fragments. Polynucleotides described herein may encode the 26B3 antibody or antigen-binding fragments thereof, capable of binding the native, nonreduced, or chemically preserved forms of FRα.

[0023] Vectors comprising the antibody- and antigen-binding fragment-encoding polynucleotides are provided, as are cells expressing the antibodies or antigen-binding fragments that specifically bind to FRα. Also provided are cells capable of expressing the described vectors. These cells may be mammalian cells (such as CHO-K1 cells), insect cells (such as Sf7 cells), yeast cells, plant cells, or bacteria cells (such as *E. coli*). The described antibodies may also be produced by hybridoma cells, as described herein.

Methods for Diagnosing Cancer

[0024] Provided herein are methods for diagnosing breast, thyroid, colorectal, endometrial, fallopian tube, ovarian or lung cancer of epithelial origin in a subject. In some embodiments the described methods involve assessing whether a subject is afflicted with FRα-expressing cancer by determining the level of FRα that is present in a sample derived from the subject; and comparing the observed level of FRα 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 either is or is not afflicted with an FRα-expressing cancer.

[0025] In some embodiments the control sample may be derived from a subject that is not afflicted with FRα-expressing cancer. In some embodiments the control sample may be derived from a subject that is afflicted with FRα-expressing cancer. In some embodiments where the control sample is derived from a subject that is not afflicted with FRα-expressing cancer, an observed increase in the amount of FRα present in the sample, relative to that observed for the control sample, is an indication that the subject being assessed is afflicted with FRα-

expressing cancer. In some embodiments where the control sample is derived from a subject that is not afflicted with FRα-expressing cancer, an observed decrease or similarity in the amount of FRα present in the test sample, relative to that observed for the control sample, is an indication that the subject being assessed is not afflicted with FRα-expressing cancer. In some embodiments where the control sample is derived from a subject that is afflicted with FRα-expressing cancer, an observed similarity in the amount of FRα present in the test sample, relative to that observed for the control sample, is an indication that the subject being assessed is afflicted with FRα-expressing cancer. In some embodiments where the control sample is derived from a subject that is afflicted with FRα-expressing cancer, an observed decrease in the amount of FRα present in the test sample, relative to that observed for the control sample, is an indication that the subject being assessed is not afflicted with FRα-expressing cancer.

[0026] In some embodiments the level of FRα in the sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα, such as the antibodies described herein. Similar methods may be used to determine if a subject is afflicted with cancer that is not associated with increased FRα production. The sample assessed for the presence of FRα may be derived from urine, blood, serum, plasma, saliva, ascites, circulating cells, circulating tumor cells, cells that are not tissue associated (i.e., free cells), tissues (e.g., surgically resected tumor tissue, biopsies, including fine needle aspiration), histological preparations, and the like.

[0027] In some embodiments the described methods involve assessing whether a subject is afflicted with FRα-expressing cancer by determining the level of FRα associated with a cell or tissue that is present in a sample derived from the subject; and comparing the observed level of FRα 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 some embodiments the level of FRα in the sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα, such as the antibodies described herein. The sample assessed for the presence of FRα may be circulating cells, circulating tumor cells, cells that are not tissue associated (i.e., free cells), tissues (e.g., surgically resected tumor tissue, biopsies, including fine needle aspiration), histological preparations, and the like.

[0028] In some embodiments the described methods involve assessing whether a subject is afflicted with FRα-expressing cancer by determining the level of FRα that is not associated with a cell or tissue that is present in a sample derived from the subject; and comparing the observed level of FRα 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 some embodiments the level of FRα in the sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα, such as the antibodies described herein. The sample assessed for the presence of FRα may be urine, blood, serum, plasma, saliva, ascites, histological preparations, and the like.

[0029] In various embodiments of the described methods, the cancer may be FRα-expressing cancer. In a particular embodiment, the FRα-expressing cancer is ovarian cancer. In some embodiments the FRα-expressing cancer is endometrial cancer. In some embodiments the FRα-expressing cancer is colorectal cancer. In some embodiments the FRα-expressing cancer is breast cancer. In some embodiments the FRα-expressing cancer is thyroid cancer. In some embodiments the FRα-expressing cancer is fallopian tube cancer. In another embodiment, the FRα-expressing cancer is non-small cell lung cancer, such as an adenocarcinoma. Alternatively, the described methods may be used to identify cancer that does not express FRα, such as squamous cell carcinoma. For example, the described methods could be used to distinguish a FRα-expressing lung cancer, such as adenocarcinoma, from a lung cancer that does not express FRα, such as squamous cell carcinoma. The described methods could be used to distinguish a FRα-expressing breast cancer, such as fibroadenoma, from breast cancer that does not express FRα, such as cystosarcoma. Furthermore, the described methods could be used to distinguish a FRα-expressing thyroid cancer, such as papillary carcinoma, from thyroid cancer that does not express FRα, such as medullary carcinoma. In some embodiments described herein detection of FRα-expressing cancer cells in a subject may be used to determine that the subject may be treated with a therapeutic agent directed against FRα. In some embodiments the therapeutic agent directed against FRα may be an antibody, such as Farletuzumab.

[0030] In various aspects, the level of FRα is determined by contacting the sample with an antibody, or antigen-binding fragment thereof, the binds FRα. In some embodiments, the sample may be contacted by more than one type of antibody, or antigen-binding fragment thereof, that binds FRα. In some embodiments, the sample may be contacted by a first antibody, or antigen-binding fragment thereof, that binds FRα and then contacted by a second antibody, or antigen-binding garment thereof, that binds FRα. When two antibodies are used one of the antibodies is as defined in any one of Claims 1 to 3 and the second antibody is selected from the group consisting of:

1. (a) an antibody, or antigen-binding fragment thereof, that binds the same epitope as any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3;
2. (b) any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3, or an antigen-binding fragment thereof;
3. (c) an antibody, or antigen-binding fragment thereof, that comprises the heavy and light chain CDRs of any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3
4. (d) an antibody, or antigen-binding fragment thereof, that comprises the heavy chain variable domain segment and light chain variable domain segment of any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3, as described in Table 1; or
5. (e) an antibody having the amino acid sequence of antibody produced by any one of the cell lines deposited with the ATCC having accession number PTA-11887, PTA-11884, PTA-11886, or PTA-11885, or an antigen binding fragment thereof.

[0031] In certain embodiments, the level of FRα is determined by western blot analysis, radioimmunoassay, immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, electrochemiluminescence (ECL) immunoassay, immunohistochemistry, fluorescence-activated cell sorting (FACS) or ELISA assay.

[0032] In various embodiments of the foregoing aspects of the invention, the control sample is a standardized control level of FRα in a healthy subject. In other embodiments the control sample may be FRα protein at a known concentration (e.g., a recombinant or purified FRα protein sample). In some embodiments, the observed FRα-levels of the tested subject may be compared with FRα levels observed in samples from subjects known to have FRα-expressing cancer or known concentrations of FRα.

Methods for Monitoring Cancer

[0033] Provided herein are methods for monitoring FRα-expressing cancer in a subject. The described methods may be used before treatment for cancer, after treatment for cancer, or both before and after treatment for cancer. In some embodiments the described methods involve assessing whether FRα-expressing cancer is progressing, regressing, or remaining stable by determining the level of FRα that is present in a test sample derived from the subject; and comparing the observed level of FRα with the level of FRα in a sample obtained from the subject at an earlier point in time, wherein a difference between the level of FRα in the test sample and the earlier sample provides an indication of whether the cancer is progressing, regressing, or remaining stable. In this regard, a test sample with an increased level of FRα, relative to the levels observed for the earlier sample, may indicate progression of an FRα-expressing cancer. Conversely, a test sample with a decreased level of FRα, relative to the levels observed for the earlier sample, may indicate regression of an FRα-expressing cancer. Accordingly, a test sample with an insignificant difference in the level of FRα, relative to the levels observed for the earlier sample, may indicate a state of stable disease for an FRα-expressing cancer. In some embodiments the level of FRα in a sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα, such as the antibodies described herein. The sample assessed for the presence of FRα may be derived from urine, blood, serum, plasma, saliva, ascites, circulating cells, circulating tumor cells, cells that are not tissue associated (i.e., free cells), tissues (e.g., surgically resected tumor tissue, biopsies, including fine needle aspiration), histological preparations, and the like.

[0034] In some embodiments the described methods involve assessing whether FRα-expressing cancer is progressing, regressing, or remaining stable by determining the level of FRα associated with a cell or tissue that is present in a test sample derived from the subject; and comparing the observed level of FRα with the level of FRα in a sample obtained from the subject, in a similar manner, at an earlier point in time, wherein a difference between the level of FRα in the test sample and the earlier sample provides an indication of whether the cancer

is progressing, regressing, or remaining stable. In this regard, a test sample with an increased level of FRα, relative to the levels observed for the earlier sample, may indicate progression of an FRα-expressing cancer. Conversely, a test sample with a decreased level of FRα, relative to the levels observed for the earlier sample, may indicate regression of an FRα-expressing cancer. Accordingly, a test sample with an insignificant difference in the level of FRα, relative to the levels observed for the earlier sample, may indicate a state of stable disease for an FRα-expressing cancer. In some embodiments the level of FRα in a sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα, such as the antibodies described herein. The sample assessed for the presence of FRα may be circulating cells, circulating tumor cells, cells that are not tissue associated (i.e., free cells), tissues (e.g., surgically resected tumor tissue, biopsies, including fine needle aspiration), histological preparations, and the like.

[0035] In some embodiments the described methods involve assessing whether FRα-expressing cancer is progressing, regressing, or remaining stable by determining the level of FRα not associated with a cell or tissue that is present in a test sample derived from the subject; and comparing the observed level of FRα with the level of FRα in a sample obtained from the subject, in a similar manner, at an earlier point in time, wherein a difference between the level of FRα in the test sample and the earlier sample provides an indication of whether the cancer is progressing, regressing, or remaining stable. In this regard, a test sample with an increased level of FRα, relative to the levels observed for the earlier sample, may indicate progression of an FRα-expressing cancer. Conversely, a test sample with a decreased level of FRα, relative to the levels observed for the earlier sample, may indicate regression of an FRα-expressing cancer. Accordingly, a test sample with an insignificant difference in the level of FRα, relative to the levels observed for the earlier sample, may indicate a state of stable disease for an FRα-expressing cancer. In some embodiments the level of FRα in a sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα, such as the antibodies described herein. The sample assessed for the presence of FRα may be urine, blood, serum, plasma, saliva, ascites, histological preparations, and the like.

[0036] In various embodiments of the described methods, the cancer may be FRα-expressing cancer. In a particular embodiment, the FRα-expressing cancer is ovarian cancer. In some embodiments the FRα-expressing cancer is endometrial cancer. In some embodiments the FRα-expressing cancer is colorectal cancer. In some embodiments the FRα-expressing cancer is breast cancer. In some embodiments the FRα-expressing cancer is thyroid cancer. In some embodiments the FRα-expressing cancer is fallopian tube cancer. In another embodiment, the FRα-expressing cancer is non-small cell lung cancer, such as an adenocarcinoma.

[0037] In various aspects, the level of FRα is determined by contacting the sample with an antibody, or antigen-binding fragment thereof, that binds FRα. In some embodiments, the sample may be contacted by more than one type of antibody, or antigen-binding fragment thereof, that binds FRα. In some embodiments, the sample may be contacted by a first antibody, or antigen-binding fragment thereof, that binds FRα and then contacted by a second antibody, or antigen-binding fragment thereof, that binds FRα. When two antibodies are used

one of the antibodies is as defined in any one of Claims 1 to 3, and the second antibody is selected from the group consisting of:

1. (a) an antibody, or antigen-binding fragment thereof, that binds the same epitope as any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3;
2. (b) any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3, or an antigen-binding fragment thereof;
3. (c) an antibody, or antigen-binding fragment thereof, that comprises the heavy and light chain CDRs of any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3.

[0038] In certain embodiments, the level of FRα is determined by western blot analysis, radioimmunoassay, immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, electrochemiluminescence (ECL) immunoassay, immunohistochemistry, fluorescence-activated cell sorting (FACS) or ELISA assay.

[0039] Additional aspects of the summarized subject matter are provided in greater detail in the detailed description and provided examples and associated figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] The present invention is defined by the claims. Any figures which fall outside the scope of the claims are provided for reference only and do not form part of the invention.

Figure 1 depicts the migratory patterns of FRα by SDS-PAGE under nonreducing, conditions. FRα was assessed in either native (lane 2) or reduced and alkylated (lane 3) form.

Figure 2 illustrates amino acid residues of FRα (SEQ ID NO: 1) that comprise the epitopes (shaded regions) for monoclonal antibodies 9F3, 24F12, and 26B3, as predicted by hydrogen/deuterium exchange mass spectrometry and docking methods.

Figure 3 shows four western blots of purified recombinant (A) and whole cell lysates (B) from CHO cells expressing FRα or FR homologs FRβ, FRΓ or FrΔ were run on SDS-PAGE gels. Proteins were prepared in sample buffer with or without reducing agents. Panel A, lane 1, molecular weight markers, lanes 2-5. 0.5µg reduced FRα, FRβ, FRΓ, and FRΔ, respectively; lane 6, blank; lanes 7-10, 0.5µg nonreduced FRα, FRβ, FRΓ, and FRΔ, respectively. The positive band represents the only reactive species in each lane and corresponds to a molecular weight of ~38kDa. Panel B, lane 1 molecular weight markers, lane 2 CHO- FRα, lane 3, CHO- FRβ, lane 4 CHO- FRΔ whole cell lysates prepared in sample buffer without reducing agents and fractionated on an SDS-PAGE gel. Each panel is probed with the designated anti-FRα mAb labeled on the right. The molecular weights for FR are: FRα ~38kDA; FRβ ~30kDa; FRΓ ~28kDa; FRΔ ~26kDa. The LK26 and BN3.2 antibodies that

recognize FRα under denatured and nonreduced or reduced conditions, respectively, were used as positive controls.

Figure 4 shows a formalin-fixed, paraffin-embedded papillary serous ovarian cancer tissue sample probed for the presence of FRα with monoclonal antibody 26B3.

Figure 5 shows FRα expression in normal tissues. Normal lung (A) and kidney (B) samples stained with antibody 26B3 demonstrate that expression of FRα is highly restricted to epithelial cells and has a predominantly apical distribution (images are 20x magnification).

Figure 6 provides a graphical representation comparing M-scores for lung adenocarcinoma generated using antibody 26B3 or antibody BN3.2.

Figure 7 shows FRα staining of histologic subtypes of non-small cell lung carcinoma: (A) lung adenocarcinoma at 20x, (B) lung adenocarcinoma at 40x, (C) lung adenosquamous at 20x, and (D) lung squamous cell carcinoma at 40x.

Figure 8 provides a graphical representation comparing M-scores for lung adenocarcinoma duplicate samples (cores) stained with antibody 26B3.

Figure 9 illustrates the M-score for FRα distribution of lung adenocarcinoma and squamous cell carcinoma. The mean M-Scores were 19.84 (±18.64) and 1.39 (±5.54), respectively ($p<0.0001$).

Figure 10 shows FRα expression in three lung adenocarcinoma fine needle aspiration (FNA) samples (A), (B), and (C). Staining of cell block material from lymph node FNAs with antibody 26B3 demonstrated successful staining of FRα, with expression limited to epithelial cells with an apical distribution.

Figure 11 illustrates the survival functions (death or censor) for subjects having lung adenocarcinoma who were deemed to be FRα positive and FRα negative by immunohistochemistry analysis of tissue samples using antibody 26B3.

Figure 12 shows representative tissue microarray (TMA) images stained with antibody 26B3 at either 20x or 40x magnification for (A) ductal carcinoma *in situ,* (B)-(D) invasive ductal carcinoma.

Figure 13 provides a graphical representation of the M-score distribution, as determined by staining with 26B3, relative to the molecular subtype (her-2 (+) and her-2 (-)) of the breast cancer sample.

Figure 14 (A-D) show representative histology samples from stage IV, her2 negative breast cancers stained with antibody 26B3 at either 20x or 40x magnification.

Figure 15 shows representative images of metastatic breast cancer samples obtained by fine needle aspiration stained with antibody 26B3.

Figure 16 shows FRα expression in ovarian serous carcinoma. (A) 3+ strong (right field) and

2+ moderate membrane staining (left upper field) are visible at 10x magnification. (B) Shows the same area as (A) at 20x magnification, confirming a 3+ strong, thick circumferential membrane staining (right field). 2+ moderate membrane staining (left upper field) has a weaker, thinner staining than 3+, and it is circumferential or localized to the luminal borders. (C) shows that 1+ weak membrane staining is limited to the luminal borders and requires 40x magnification to visualize. (D) Ovarian surface epithelial cells and the underlying cortical stromal cells are entirely negative (20x magnification).

Figure 17 shows FRα expression is limited to the luminal borders normal endometrium with weak 1+ and moderate 2+ intensity at 40x magnification (A). Strong (+3) membrane staining can be observed on the luminal borders of atypical complex hyperplasia at 20x magnification (B).

Figure 18 shows strong (+3) FRα membrane staining on the luminal borders of grade 1 adenocarcinoma of endometrium (A). In addition, many tumor cells have 2+ or 3+ cytoplasmic staining (20x magnification). FRα membrane staining (2+ and 3+) is present on the luminal borders of grade 2 adenocarcinoma of endometrium; cytoplasmic staining is weak (20x magnification (B). About 50% of the tumor cells of grade 3 adenocarcinoma of endometrium demonstrate 3+ strong, circumferential membrane staining with weak cytoplasmic staining at 40x magnification (C).

Figure 19 shows adenocarcinoma with squamous metaplasia with about 80% of metaplastic squamous cells with 2+ and 3+ FRα membrane staining and 1+ and 2+ FRα cytoplasmic staining at 20x magnification (A). Clear cell carcinoma of endometrium tumor cells have large irregular nuclei, prominent nucleoli and abundant clear cytoplasm. The majority of these tumor cells have 2+ or 3+ FRα membrane staining at 40x magnification (B).

Figure 20 shows that ciliated and non-ciliated cells of normal fallopian tube have 3+ FRα membrane staining on the luminal and lateral cell borders (A). Cytoplasmic staining is also evident (20x magnification). (B) Chronic salpingitis with abundant lymphocytes and plasma cells in the stroma. Mucosal cells retain 3+ FRα staining on the luminal borders (20x magnification). (C) Grade 2 tubal serous adenocarcinoma tumor cells form complex papillary projections and show 3+ FRα membrane staining on the luminal and lateral cell borders, with cytoplasmic staining also evident (20x magnification).

Figure 21 depicts ovarian cortical serous/tubal cysts. Lining cells reveal 3+, strong membrane and cytoplasmic staining (20x magnification).

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0041] The following description characterizes antibodies, and antigen-binding fragments thereof, that specifically bind to FRα. Also described are related polynucleotides capable of encoding these antibodies, and antigen-binding fragments, cells expressing the antibodies,

and antigen-binding fragments, as well as associated vectors and detectable antibody labels. In addition, methods of using the antibodies, and antigen-binding fragments, are described. For example, the provided antibodies, and antigen-binding fragments, may be used to diagnose ovarian, breast, thyroid, colorectal, endometrial, fallopian tube, or lung cancer; monitor ovarian, breast, thyroid, colorectal, endometrial, fallopian tube, or lung cancer progression, regression, or stable disease; to determine whether or not a patient should be treated for cancer, or to determine whether or not a subject is afflicted with FRα-expressing cancer and thus may be amenable to treatment with a FRα-specific anti-cancer therapeutic.

Definitions

[0042] Various terms relating to aspects of the description are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

[0043] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a cell" includes a combination of two or more cells, and the like.

[0044] The term "about" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of up to ±10% from the specified value, as such variations are appropriate to perform the disclosed methods. Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0045] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0046] "Isolated" means a biological component (such as a nucleic acid, peptide or protein) has been substantially separated, produced apart from, or purified away from other biological components of the organism in which the component naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA, and proteins. Nucleic acids, peptides and proteins that

have been "isolated" thus include nucleic acids and proteins purified by standard purification methods. "Isolated" nucleic acids, peptides and proteins that can be part of a composition and still be isolated if such composition is not part of the native environment of the nucleic acid, peptide, or protein. The term also embraces nucleic acids, peptides and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

[0047] "Polynucleotide," synonymously referred to as "nucleic acid molecule" or "nucleic acids," refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short nucleic acid chains, often referred to as oligonucleotides.

[0048] The meaning of "substantially the same" can differ depending on the context in which the term is used. Because of the natural sequence variation likely to exist among heavy and light chains and the genes encoding them, one would expect to find some level of variation within the amino acid sequences or the genes encoding the antibodies or antigen-binding fragments described herein, with little or no impact on their unique binding properties *(e.g.,* specificity and affinity). Such an expectation is due in part to the degeneracy of the genetic code, as well as to the evolutionary success of conservative amino acid sequence variations, which do not appreciably alter the nature of the encoded protein. Accordingly, in the context of nucleic acid sequences, "substantially the same" means at least 65% identity between two or more sequences. Preferably, the term refers to at least 70% identity between two or more sequences, more preferably at least 75% identity, more preferably at least 80% identity, more preferably at least 85% identity, more preferably at least 90% identity, more preferably at least 91% identity, more preferably at least 92% identity, more preferably at least 93% identity, more preferably at least 94% identity, more preferably at least 95% identity, more preferably at least 96% identity, more preferably at least 97% identity, more preferably at least 98% identity, and more preferably at least 99% or greater identity. Such identity may be determined using nBLAST algorithm (Altschul et al., (1990) Proc. Natl. Acad. Sci. USA 87:2264-8; Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-7).

[0049] The degree of variation that may occur within the amino acid sequence of a protein without having a substantial effect on protein function is much lower than that of a nucleic acid sequence, since the same degeneracy principles do not apply to amino acid sequences.

Accordingly, in the context of an antibody or antigen-binding fragment, "substantially the same" means antibodies or antigen-binding fragments having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the antibodies or antigen-binding fragments described. Other embodiments include FRα specific antibodies, or antigen-binding fragments, that have framework, scaffold, or other non-binding regions that do not share significant identity with the antibodies and antigen-binding fragments described herein, but do incorporate one or more CDRs or other sequences needed to confer binding that are 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to such sequences described herein.

[0050] A "vector" is a replicon, such as plasmid, phage, cosmid, or virus in which another nucleic acid segment may be operably inserted so as to bring about the replication or expression of the segment.

[0051] A cell has been "transformed" when exogenous or heterologous nucleic acids such as DNA have been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell, or "stable cell" is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations. In some examples provided herein, cells are transformed by transfecting the cells with DNA.

[0052] The terms "express" and "produce" are used synonymously herein, and refer to the biosynthesis of a gene product. These terms encompass the transcription of a gene into RNA. These terms also encompass translation of RNA into one or more polypeptides, and further encompass all naturally occurring post-transcriptional and post-translational modifications. The expression or production of an antibody or antigen-binding fragment thereof may be within the cytoplasm of the cell, or into the extracellular milieu such as the growth medium of a cell culture.

[0053] The terms "treating" or "treatment" refer to any success or indicia of success in the attenuation or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement, remission, diminishing of symptoms or making the condition more tolerable to the patient, slowing in the rate of degeneration or decline, making the final point of degeneration less debilitating, improving a subject's physical or mental well-being, or prolonging the length of survival. The treatment may be assessed by objective or subjective parameters; including the results of a physical examination, neurological examination, or psychiatric evaluations.

[0054] "Antibody" refers to all isotypes of immunoglobulins (IgG, IgA, IgE, IgM, IgD, and IgY) including various monomeric and polymeric forms of each isotype, unless otherwise specified.

[0055] Antigen-binding fragments are any proteinaceous structure that may exhibit binding affinity for a particular antigen. Some antigen-binding fragments are composed of portions of intact antibodies that retain antigen-binding specificity of the parent antibody molecule. For example, antigen-binding fragments may comprise at least one variable region (either a heavy chain or light chain variable region) or one or more CDRs of an antibody known to bind a particular antigen. Examples of suitable antigen-binding fragments include, without limitation diabodies and single-chain molecules as well as Fab, F(ab')2, Fc, Fabc, and Fv molecules, single chain (Sc) antibodies, individual antibody light chains, individual antibody heavy chains, chimeric fusions between antibody chains or CDRs and other proteins, protein scaffolds, heavy chain monomers or dimers, light chain monomers or dimers, dimers consisting of one heavy and one light chain, and the like. All antibody isotypes may be used to produce antigen-binding fragments. Additionally, antigen-binding fragments may include non-antibody proteinaceous frameworks that may successfully incorporate polypeptide segments in an orientation that confers affinity for a given antigen of interest, such as protein scaffolds. Antigen-binding fragments may be recombinantly produced or produced by enzymatic or chemical cleavage of intact antibodies. The phrase "an antibody or antigen-binding fragment thereof" may be used to denote that a given antigen-binding fragment incorporates one or more amino acid segments of the antibody referred to in the phrase.

[0056] "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.

[0057] The term "subject" refers to human and non-human animals, including all vertebrates, e.g., mammals and non-mammals, such as non-human primates, mice, rabbits, sheep, dogs, cats, horses, cows, chickens, amphibians, and reptiles. In many embodiments of the described methods, the subject is a human.

[0058] 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. Soluble forms of FRα may be derived by the action of proteases or phospholipase on membrane anchored folate receptors. The amino acid sequence for human FRα is set forth herein as SEQ ID NO: 1. 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 will be appreciated by those skilled in the art, cell associated and non-cell associated forms of human FRα may encompass variant forms of SEQ ID NO: 1.

[0059] The term "sample" as used herein refers to a collection of similar fluids, cells, or tissues (e.g., surgically resected tumor tissue, biopsies, including fine needle aspiration), isolated from a subject, as well as fluids, cells, or tissues present within a subject. In some embodiments the

sample is a biological fluid. 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, saliva, cystic fluid, tear drops, feces, sputum, mucosal secretions of the secretory tissues and organs, vaginal secretions, 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.

[0060] 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. The term "sample," as used herein, encompasses materials removed from a subject or materials present in a subject.

[0061] The term "progression," as used in the context of progression of an FRα-expressing cancer, includes the change of 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 or 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.

[0062] The term "regression," as used in the context of regression of an FRα-expressing cancer, includes the change of a cancer from a more severe to a less severe state. This could include a decrease in the number or severity of tumors, the degree of metastasis, the speed with which the cancer is growing or spreading, and the like. For example, "the regression of ovarian cancer" includes the regression of such a cancer from a more severe to a less severe state, such as the progression from stage III to stage II, from stage II to stage I, etc.

[0063] The term "stable" as used in the context of stable FRα-expressing cancer, is intended to describe a disease condition that is not, or has not, changed significantly enough over a clinically relevant period of time to be considered a progressing cancer or a regressing cancer.

[0064] The embodiments described herein are not limited to particular methods, reagents, compounds, compositions or biological systems, which can, of course, vary.

FRoc-Specific Antibodies and Antigen-Binding Fragments

[0065] Described herein are isolated monoclonal antibodies or antigen-binding fragments that specifically bind FRα. The general structure of an antibody molecule comprises an antigen binding domain, which includes heavy and light chains, and the Fc domain, which serves a variety of functions, including complement fixation and binding antibody receptors.

[0066] The described antibodies or antigen-binding fragments include all isotypes, IgA, IgD, IgE, IgG and IgM, and synthetic multimers of the four-chain immunoglobulin structure. The described antibodies or antigen-binding fragments also include the IgY isotype generally found in hen or turkey serum and hen or turkey egg yolk.

[0067] The antibodies or antigen-binding fragments disclosed in the examples section are derived from mice. Similar antibodies may be derived from any species by recombinant means. For example, the antibodies or antigen-binding fragments may be chimeric rat, goat, horse, swine, bovine, chicken, rabbit, camelid, donkey, human, and the like. For use in administration to humans, non-human derived antibodies or antigen-binding fragments may be genetically or structurally altered to be less antigenic upon administration to a human patient.

[0068] In some embodiments, the antibodies or antigen-binding fragments are chimeric. As used herein, the term "chimeric" refers to an antibody, or antigen-binding fragment thereof, having at least some portion of at least one variable domain derived from the antibody amino acid sequence of a non-human mammal, a rodent, or a reptile, while the remaining portions of the antibody, or antigen-binding fragment thereof, are derived from a human. For example, a chimeric antibody may comprise a mouse antigen binding domain with a human Fc or other such structural domain.

[0069] In some embodiments, the antibodies are humanized antibodies. Humanized antibodies may be chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin sequence. The humanized antibody may include at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

[0070] The antibodies or antigen-binding fragments described herein can occur in a variety of forms, but will include one or more of the antibody variable domain segments or CDRs shown in Table 1. The isotypes of the antibodies described in Table 1 are shown in parentheses to describe the constant region of each antibody, which are known to have conserved sequences. Table 1. Antibody segments of the described antibodies and antigen-binding fragments thereof ("Lc" denotes light chain and "Hc" denotes heavy chain).

Antibody Segment	SEQ ID NO.	Sequence
Monoclonal antibody 9F3 (murine IgG2a constant region)		
Lc CDR1	2	RASSTVSYSYLH
Lc CDR2	3	GTSNLAS
Lc CDR3	4	QQYSGYPLT
Lc variable domain segment	5	PAIMSASPGEKVTMTCRASSTVSYSYLHWYQQ KSGASPQLWIYGTSNLASGVPARFSGSGSGTSY SLTISSVEAEDAATYYCQQYSGYPLTFGAGTKL ELKRADAAP
Hc CDR1	6	SGYYWN
Hc CDR2	7	YIKSDGSNNYNPSLKN
Hc CDR3	8	EWKAMDY
Hc variable domain segment	9	ESGPGLVRPSQSLSLTCSVTGYSITSGYYWNWIR QFPGSRLEWMGYIKSDGSNNYNPSLKNRISITR DTSKNQFFLKLNSVTTEDTATYFCTREWKAMD YWGQGTSVTVSSAKTTPPSVYPLAPGCGDT
Monoclonal antibody 19D4 (murine IgG2a constant region)		
Lc CDR1	10	RASESVDTYGNNFIH
Lc CDR2	11	LASNLES
Lc CDR3	12	QQNNGDPWT
Lc variable domain segment	13	PASLAVSLGQRATISCRASESVDTYGNNFIHWY QQKPGQPPKLLIYLASNLESGVPARFSGSGSRTD FTLTIDPVEADDAATYYCQQNNGDPWTFGGGT KLEIKRADAAP
Hc CDR1	14	HPYMH
Hc CDR2	15	RIDPANGNTKYDPKFQG
Hc CDR3	16	EEVADYTMDY
Hc variable domain segment	17	GAELVKPGASVKLSCTASGFNIKHPYMHWVKQ RPDQGLEWIGRIDPANGNTKYDPKFQGKATITA DTSSNTAYLQLSSLTSEDTAVYYCGREEVADYT MDYWGQGTSVTVSSAKTTAPSVYPLAPV
Monoclonal antibody 24F12 (murine IgG1 constant region)		
Lc CDR1	18	SASQGINNFLN
Lc CDR2	19	YTSSLHS
Lc CDR3	20	QHFSKLPWT

Monoclonal antibody 24F12 (murine IgG1 constant region)		
Lc variable domain	21	TSSLSASLGDRVTISCSASQGINNFLNWYQQKP DGTVKLLIYYTSSLHSGVPSRFSGSGSGTDYSLT ISNLEPEDIATYYCQHFSKLPWTFGGGTKLEIKR ADAAP
Hc CDR1	22	SYAMS
Hc CDR2	23	EIGSGGSYTYYPDTVTG
Hc CDR3	24	ETTAGYFDY
Hc variable domain	25	SGGGLVRPGGSLKLSCAASGFTFSSYAMSWVR QSPEKRLEWVAEIGSGGSYTYYPDTVTGRFTISR DNAKSTLYLEMSSLRSEDTAIYYCARETTAGYF DYWGQGTTLTVSS
Monoclonal antibody 26B3 (murine IgG1 constant region)		
Lc CDR1	26	RTSENIFSYLA
Lc CDR2	27	NAKTLAE
Lc CDR3	28	QHHYAFPWT
Lc variable domain segment	29	PASLSASVGETVTITCRTSENIFSYLAWYQQKQ GISPQLLVYNAKTLAEGVPSRFSGSGSGTQFSLK INSLQPEDFGSYYCQHHYAFPWTFGGGSKLEIK RADAAP
Hc CDR1	30	GYFMN
Hc CDR2	31	RIFPYNGDTFYNQKFKG
Hc CDR3	32	GTHYFDY
Hc variable domain segment	33	GPELVKPGASVKISCKASDYSFTGYFMNWVMQ SHGKSLEWIGRIFPYNGDTFYNQKFKGRATLTV DKSSSTAHMELRSLASEDSAVYFCARGTHYFD YWGQGTTLTVSSAKTTPPSVYPLAPGSAAQT
Monoclonal antibody 9F3 (murine IgG2a constant region)		
Lc CDR1	34	AGGGCCAGCTCAACTGTAAGTTACAGTTACTT GCAC
Lc CDR2	35	GGCACATCCAACTTGGCTTCT
Lc CDR3	36	CAGCAGTACAGTGGTTACCCACTCACG
Lc variable domain segment	37	CCAGCAATCATGTCTGCATCTCCAGGGGAAA AGGTCACCATGACCTGCAGGGCCAGCTCAAC TGTAAGTTACAGTTACTTGCACTGGTACCAGC

Monoclonal antibody 9F3 (murine IgG2a constant region)		
		AGAAGTCAGGTGCCTCCCCCCAACTCTGGATT TATGGCACATCCAACTTGGCTTCTGGAGTCCC TGCTCGCTTCAGTGGCAGTGGGTCTGGGACCT CTTACTCTCTCACAATCAGCAGTGTGGAGGCT GAAGATGCTGCCACTTATTACTGCCAGCAGTA CAGTGGTTACCCACTCACGTTCGGTGCTGGGA CCAAGCTGGAGCTGAAACGGGCTGATGCTGC ACCAAC
Hc CDR1	38	AGTGGTTATTACTGGAAC
Hc CDR2	39	TACATAAAGTCCGACGGTAGCAATAATTACA ACCCATCTCTCAAAAAT
Hc CDR3	40	GAGTGGAAGGCTATGGACTAC
Hc variable domain segment	41	GAGTCAGGACCTGGCCTCGTGAGACCTTCTCA GTCTCTGTCTCTCACCTGCTCTGTCACTGGCT ACTCCATCACCAGTGGTTATTACTGGAACTGG ATCCGGCAGTTTCCAGGAAGCAGACTGGAAT GGATGGGCTACATAAAGTCCGACGGTAGCAA TAATTACAACCCATCTCTCAAAAATCGAATCT CCATCACTCGTGACACATCTAAGAACCAGTTT TTCCTGAAGTTGAATTCTGTGACTACTGAGGA CACAGCTACATATTTCTGTACAAGGGAGTGG AAGGCTATGGACTACTGGGGTCAGGGAACCT CAGTCACCGTCTCCTCAGCCAAAACAACACC CCCATCAGTCTATCCACTGGCCCCTGGGTGTG GAGATACAAC
Monoclonal antibody 19D4 (murine IgG2a constant region)		
Lc CDR1	42	AGAGCCAGTGAAAGTGTTGATACTTATGGCA ATAATTTTATACAC
Lc CDR2	43	CTTGCATCCAACCTAGAATCT
Lc CDR3	44	CAGCAAAATAATGGGGATCCGTGGACG
Lc variable domain segment	45	CCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAG GGCCACCATATCCTGCAGAGCCAGTGAAAGT GTTGATACTTATGGCAATAATTTTATACACTG GTACCAGCAGAAACCAGGACAGCCACCCAAA CTCCTCATTTATCTTGCATCCAACCTAGAATC TGGGGTCCCTGCCAGGTTCAGTGGCAGTGGG TCTAGGACAGACTTCACCCTCACCATTGATCC TGTGGAGGCTGATGATGCTGCAACCTATTACT GTCAGCAAAATAATGGGGATCCGTGGACGTT CGGTGGAGGCACCAAGCTGGAGATCAAACGG GCTGATGCTGCACCAA

Monoclonal antibody 19D4 (murine IgG2a constant region)		
Hc CDR1	46	CACCCCTATATGCAC
Hc CDR2	47	AGGATTGATCCTGCGAATGGTAATACTAAAT ATGACCCGAAGTTCCAGGGC
Hc CDR3	48	GAGGAGGTGGCGGACTATACTATGGACTAC
Hc variable domain segment	49	GGGGCAGAGCTTGTGAAGCCAGGGGCCTCAG TCAAGTTGTCCTGCACAGCTTCTGGCTTCAAC ATTAAACACCCCTATATGCACTGGGTGAAGC AGAGGCCTGACCAGGGCCTGGAGTGGATTGG AAGGATTGATCCTGCGAATGGTAATACTAAA TATGACCCGAAGTTCCAGGGCAAGGCCACTA TAACAGCAGACACATCCTCCAACACAGCCTA CCTACAGCTCAGCAGCCTGACATCTGAGGAC ACTGCCGTCTATTACTGTGGTAGAGAGGAGG TGGCGGACTATACTATGGACTACTGGGGTCA AGGAACCTCAGTCACCGTCTCCTCAGCCAAA ACAACAGCCCCATCGGTCTATCCACTGGCCCC TGTGTG
Monoclonal antibody 24F12 (murine IgG1 constant region)		
Lc CDR1	50	AGTGCAAGTCAGGGCATTAACAATTTTTTAAA C
Lc CDR2	51	TACACATCAAGTTTACACTCA
Lc CDR3	52	CAGCACTTTAGTAAGCTTCCGTGGACG
Lc variable domain segment	53	ACATCCTCCCTGTCTGCCTCTCTGGGAGACAG AGTCACCATCAGTTGCAGTGCAAGTCAGGGC ATTAACAATTTTTTAAACTGGTATCAGCAGAA ACCAGATGGCACTGTTAAACTCCTGATCTATT ACACATCAAGTTTACACTCAGGAGTCCCATCA AGGTTCAGTGGCAGTGGGTCTGGGACAGATT ATTCTCTCACCATCAGCAACCTGGAACCTGAA GATATTGCCATATACTATTGTCAGCACTTTAG TAAGCTTCCGTGGACGTTCGGTGGAGGCACC AAGCTGGAAATCAAACGGGCTGATGCTGCAC CAAC
Hc CDR1	54	AGCTATGCCATGTCT
Hc CDR2	55	GAAATTGGTAGTGGTGGTAGTTACACCTACTA TCCAGACACTGTGACGGGC
Hc CDR3	56	GAAACTACGGCGGGCTACTTTGACTAC
Hc variable domain segment	57	TCTGGGGGAGGCTTAGTGAGGCCTGGAGGGT CCCTGAAACTCTCCTGTGCAGCCTCTGGATTC

Monoclonal antibody 24F12 (murine IgG1 constant region)		
		ACTTTCAGTAGCTATGCCATGTCTTGGGTTCG CCAGTCTCCAGAGAAGAGGCTGGAGTGGGTC GCAGAAATTGGTAGTGGTGGTAGTTACACCT ACTATCCAGACACTGTGACGGGCCGATTCAC CATCTCCAGAGACAATGCCAAGAGCACCCTG TACCTGGAAATGAGCAGTCTGAGGTCTGAGG ACACGGCCATCTATTACTGTGCAAGGGAAAC TACGGCGGGCTACTTTGACTACTGGGGCCAA
		GGCACCACTCTCACAGTCTCCTCA
Monoclonal antibody 26B3 (murine IgG1 constant region)		
Lc CDR1	58	CGAACAAGTGAGAATATTTTCAGTTATTTAGC A
Lc CDR2	59	AATGCAAAAACCTTAGCAGAG
Lc CDR3	60	CAACATCATTATGCTTTTCCGTGGACG
Lc variable domain segment	61	CCAGCCTCCCTATCTGCATCTGTGGGAGAAAC TGTCACCATCACATGTCGAACAAGTGAGAAT ATTTTCAGTTATTTAGCATGGTATCAGCAGAA ACAGGGAATATCTCCTCAGCTCCTGGTCTATA ATGCAAAAACCTTAGCAGAGGGTGTGCCATC AAGGTTCAGTGGCAGTGGATCAGGCACACAG TTTTCTCTGAAGATCAACAGCCTGCAGCCTGA AGATTTTGGGAGTTATTACTGTCAACATCATT ATGCTTTTCCGTGGACGTTCGGTGGAGGCTCC AAGCTGGAAATCAAACGGGCTGATGCTGCAC CAAC
Hc CDR1	62	GGCTACTTTATGAAC
Hc CDR2	63	CGTATTTTTCCTTACAATGGTGATACTTTCTAC AACCAGAAGTTCAAGGGC
Hc CDR3	64	GGGACTCATTACTTTGACTAC
Hc variable domain segment	65	GGACCTGAGCTGGTGAAGCCTGGGGCTTCAG TGAAGATATCCTGCAAGGCTTCTGATTACTCT TTTACTGGCTACTTTATGAACTGGGTGATGCA GAGCCATGGAAAGAGCCTTGAGTGGATTGGA CGTATTTTTCCTTACAATGGTGATACTTTCTAC AACCAGAAGTTCAAGGGCAGGGCCACATTGA CTGTAGACAAATCCTCTAGCACAGCCCACAT GGAGCTCCGGAGCCTGGCATCTGAGGACTCT GCAGTCTATTTTTGTGCAAGAGGGACTCATTA CTTTGACTACTGGGGCCAAGGCACCACTCTCA CTGTCTCCTCAGCCAAAACGACACCCCCATCT GTCTATCCACTGGCCCCTGGATCTGCTGCCCA AACTAA

[0071] Described herein are isolated antibodies and antigen-binding fragments that specifically bind to FRα. In some embodiments, the antibodies or antigen-binding fragments are murine IgG, or derivatives thereof. While the antibodies or antigen-binding fragments may be human, humanized, or chimeric, the antibodies or antigen-binding fragments exemplified herein are murine. In some embodiments, antibodies or antigen-binding fragments may include a light chain CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 26. In some embodiments, antibodies or antigen-binding fragments may include a light chain CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 27. In some embodiments, antibodies or antigen-binding fragments may include a light chain CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 28. In some embodiments, antibodies or antigen-binding fragments may include a heavy chain CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 30. In some embodiments, antibodies or antigen-binding fragments may include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 31. In some embodiments, antibodies or antigen-binding fragments may include a heavy chain CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 32. The antibodies or antigen-binding fragments may include a light chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 26; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 27; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 28. The antibodies or antigen-binding fragments may include a heavy chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 30; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 31; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 32. The antibodies or antigen-binding fragments may include a light chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 26; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 27; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 28, and also have a heavy chain having a CDR1 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 30; a CDR2 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 31; and a CDR3 amino acid sequence substantially the same as, or identical to, SEQ ID NO: 32.

[0072] The described antibodies or antigen-binding fragments may include a light chain variable domain that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 29. In some embodiments, an isolated polynucleotide that includes a sequence substantially the same as, or identical to, SEQ ID NO: 61 may encode this light chain variable domain amino acid sequence. The described antibodies or antigen-binding fragments may include a heavy chain variable domain that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 33. In some embodiments, an isolated polynucleotide that includes a sequence substantially the same as, or identical to, SEQ ID NO: 65 may encode this heavy chain variable domain amino acid sequence. The described antibodies or antigen-binding fragments 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: 29, and the heavy chain variable domain includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 33.

[0073] In some embodiments, the antibodies are 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 antibodies, or antigen-binding fragments thereof, have the binding affinity for FRα of the antibodies produced by the deposited antibody-producing cells. In some embodiments, the disclosed antibodies, or antigen-binding fragments thereof, comprise the light and heavy chain CDRs of the antibodies produced by the deposited antibody-producing cells. In some embodiments, the antibodies, or antigen-binding fragments thereof, comprise the light and heavy chain variable regions of the antibodies produced by the deposited antibody-producing cells.

[0074] Also disclosed are polynucleotides that encode antibodies or antigen-binding fragments that specifically bind to FRα. In some embodiments, the isolated polynucleotides encode an antibody or antigen-binding fragment thereof having a light chain CDR1 sequence substantially the same as, or identical to, SEQ ID NO: 26, for example SEQ ID NO: 58. In some embodiments, the isolated polynucleotides encode an antibody or antigen-binding fragment thereof having a light chain CDR2 substantially the same as, or identical to, SEQ ID NO: 27, for example SEQ ID NO: 59. In some embodiments, the isolated polynucleotides encode an antibody or antigen-binding fragment thereof having a light chain CDR3 substantially the same as, or identical to, SEQ ID NO: 28, for example SEQ ID NO: 60. In some embodiments, the isolated polynucleotides encode an antibody or antigen-binding fragment thereof having a heavy chain CDR1 substantially the same as, or identical to, SEQ ID NO: 30, for example SEQ ID NO: 62. In some embodiments, the isolated polynucleotides encode an antibody or antigen-binding fragment thereof having a heavy chain CDR2 substantially the same as, or identical to, SEQ ID NO: 31, for example SEQ ID NO: 63. In some embodiments, the isolated polynucleotides encode an antibody or antigen-binding fragment thereof having a heavy chain CDR3 substantially the same as, or identical to, SEQ ID NO: 32, for example SEQ ID NO: 64. The polynucleotides may encode an antibody or antigen-binding fragment thereof having a light chain with a CDR1 substantially the same as, or identical to, SEQ ID NO: 26, for example SEQ ID NO: 58; a CDR2 substantially the same as, or identical to, SEQ ID NO: 27, for example SEQ ID NO: 59; and a CDR3 substantially the same as, or identical to, SEQ ID NO: 28, for example SEQ ID NO: 60. The polynucleotides may encode an antibody or antigen-binding fragment thereof having a heavy chain CDR1 substantially the same as, or identical to, SEQ ID NO: 30, for example SEQ ID NO: 62; a CDR2 substantially the same as, or identical to, SEQ ID NO: 31, for example SEQ ID NO: 63; and a CDR3 substantially the same as, or identical to, SEQ ID NO: 32, for example SEQ ID NO: 64. The polynucleotides may encode an antibody or antigen-binding fragment thereof having a light chain CDR1 substantially the same as, or identical to, SEQ ID NO: 26, for example SEQ ID NO: 58; a CDR2 encoded by a nucleotide sequence substantially the same as, or identical to, SEQ ID NO: 27, for example SEQ ID NO: 59; and a CDR3 encoded by a nucleotide sequence substantially the same as, or identical to,

SEQ ID NO: 28, for example SEQ ID NO: 60; and a heavy chain CDR1 substantially the same as, or identical to, SEQ ID NO: 30, for example SEQ ID NO: 62; a CDR2 substantially the same as, or identical to, SEQ ID NO: 31, for example SEQ ID NO: 63; and a CDR3 substantially the same as, or identical to, SEQ ID NO: 32, for example SEQ ID NO: 64. Antigen-binding arrangements of CDRs may also be engineered using antibody-like proteins as CDR scaffolding. Such engineered antigen-binding proteins are within the scope of the disclosure.

[0075] Polynucleotides described herein may encode antibodies or antigen-binding fragments that have a light chain variable domain segment that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 29, for example SEQ ID NO: 61. In some embodiments the described polynucleotides may encode antibodies or antigen-binding fragments that have a heavy chain variable domain segment that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 33, for example SEQ ID NO: 65. In some embodiments the described polynucleotides may encode antibodies or antigen-binding fragments that have a light chain variable domain segment that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 29, for example SEQ ID NO: 61; and a heavy chain variable domain segment that includes an amino acid sequence substantially the same as, or identical to, SEQ ID NO: 33, for example SEQ ID NO: 65. The polynucleotides capable of encoding the variable domain segments provided herein may be included on the same, or different, vectors to produce antibodies or antigen-binding fragments.

[0076] Polynucleotides encoding engineered antigen-binding proteins also are within the scope of the disclosure. In some embodiments, the polynucleotides described (and the peptides they encode) include a leader sequence. Any leader sequence known in the art may be employed. The leader sequence may include, but is not limited to, a restriction site or a translation start site.

[0077] The antibodies or antigen-binding fragments described herein include variants having single or multiple amino acid substitutions, deletions, or additions that retain the biological properties (*e.g.*, binding affinity or immune effector activity) of the described antibodies or antigen-binding fragments. The skilled person may produce variants having single or multiple amino acid substitutions, deletions, or additions. These variants may include: (a) variants in which one or more amino acid residues are substituted with conservative or nonconservative amino acids, (b) variants in which one or more amino acids are added to or deleted from the polypeptide, (c) variants in which one or more amino acids include a substituent group, and (d) variants in which the polypeptide is fused with another peptide or polypeptide such as a fusion partner, a protein tag or other chemical moiety, that may confer useful properties to the polypeptide, such as, for example, an epitope for an antibody, a polyhistidine sequence, a biotin moiety and the like. Antibodies or antigen-binding fragments described herein may include variants in which amino acid residues from one species are substituted for the corresponding residue in another species, either at the conserved or nonconserved positions. In other embodiments, amino acid residues at nonconserved positions are substituted with conservative or nonconservative residues. The techniques for obtaining these variants, including genetic (suppressions, deletions, mutations, etc.), chemical, and enzymatic

techniques, are known to the person having ordinary skill in the art.

[0078] The antibodies or antigen-binding fragments described herein may embody several antibody isotypes, such as IgM, IgD, IgG, IgA and IgE. Antibody or antigen-binding fragment thereof specificity is largely determined by the amino acid sequence, and arrangement, of the CDRs. Therefore, the CDRs of one isotype may be transferred to another isotype without altering antigen specificity. Alternatively, techniques have been established to cause hybridomas to switch from producing one antibody isotype to another (isotype switching) without altering antigen specificity. Accordingly, such antibody isotypes are within the scope of the described antibodies or antigen-binding fragments

[0079] The antibodies or antigen-binding fragments described herein have binding affinities (in M) for FRα that include a dissociation constant (K_D) of less than about $1x10^{-8}$ M. In one embodiment the antibody 9F3 has an affinity for FRα of $7.15x10^{-10}$ M. In one embodiment the antibody 19D4 has an affinity for FRα of $5.67x10^{-10}$ M. In one embodiment the antibody 24F12 has an affinity for FRα of $1.02x10^{-10}$ M. In one embodiment the antibody 26B3 has an affinity for FRα of $2.73x10^{-11}$ M. In one embodiment the antibody 9F3 has an affinity for FRα of about $6.5x10^{-10}$ M to about $8x10^{-10}$ M. In one embodiment the antibody 19D4 has an affinity for FRα of about $5x10^{-10}$ M to about $6.5x10^{-10}$ M. In one embodiment the antibody 24F12 has an affinity for FRα of about $0.5x10^{-10}$ M to about $2x10^{-10}$ M. In one embodiment the antibody 26B3 has an affinity for FRα of about $1x10^{-11}$ M to about $3.5x10^{-11}$ M.

[0080] Also provided are vectors comprising the polynucleotides described herein. The vectors can be expression vectors. Recombinant expression vectors containing a sequence encoding a polypeptide of interest are thus contemplated as within the scope of this disclosure. The expression vector may contain one or more additional sequences such as but not limited to regulatory sequences (e.g., promoter, enhancer), a selection marker, and a polyadenylation signal. Vectors for transforming a wide variety of host cells are well known and include, but are not limited to, plasmids, phagemids, cosmids, baculoviruses, bacmids, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs), as well as other bacterial, yeast and viral vectors.

[0081] Recombinant expression vectors within the scope of the description include synthetic, genomic, or cDNA-derived nucleic acid fragments that encode at least one recombinant protein which may be operably linked to suitable regulatory elements. Such regulatory elements may include a transcriptional promoter, sequences encoding suitable mRNA ribosomal binding sites, and sequences that control the termination of transcription and translation. Expression vectors, especially mammalian expression vectors, may also include one or more nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, other 5' or 3' flanking nontranscribed sequences, 5' or 3' nontranslated sequences (such as necessary ribosome binding sites), a polyadenylation site, splice donor and acceptor sites, or transcriptional termination sequences. An origin of

replication that confers the ability to replicate in a host may also be incorporated.

[0082] The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. Exemplary vectors may be constructed as described by Okayama and Berg, 3 Mol. Cell. Biol. 280 (1983).

[0083] In some embodiments, the antibody- or antigen-binding fragment-coding sequence is placed under control of a powerful constitutive promoter, such as the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, beta-actin, human myosin, human hemoglobin, human muscle creatine, and others. In addition, many viral promoters function constitutively in eukaryotic cells and are suitable for use with the described embodiments. Such viral promoters include without limitation, Cytomegalovirus (CMV) immediate early promoter, the early and late promoters of SV40, the Mouse Mammary Tumor Virus (MMTV) promoter, the long terminal repeats (LTRs) of Maloney leukemia virus, Human Immunodeficiency Virus (HIV), Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV), and other retroviruses, and the thymidine kinase promoter of Herpes Simplex Virus. In one embodiment, the antibody or antigen-binding fragment thereof coding sequence is placed under control of an inducible promoter such as the metallothionein promoter, tetracycline-inducible promoter, doxycycline-inducible promoter, promoters that contain one or more interferon-stimulated response elements (ISRE) such as protein kinase R 2',5'-oligoadenylate synthetases, Mx genes, ADAR1, and the like.

[0084] Vectors described herein may contain one or more Internal Ribosome Entry Site(s) (IRES). Inclusion of an IRES sequence into fusion vectors may be beneficial for enhancing expression of some proteins. In some embodiments the vector system will include one or more polyadenylation sites (*e.g.,* SV40), which may be upstream or downstream of any of the aforementioned nucleic acid sequences. Vector components may be contiguously linked, or arranged in a manner that provides optimal spacing for expressing the gene products (i.e., by the introduction of "spacer" nucleotides between the ORFs), or positioned in another way. Regulatory elements, such as the IRES motif, may also be arranged to provide optimal spacing for expression.

[0085] The vectors may comprise selection markers, which are well known in the art. Selection markers include positive and negative selection markers, for example, antibiotic resistance genes (*e.g.*, neomycin resistance gene, a hygromycin resistance gene, a kanamycin resistance gene, a tetracycline resistance gene, a penicillin resistance gene), glutamate sythase genes, HSV-TK, HSV-TK derivatives for ganciclovir selection, or bacterial purine nucleoside phosphorylase gene for 6-methylpurine selection (Gadi et al., 7 Gene Ther. 1738-1743 (2000)). A nucleic acid sequence encoding a selection marker or the cloning site may be upstream or downstream of a nucleic acid sequence encoding a polypeptide of interest or cloning site.

[0086] The vectors described herein may be used to transform various cells with the genes encoding the described antibodies or antigen-binding fragments. For example, the vectors may

be used to generate antibody or antigen-binding fragment-producing cells. Thus, another aspect features host cells transformed with vectors comprising a nucleic acid sequence encoding an antibody or antigen-binding fragment thereof that specifically binds FRα, such as the antibodies or antigen-binding fragments described and exemplified herein.

[0087] Numerous techniques are known in the art for the introduction of foreign genes into cells and may be used to construct the recombinant cells for purposes of carrying out the described methods, in accordance with the various embodiments described and exemplified herein. The technique used should provide for the stable transfer of the heterologous gene sequence to the host cell, such that the heterologous gene sequence is heritable and expressible by the cell progeny, and so that the necessary development and physiological functions of the recipient cells are not disrupted. Techniques which may be used include but are not limited to chromosome transfer (*e.g.*, cell fusion, chromosome mediated gene transfer, micro cell mediated gene transfer), physical methods (*e.g.*, transfection, spheroplast fusion, microinjection, electroporation, liposome carrier), viral vector transfer (*e.g.*, recombinant DNA viruses, recombinant RNA viruses) and the like (described in Cline, 29 Pharmac. Ther. 69-92 (1985)). Calcium phosphate precipitation and polyethylene glycol (PEG)-induced fusion of bacterial protoplasts with mammalian cells may also be used to transform cells.

[0088] Cells suitable for use in the expression of the antibodies or antigen-binding fragments described herein are preferably eukaryotic cells, more preferably cells of plant, rodent, or human origin, for example but not limited to NSO, CHO, CHOK1, perC.6, Tk-ts13, BHK, HEK293 cells, COS-7, T98G, CV-1/EBNA, L cells, C127, 3T3, HeLa, NS1, Sp2/0 myeloma cells, and BHK cell lines, among others. In addition, expression of antibodies may be accomplished using hybridoma cells. Methods for producing hybridomas are well established in the art.

[0089] Cells transformed with expression vectors described herein may be selected or screened for recombinant expression of the antibodies or antigen-binding fragments described herein. Recombinant-positive cells are expanded and screened for subclones exhibiting a desired phenotype, such as high level expression, enhanced growth properties, or the ability to yield proteins with desired biochemical characteristics, for example, due to protein modification or altered post-translational modifications. These phenotypes may be due to inherent properties of a given subclone or to mutation. Mutations may be effected through the use of chemicals, UV-wavelength light, radiation, viruses, insertional mutagens, inhibition of DNA mismatch repair, or a combination of such methods.

[0090] Provided herein are methods for detecting FRα in a sample by contacting the sample with an antibody, or antigen-binding fragment thereof, described herein. As described herein, the sample may be derived from urine, blood, serum, plasma, saliva, ascites, circulating cells, circulating tumor cells, cells that are not tissue associated (*i.e.,* free cells), tissues (*e.g.,* surgically resected tumor tissue, biopsies, including fine needle aspiration), histological preparations, and the like. In some embodiments the described methods include detecting FRα in a sample by contacting the sample with an antibody or antibody fragment thereof, specific

for folate receptor alpha, as defined in the claims. In embodiments where two antibodies, or an antigen binding fragment thereof, are used, one of the antibodies or antigen binding fragments thereof are as defined in the claims and the second antibody is selected from

1. (a) an antibody, or antigen-binding fragment thereof, that binds the same epitope as any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3;
2. (b) any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3, or an antigen-binding fragment thereof;
3. (c) an antibody, or antigen-binding fragment thereof, that comprises heavy chain CDR1, CDR2, and CDR3 amino acid sequences and light chain CDR1, CDR2, and CDR3 amino acid sequences of any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3, as described in Table 1;
4. (d) an antibody, or antigen-binding fragment thereof, that comprises the heavy chain variable domain segment and light chain variable domain segment of any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3, as described in Table 1; or
5. (e) an antibody having the amino acid sequence of antibody produced by any one of the cell lines deposited with the ATCC having accession number PTA-11887, PTA-11884, PTA-11886, or PTA-11885, or an antigen binding fragment thereof.

[0091] In some embodiments the sample may be contacted with more than one of the antibodies, or antigen-binding fragments described herein. For example, a sample may be contacted with a first antibody, or antigen-binding fragment thereof, and then contacted with a second antibody, or antigen-binding fragment thereof, wherein the first antibody or antigen-binding fragment and the second antibody or antigen-binding fragment are not the same antibody or antigen-binding fragment. In some embodiments, the first antibody, or antigen-binding fragment thereof, may be affixed to a surface, such as a multiwell plate, chip, or similar substrate prior to contacting the sample. In other embodiments the first antibody, or antigen-binding fragment thereof, may not be affixed, or attached, to anything at all prior to contacting the sample.

[0092] Various combinations of the antibodies, or antigen-binding fragments thereof, may be used to detect FRα in a sample.

[0093] In one embodiment the sample may be first contacted with an antibody, or antigen-binding fragment thereof, that comprises heavy chain CDR1, CDR2, and CDR3 amino acid sequences and light chain CDR1, CDR2, and CDR3 amino acid sequences of antibody 26B3 (as provided in Table 1), and then separately contacted with a second antibody, or antigen-binding fragment thereof, that comprises heavy chain CDR1, CDR2, and CDR3 amino acid sequences and light chain CDR1, CDR2, and CDR3 amino acid sequences of antibody 9F3 (as provided in Table 1). In one embodiment the sample may be first contacted with an antibody, or antigen-binding fragment thereof, that comprises heavy chain CDR1, CDR2, and CDR3 amino acid sequences and light chain CDR1, CDR2, and CDR3 amino acid sequences of

antibody 26B3 (as provided in Table 1), and then separately contacted with a second antibody, or antigen-binding fragment thereof, that comprises heavy chain CDR1, CDR2, and CDR3 amino acid sequences and light chain CDR1, CDR2, and CDR3 amino acid sequences of antibody 24F12 (as provided in Table 1). In one embodiment the sample may be first contacted with an antibody, or antigen-binding fragment thereof, that comprises heavy chain CDR1, CDR2, and CDR3 amino acid sequences and light chain CDR1, CDR2, and CDR3 amino acid sequences of antibody 26B3 (as provided in Table 1), and then separately contacted with a second antibody, or antigen-binding fragment thereof, that comprises heavy chain CDR1, CDR2, and CDR3 amino acid sequences and light chain CDR1, CDR2, and CDR3 amino acid sequences of antibody 19D4 (as provided in Table 1).

[0094] In one embodiment the sample may be first contacted with an antibody, or antigen-binding fragment thereof, that comprises the heavy chain variable domain segment and light chain variable domain segment amino acid sequences of antibody 26B3 (as provided in Table 1), and then separately contacted with a second antibody, or antigen-binding fragment thereof, that comprises the heavy chain variable domain segment and light chain variable domain segment amino acid sequences of antibody 9F3 (as provided in Table 1). In one embodiment the sample may be first contacted with an antibody, or antigen-binding fragment thereof, that comprises the heavy chain variable domain segment and light chain variable domain segment amino acid sequences of antibody 26B3 (as provided in Table 1), and then separately contacted with a second antibody, or antigen-binding fragment thereof, that comprises the heavy chain variable domain segment and light chain variable domain segment amino acid sequences of antibody 24F12 (as provided in Table 1). In one embodiment the sample may be first contacted with an antibody, or antigen-binding fragment thereof, that comprises the heavy chain variable domain segment and light chain variable domain segment amino acid sequences of antibody 26B3 (as provided in Table 1), and then separately contacted with a second antibody, or antigen-binding fragment thereof, that comprises the heavy chain variable domain segment and light chain variable domain segment amino acid sequences of antibody 19D4 (as provided in Table 1).

[0095] In one embodiment the sample may be first contacted with the antibody produced by the cell line having ATCC accession number PTA-11885, or an antigen-binding fragment thereof, and then separately contacted with a second antibody produced by the cell line having ATCC accession number PTA-11884, or an antigen-binding fragment thereof. In one embodiment the sample may be first contacted with the antibody produced by the cell line having ATCC accession number PTA-11885, or an antigen-binding fragment thereof, and then separately contacted with a second antibody produced by the cell line having ATCC accession number PTA-11887, or an antigen-binding fragment thereof. In one embodiment the sample may be first contacted with the antibody produced by the cell line having ATCC accession number PTA-11885, or an antigen-binding fragment thereof, and then separately contacted with a second antibody produced by the cell line having ATCC accession number PTA-11886, or an antigen-binding fragment thereof.

[0096] The described antibodies and antigen-binding fragments may be detectably labeled. In

some embodiments labeled antibodies and antigen-binding fragments may facilitate the detection FRα via the methods described herein. Many such labels are readily known to those skilled in the art. For example, suitable labels include, but should not be considered limited to, radiolabels, fluorescent labels (such as DyLight® 649), epitope tags, biotin, chromophore labels, ECL labels, or enzymes. More specifically, the described labels include ruthenium, ^{111}In-DOTA, ^{111}In- diethylenetriaminepentaacetic acid (DTPA), horseradish peroxidase, alkaline phosphatase and beta-galactosidase, poly-histidine (HIS tag), acridine dyes, cyanine dyes, fluorone dyes, oxazin dyes, phenanthridine dyes, rhodamine dyes, Alexafluor® dyes, and the like.

[0097] The described antibodies and antigen-binding fragments may be used in a variety of assays to detect FRα in a sample. Some suitable assays include, but should not be considered limited to, western blot analysis, radioimmunoassay, immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, electrochemiluminescence (ECL) immunoassay, immunohistochemistry, fluorescence-activated cell sorting (FACS) or ELISA assay.

[0098] In some embodiments described herein detection of FRα-expressing cancer cells in a subject may be used to determine that the subject may be treated with a therapeutic agent directed against FRα. In some embodiments the therapeutic agent directed against FRα may be an antibody, such as Farletuzumab.

Methods for Diagnosing Cancer

[0099] Provided herein are methods for diagnosing ovarian, breast, thyroid, colorectal, endometrial, fallopian tube, or lung cancer of epithelial origin in a subject. In some embodiments, as described above, detecting FRα in a sample, such as a histological sample, a fine needle aspirate sample, resected tumor tissue, circulating cells, circulating tumor cells, and the like, provides the ability to diagnose cancer in the subject from whom the sample was obtained. In some embodiments, it may already be known that the subject from whom the sample was obtained has cancer, but the type of cancer afflicting the subject may not yet have been diagnosed or a preliminary diagnosis may be unclear, thus detecting FRα in a sample obtained from the subject can allow for, or clarify, diagnosis of the cancer.

[0100] In some embodiments the described methods involve assessing whether a subject is afflicted with FRα-expressing cancer by determining the amount of FRα that is present in a sample derived from the subject; and comparing the observed amount of FRα with the amount of FRα in a control sample, wherein a difference between the amount of FRα in the sample derived from the subject and the amount of FRα in the control sample is an indication that the subject is afflicted with an FRα-expressing cancer. In some embodiments the amount of FRα in the sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα, such as the antibodies described herein. Similar methods may be used to determine if a subject is afflicted with cancer that is not associated with increased FRα

production. The sample assessed for the presence of FRα may be derived from urine, blood, serum, plasma, saliva, ascites, circulating cells, circulating tumor cells, cells that are not tissue associated (*i.e.,* free cells), tissues (*e.g.,* surgically resected tumor tissue, biopsies, including fine needle aspiration), histological preparations, and the like. In some embodiments the subject is a human.

[0101] In some embodiments the method of diagnosing an FRα-expressing cancer will involve: contacting a biological sample of a subject with an FRα-specific antibody, or antigen-binding fragment thereof (such as those derivable from the antibodies and fragments provided in Table 1), quantifying the amount of FRα present in the sample that is bound by the antibody or antigen-binding fragment thereof, comparing the amount of FRα present in the sample to a known standard; and determining whether the subject's FRα levels fall within the levels of FRα associated with cancer. In an additional embodiment, the diagnostic method can be followed with an additional step of administering or prescribing a cancer-specific treatment. In some embodiments the cancer-specific treatment may be directed against FRα-expressing cancers, such as Farletuzumab.

[0102] In some embodiments the described methods involve assessing whether a subject is afflicted with FRα-expressing cancer by determining the amount of FRα associated with a cell or tissue that is present in a sample derived from the subject; and comparing the observed amount of FRα with the amount of FRα in a control sample, wherein a difference between the amount of FRα in the sample derived from the subject and the amount of FRα in the control sample is an indication that the subject is afflicted with an FRα-expressing cancer.

[0103] In some embodiments the control sample may be derived from a subject that is not afflicted with FRα-expressing cancer. In some embodiments the control sample may be derived from a subject that is afflicted with FRα-expressing cancer. In some embodiments where the control sample is derived from a subject that is not afflicted with FRα-expressing cancer, an observed increase in the amount of FRα present in the sample, relative to that observed for the control sample, is an indication that the subject being assessed is afflicted with FRα-expressing cancer. In some embodiments where the control sample is derived from a subject that is not afflicted with FRα-expressing cancer, an observed decrease or similarity in the amount of FRα present in the test sample, relative to that observed for the control sample, is an indication that the subject being assessed is not afflicted with FRα-expressing cancer. In some embodiments where the control sample is derived from a subject that is afflicted with FRα-expressing cancer, an observed similarity in the amount of FRα present in the test sample, relative to that observed for the control sample, is an indication that the subject being assessed is afflicted with FRα-expressing cancer. In some embodiments where the control sample is derived from a subject that is afflicted with FRα-expressing cancer, an observed decrease in the amount of FRα present in the test sample, relative to that observed for the control sample, is an indication that the subject being assessed is not afflicted with FRα-expressing cancer.

[0104] In some embodiments the amount of FRα in the sample derived from the subject is

assessed by contacting the sample with an antibody that binds FRα, such as the antibodies described herein. The sample assessed for the presence of FRα may be circulating cells, circulating tumor cells, cells that are not tissue associated (*i.e.,* free cells), tissues (*e.g.,* surgically resected tumor tissue, biopsies, including fine needle aspiration), histological preparations, and the like.

[0105] In some embodiments the described methods involve assessing whether a subject is afflicted with FRα-expressing cancer by determining the amount of FRα that is not associated with a cell or tissue that is present in a sample derived from the subject; and comparing the observed amount of FRα with the amount of FRα in a control sample, wherein a difference between the amount of FRα in the sample derived from the subject and the amount of FRα in the control sample is an indication that the subject is afflicted with an FRα-expressing cancer. In some embodiments the amount of FRα in the sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα, such as the antibodies described herein. The sample assessed for the presence of FRα may be urine, blood, serum, plasma, saliva, ascites, histological preparations, and the like.

[0106] In various embodiments of the described methods, the cancer may be FRα-expressing cancer. In a particular embodiment, the FRα-expressing cancer is ovarian cancer. In some embodiments the FRα-expressing cancer is endometrial cancer. In some embodiments the FRα-expressing cancer is colorectal cancer. In some embodiments the FRα-expressing cancer is breast cancer. In some embodiments the FRα-expressing cancer is thyroid cancer. In some embodiments the FRα-expressing cancer is fallopian tube cancer. In another embodiment, the FRα-expressing cancer is non-small cell lung cancer, such as an adenocarcinoma. Alternatively, the described methods may be used to diagnose cancer that does not express FRα, such as squamous cell carcinoma.

[0107] In various aspects, the amount of FRα is determined by contacting the sample with an antibody, or antigen-binding fragment thereof, that binds FRα. In some embodiments, the sample may be contacted by more than one type of antibody, or antigen-binding fragment thereof, that binds FRα. In some embodiments, the sample may be contacted by a first antibody, or antigen fragment thereof, that binds FRα as defined in Claims 1 to 3 and then contacted by a second antibody, or antigen-binding fragment thereof, that binds FRα. The second antibody is selected from the group consisting of:

1. (a) an antibody, or antigen-binding fragment thereof, that binds the same epitope as any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3;
2. (b) any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3, or an antigen-binding fragment thereof;
3. (c) an antibody, or antigen-binding fragment thereof, that comprises heavy chain CDR11, CDR2, and CDR3 amino acid sequences and light chain CDR1, CDR2, and CDR3 amino acid sequences of any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3, as described in Table 1;
4. (d) an antibody, or antigen-binding fragment thereof, that comprises the heavy chain

variable domain segment and light chain variable domain segment of any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3, as described in Table 1; or

5. (e) an antibody having the amino acid sequence of antibody produced by any one of the cell lines deposited with the ATCC having accession number PTA-11887, PTA-11884, PTA-11886, or PTA-11885,or an antigen binding fragment thereof.

Various combinations of the antibodies and antigen-binding fragments described in (a)-(e), as detailed above in the general section describing methods of detection, can be used to provide a "first" and "second" antibody or antigen-binding fragment to carry out the described diagnostic methods.

[0108] In certain embodiments, the amount of FRα is determined by western blot analysis, radioimmunoassay, immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion, electrochemiluminescence (ECL) immunoassay, immunohistochemistry, fluorescence-activated cell sorting (FACS) or ELISA assay.

[0109] In various embodiments of the described diagnostic methods a control sample is used. The control sample may be a positive or negative assay control that ensures the assay used is working properly; for example, an assay control of this nature might be commonly used for immunohistochemistry assays. Alternatively, the control sample may be a standardized control amount of FRα in a healthy subject. In some embodiments, the observed FRα levels of the tested subject may be compared with FRα levels observed in samples from control subjects known to have FRα-expressing cancer. In some embodiments, the control subject's FRα-expressing cancer is ovarian cancer, endometrial cancer, colorectal cancer, breast cancer, thyroid cancer, fallopian tube cancer, or lung cancer, such as adenocarcinoma. In some embodiments, the control subject is known to have early stage FRα-expressing cancer, such as stage I ovarian cancer, endometrial cancer, colorectal cancer, breast cancer, thyroid cancer, fallopian tube cancer, or lung cancer (e.g., adenocarcinoma). In some embodiments, the control subject is known to have intermediate stage FRα-expressing cancer, such as stage II ovarian cancer, endometrial cancer, colorectal cancer, breast cancer, thyroid cancer, fallopian tube cancer, or lung cancer (e.g., adenocarcinoma). In some embodiments, the control subject is known to have late stage FRα-expressing cancer, such as stage III or stage IV ovarian cancer, endometrial cancer, colorectal cancer, breast cancer, thyroid cancer, fallopian tube cancer, or lung cancer (e.g., adenocarcinoma).

[0110] The diagnostic methods provided herein also provide a basis upon which it may be possible to predict whether a subject has a relatively higher or lower likelihood of surviving 5 years following diagnosis. In some embodiments, the described method may be used to predict a favorable outcome for a subject having adenocarcinoma, wherein a favorable outcome is defined as having an increased 5-year survival rate. As data provided herein indicate, subjects determined to have stage I or stage II adenocarcinoma that does not express FRα are about 2 times more likely to die within five years than subjects determined to have stage I or stage II adenocarcinoma that does express FRα. Accordingly, the diagnostic

methods described herein may be combined with this knowledge to allow for a method of predicting 5-year survivorship likelihood for subjects determined to have cancer. In some embodiments the method is used to predict the 5-year survivorship likelihood for subjects determined to have adenocarcinoma.

[0111] In some embodiments the described prognostic method will involve: contacting a biological sample of a subject with an FRα-specific antibody, or antigen-binding fragment thereof (such as those derivable from the antibodies and fragments provided in Table 1), quantifying the amount of FRα present in the sample that is bound by the antibody or antigen-binding fragment thereof, comparing the amount of FRα present in the sample to a known standard; and determining whether the subject's FRα levels indicate the presence of a FRα expressing cancer, thereby allowing for a prediction to be made as to the likelihood the subject will survive five years after being diagnosed with cancer. In some embodiments the subject is known to have or determined to have adenocarcinoma. In some embodiments the subject is a human.

Methods for Monitoring Cancer

[0112] Provided herein are methods for monitoring cancer of epithelial origin in a subject. In some embodiments the described methods involve assessing whether FRα-expressing cancer is progressing, regressing, or remaining stable by determining the amount of FRα that is present in a test sample derived from the subject; and comparing the observed amount of FRα with the amount of FRα in a sample obtained from the subject at an earlier point in time, wherein a difference between the amount of FRα in the test sample and the earlier sample provides an indication of whether the cancer is progressing, regressing, or remaining stable. In this regard, a test sample with an increased amount of FRα, relative to the amount observed for the earlier sample, may indicate progression of an FRα-expressing cancer. Conversely, a test sample with a decreased amount of FRα, relative to the amount observed for the earlier sample, may indicate regression of an FRα-expressing cancer. Accordingly, a test sample with an insignificant difference in the amount of FRα, relative to the amount observed for the earlier sample, may indicate a state of stable disease for an FRα-expressing cancer. In some embodiments the amount of FRα in a sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα, such as the antibodies described herein. The sample assessed for the presence of FRα may be derived from urine, blood, serum, plasma, saliva, ascites, circulating cells, circulating tumor cells, cells that are not tissue associated (i.e., free cells), tissues (e.g., surgically resected tumor tissue, biopsies, including fine needle aspiration), histological preparations, and the like. In some embodiments the subject is a human.

[0113] In some embodiments the method of monitoring an FRα-expressing cancer will involve: contacting a biological sample of a subject with an FRα-specific antibody, or antigen-binding fragment thereof (such as those derivable from the antibodies and fragments provided in Table 1), quantifying the amount of FRα present in the sample that is bound by the antibody or

antigen-binding fragment thereof, comparing the amount of FRα present in the sample to the amount of FRα determined to be in a sample from the same subject at an earlier point in time; and determining whether the subject's FRα levels have changed over time. A test sample with an increased amount of FRα, relative to the amount observed for the earlier sample, may indicate progression of an FRα-expressing cancer. Conversely, a test sample with a decreased amount of FRα, relative to the amount observed for the earlier sample, may indicate regression of an FRα-expressing cancer. Accordingly, a test sample with an insignificant difference in the amount of FRα, relative to the amount observed for the earlier sample, may indicate a state of stable disease for an FRα-expressing cancer. In some embodiments, the FRα levels of the sample may be compared to a known standard, alone or in addition to the FRα levels observed for a sample assessed at an earlier point in time. In some embodiments the known standard may be FRα protein at a known concentration (e.g., a recombinant or purified FRα protein sample). In an additional embodiment, the diagnostic method can be followed with an additional step of administering a cancer-specific treatment. In some embodiments the cancer-specific treatment may be directed against FRα-expressing cancers, such as Farletuzumab.

[0114] In some embodiments the described methods involve assessing whether FRα-expressing cancer is progressing, regressing, or remaining stable by determining the amount of FRα associated with a cell or tissue that is present in a test sample derived from the subject; and comparing the observed amount of FRα with the amount of FRα in a sample obtained from the subject, in a similar manner, at an earlier point in time, wherein a difference between the amount of FRα in the test sample and the earlier sample provides an indication of whether the cancer is progressing, regressing, or remaining stable. In this regard, a test sample with an increased amount of FRα, relative to the amount observed for the earlier sample, may indicate progression of an FRα-expressing cancer. Conversely, a test sample with a decreased amount of FRα, relative to the amount observed for the earlier sample, may indicate regression of an FRα-expressing cancer. Accordingly, a test sample with an insignificant difference in the amount of FRα, relative to the amount observed for the earlier sample, may indicate a state of stable disease for an FRα-expressing cancer. In some embodiments the amount of FRα in a sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα, such as the antibodies described herein. The sample assessed for the presence of FRα may be circulating cells, circulating tumor cells, cells that are not tissue associated (*i.e.,* free cells), tissues *(e.g.,* surgically resected tumor tissue, biopsies, including fine needle aspiration), histological preparations, and the like.

[0115] In some embodiments the described methods involve assessing whether FRα-expressing cancer is progressing, regressing, or remaining stable by determining the amount of FRα not associated with a cell or tissue that is present in a test sample derived from the subject; and comparing the observed amount of FRα with the amount of FRα in a sample obtained from the subject, in a similar manner, at an earlier point in time, wherein a difference between the amount of FRα in the test sample and the earlier sample provides an indication of whether the cancer is progressing, regressing, or remaining stable. In this regard, a test sample with an increased amount of FRα, relative to the amount observed for the earlier

sample, may indicate progression of an FRα-expressing cancer. Conversely, a test sample with a decreased amount of FRα, relative to the amount observed for the earlier sample, may indicate regression of an FRα-expressing cancer. Accordingly, a test sample with an insignificant difference in the amount of FRα, relative to the amount observed for the earlier sample, may indicate a state of stable disease for an FRα-expressing cancer. In some embodiments the amount of FRα in a sample derived from the subject is assessed by contacting the sample with an antibody that binds FRα, such as the antibodies described herein. The sample assessed for the presence of FRα may be urine, blood, serum, plasma, saliva, ascites, histological preparations, and the like.

[0116] In various embodiments of the described methods, the cancer may be FRα-expressing cancer. In a particular embodiment, the FRα-expressing cancer is ovarian cancer. In some embodiments the FRα-expressing cancer is endometrial cancer. In some embodiments the FRα-expressing cancer is colorectal cancer. In some embodiments the FRα-expressing cancer is breast cancer. In some embodiments the FRα-expressing cancer is thyroid cancer. In some embodiments the FRα-expressing cancer is fallopian tube cancer. In another embodiment, the FRα-expressing cancer is non-small cell lung cancer, such as an adenocarcinoma.

[0117] In various aspects, the amount of FRα is determined by contacting the sample with an antibody, or antigen-binding fragment thereof, that binds FRα. In some embodiments, the sample may be contacted by more than one type of antibody, or antigen-binding fragment thereof, that binds FRα. In some embodiments, the sample may be contacted by a first antibody, or antigen-binding fragment thereof, that binds FRα as defined in Claims 1 to 3 and then contacted by a second antibody, or antigen-binding fragment thereof, that binds FRα. The second antibody may be selected from among:

1. (a) an antibody, or antigen-binding fragment thereof, that binds the same epitope as any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3;
2. (b) any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3, or an antigen-binding fragment thereof;
3. (c) an antibody, or antigen-binding fragment thereof, that comprises heavy chain CDR1, CDR2, and CDR3 amino acid sequences and light chain CDR1, CDR2, and CDR3 amino acid sequences of any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3, as described in Table 1;
4. (d) an antibody, or antigen-binding fragment thereof, that comprises the heavy chain variable domain segment and light chain variable domain segment of any one of antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3, as described in Table 1; or
5. (e) an antibody having the amino acid sequence of antibody produced by any one of the cell lines deposited with the ATCC having accession number PTA-11887, PTA-11884, PTA-11886, or PTA-11885, or an antigen binding fragment thereof.

Various combinations of the antibodies and antigen-binding fragments described in (a)-(e), as detailed above in the general section describing methods of detection, can be used to provide a "first" and "second" antibody or antigen-binding fragment to carry out the described

monitoring methods.

[0118] In certain embodiments, the amount of FRα is determined by western blot analysis, radioimmunoassay, immunofluorimetry, immunoprecipitation, equilibrium dialysis, iinmunodiffusion, electrochemiluminescence (ECL) immunoassay, immunohistochemistry, fluorescence-activated cell sorting (FACS) or ELISA assay.

[0119] Additional aspects of the summarized subject matter are provided in greater detail in the detailed description and provided examples and associated figures.

Kits for Detecting the FRα

[0120] Provided herein are kits for detecting FRα in a sample as defined in Claim 29.

[0121] The provided antibody, or antigen-binding fragment, may be in solution; lyophilized; affixed to a substrate, carrier, or plate; or conjugated to a detectable label.

[0122] The described kits may also include additional components useful for performing the methods described herein. 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/or instructional material which describes performance of a method of the invention and tissue specific controls/standards.

[0123] 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.

[0124] The described kits 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.

[0125] The described kits may also include a blocking reagent that can be applied to a sample to decrease nonspecific binding of a primary or secondary antibody. An example of a blocking reagent is bovine serum albumin (BSA), which may be diluted in a buffer prior to use. Other commercial blocking reagents, such as Block Ace and ELISA Synblock (AbD serotec), Background Punisher (BIOCARE MEDICAL), and StartingBlock (Thermo Fisher Scientific) are known in the art. The described kits may also include a negative control primary antibody that does not bind to FRα sufficiently to yield a positive result in an antibody-based detection assay. In addition, the described kits may include a secondary antibody capable of binding to a FRα

primary antibody, such as antibody 9F3, antibody 19D4, antibody 24F12, or antibody 26B3. In some embodiments the secondary antibody may be conjugated to a detectable label, such as horse radish peroxidase (HRP) or a fluorophore, to allow for detection of the primary antibody bound to a sample. The described kits may also include a colorimetric or chemiluminescent substrate that allows the presence of a bound secondary antibody to be detected on a sample. In some embodiments the colorimetric or chemiluminescent substrate may be 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS); 3,3',5,5'-Tetramethylbenzidine (TMB); 3,3'-Diaminobenzidine (DAB); SuperSignal (Thermo Fisher Scientific); ECL reagent (Thermo Fisher Scientific) or other such reagents known to those of ordinary skill in the art.

[0126] The following examples are provided to supplement the prior disclosure and to provide a better understanding of the subject matter described herein. These examples should not be considered to limit the described subject matter.

[0127] The present invention is defined by the claims. Any examples which fall outside the scope of the claims are provided for reference only and do not form part of the invention.

EXAMPLE 1 - Expression and Purification of Recombinant, Human FRα

[0128] To conduct the experiments associated with the studies described herein, several folate receptor alpha (FRα)-expressing cell systems or lines were created to generate FRα-expressing cell substrates or to generate purified recombinant human FRα protein. One expression system used was an Sf9 insect cell line that expressed recombinant human FRα via baculovirus. This system was prepared using a human FRα sequence, containing a leader sequence optimized for insect cell expression, a N-terminal 6x histidine (6xhis) epitope tag, and the native GPI attachment site intact. The cells were then incubated in a 1L shake flask and log-phase cultures of Sf9 insect cells were infected with the recombinant baculovirus at a multiplicity of infection (MOI) of <1. Cells from 30L of culture were harvested, lysed and extracted 2x with 1X phosphate-buffered saline (PBS) containing 10 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). The NaCl concentration was adjusted to 300 mM and filtered through a 0.2 um membrane. The clarified supernatant was purified by affinity chromatography, using 1X PBS with 2M NaCl, 1 mM CHAPS, pH 7.4 as wash buffer, followed by elution with 10 mM 3-(N-morpholino)propanesulfonic acid (MOPS), 3M $MgCl_2$, 1 mM CHAPS, pH 6.8. Peak fractions were dialyzed extensively against 1X PBS, pH 7.4, analyzed for purity by SDS-PAGE, quantitated by bicinchoninic acid assay (BCA) assay, aliquoted and stored at -80 degrees Celsius.

[0129] A Chinese hamster ovary (CHO) cell line stably expressing and secreting human FRα was produced using a human folate receptor alpha (FRα) sequence, containing a human immunoglobulin kappa leader sequence and a C-terminal 6xhis epitope tag replacing the GPI attachment site. Once produced, the FRα-expressing CHO cells were grown at 25L-scale in wave bags. To purify the secreted FRα protein, cell supernatant was cleared of cellular debris

by depth filtration and then concentrated 10-fold by tangential flow filtration and diafiltered into 50 mM sodium phosphate, 300 mM NaCl, 1 mM imidazole, pH 8.0. This was loaded onto a pre-packed Talon® IMAC column using an FPLC. Unbound material was washed out using 50 mM sodium phosphate, 300 mM NaCl, 5 mM imidazole, pH 8.0 and bound protein was eluted using a linear gradient of 5 mM -100 mM imidazole in 50 mM sodium phosphate, 300 mM NaCl, pH 8.0. Peak fractions were dialyzed extensively against 1X PBS, pH 7.4, analyzed for purity by SDS-PAGE, quantitated by BCA assay, aliquoted and stored at -80 degrees Celsius.

[0130] A similar cell system was also produced for human folate receptor beta (FRβ), human folate receptor gamma (FRγ), and human folate receptor delta (FRδ). Briefly, constructs of either FRβ, FRγ, or FRδ containing a human immunoglobulin kappa leader sequence and a C-terminal 6xhis epitope tag replacing the GPI attachment site, were used to transiently transfect 1L cultures of 293F cells. Recombinant FR proteins were purified as described above for human FRα.

[0131] A Chinese hamster ovary (CHO) cell line stably expressing and secreting a human mesothelin sequence, containing a human immunoglobulin kappa leader sequence and a C-terminal 6xhis epitope tag replacing the GPI attachment site, was also prepared, as mesothelin served as a negative control for many studies. Human mesothelin-expressing CHO cells were grown at 25L-scale in wave bags. To purify the secreted mesothelin protein, cell supernatant was cleared of debris by hollow-fiber filtration and clarified supernatant was concentrated 10-fold by tangential flow filtration. Supernatant NaCl concentration was adjusted to 300 mM NaCl and 0.5 mM imidazole. This was loaded onto a pre-packed Talon® IMAC column using an FPLC. Unbound material was washed out using 50 mM sodium phosphate, 300 mM NaCl, 3 mM imidazole, pH 8.0 and bound protein was eluted using 50 mM sodium phosphate, 300 mM NaCl, 150 mM imidazole, pH 8.0. Peak fractions were dialyzed extensively against 50 mM potassium phosphate, pH 7.5. Ammonium sulfate was added to a final concentration of 1M, and final purification was then done on a pre-packed phenyl sepharose column using a step gradient of 1M - 0M ammonium sulfate in 50 mM potassium phosphate, pH 7.5. Peak fractions were dialyzed extensively against 1X PBS, pH 7.4, analyzed for purity by SDS-PAGE, quantitated by BCA assay, aliquoted and stored at -80 degrees Celsius.

EXAMPLE 2 - Production of Purified Reduced and Alkylated FRα

[0132] Efforts were undertaken to produce a reduced and alkylated antigenic form of FRα. To reduce the protein, purified FRα was concentrated to 2 mg/mL in phosphate buffered saline (pH 7.4) using centrifugal filters (Amicon Ultra, 3 kD MW limit). The protein concentration was determined using a BCA assay (Thermo Scientific). The resultant FRα was diluted 1:1 in 8M urea/ PBS to generate a final concentration of 1 mg/mL FRα in PBS containing 4M urea. Dithiothreitol solution (500 mM in PBS) was added to a final concentration of 10 mM. The solution was incubated at 65 degrees Celsius for one hour, and cooled to room temperature.

[0133] Next 1M of iodoacetamide solution in phosphate buffer saline was added into the

reduced folate receptor solution to a final concentration of 10 mM, and the reaction was kept in dark at room temperature for 30 minutes. The protein remained soluble under these conditions. The final reduced FRα to be used for immunization was stored in phosphate buffer saline containing 4M of urea, 10mM of DTT, and 10 mM of iodoacetamide.

[0134] Figure 1 shows the differential migration of native FRα protein and a reduced and alkylated form of the protein analyzed by SDS-PAGE under nonreducing conditions.

EXAMPLE 3 - Production of Hybridomas using FRα

[0135] Eight week old female Balb/c mice were immunized with hexa-histidine tagged FRα protein (n=5) or reduced and alkylated FRα protein (n=5). Initial intraperitoneal immunizations administered on day 0 comprised 50µg of the respective immunogen mixed 1:1 (v:v) with complete Freund's adjuvant (Rockland, Cat# D614-0050). Mice were then boosted with 50µg immunogen mixed 1:1 (v:v) with incomplete Freund's adjuvant (Rockland, Cat# D615-0050) administered intraperitoneally 14 days later and every 21 days thereafter. Blood samples were collected from immunized mice 24 days after the initial immunization and every 21 days thereafter.

[0136] Collected blood samples were analyzed by direct enzyme-linked immunoassay (EIA) against FRα. Plates were coated with FRα protein (100ml of a 1mg/mL solution in PBS, 0.02 M potassium phosphate, 0.15 M Sodium Chloride, pH 7.2) and incubated overnight at 4°C, washed with PBS containing 0.2% Tween®-20 (PBST; Rockland, Cat# MB-075-1000) and blocked with 3% fish gel (Sigma) for 1hr at room temperature. A 3-fold dilution series of individual mouse serum samples were allowed to bind for 1hr at room temperature, plates were then washed 3 times with PBST and subsequently probed with an HRP-conjugated rabbit-anti-mouse antibody (Rockland, Cat#610-4320) at 1:2500 for 30 minutes at 37°C. TMB substrate (Rockland, Cat# TMBE-100) was added and the reaction was stopped after 30 minutes by addition of 100mL of 1M HCl prior to absorbance reading at 450nm (Microplate Reader "Benchmark"; Biorad). All samples were counter-screened against hexa-histidine tagged recombinant mesothelin (mesothelin-His_6) protein as a negative control.

[0137] Spleens from mice showing the highest antigen-specific titers were harvested and hybridomas were prepared by electrofusion (Hybrimune™ Model CEEF-50B Waveform Generator; Cellectis, Romainville, France) of splenocytes with Sp2/0 Ag14 myeloma cells (ATTC CRL1581). Subsequently, hybridoma supernatants were screened by ELISA against FRα and recombinant Mesothelin-His_6 as described above to select positive parental fusion cell lines.

[0138] Selected parental cell lines determined to produce antibodies reactive to recombinant human FRα (rhFRα) were then subcloned by limiting dilution. The antibodies produced by these cells were then retested for FRα binding and isotyped using the Clonetyping™ System

(SouthernBiotech, Birmingham, AL). Supernatants from these clones were further screened by direct ELISA against three additional isoforms of the human folate receptor (FRβ, FRγ and FRδ) to determine receptor specificity. Plates were coated overnight with 100µL of a 1µg/mL solution of the respective FRα isoform at 4°C, washed with PBS containing 0.2% Tween®-20 (Rockland, Cat# MB-075-1000) and blocked with 3% fish gel (Sigma). A 3-fold dilution series of culture supernatants was allowed to bind for 1hr at room temperature, before plates were washed and probed with an HRP-conjugated anti-mouse antibody as described above. Clones producing antibodies reactive to FRβ, FRγ and FRδ were not selected for further analysis.

[0139] Four selected hybridoma clones, 19D4.B7, 26B3.F2, 24F12.B1, and 9F3.H9.H3.H3.B5.G2, were deposited with the American Type Culture Collection on May 19, 2011 and were assigned ATCC accession numbers PTA-11884, PTA-11885, PTA-11886, and PTA-11887, respectively.

EXAMPLE 4 - Production of Purified Monoclonal Antibodies to FRα

[0140] Selected cell lines were tested for mycoplasma using a mycoplasma test kit (Rockland, Cat# MAB-012) before seeding into 1L roller bottles containing serum free medium (Invitrogen, Cat#12045-076) and 5% low IgG FBS (0.1µg/ml) (Gibco, Cat# 16250-078) at $0.5x10^5$ cells/mL. Cultures were allowed to grow at 37°C for either 14 or 21 days, after which supernatant was harvested and concentrated approximately 10-fold through a 50kDa filtration membrane (Spectrum Labs, Rancho Dominguez CA) and then purified using protein A chromatography (Rockland, Cat# PA50-00-0025). Bound antibody was eluted with 0.1M sodium citrate, pH 3.5/4.5 depending on antibody isotype, and buffer was exchanged against PBS by dialysis using a 12-14kDa membranous tubing (Spectrum Labs, Rancho Dominguez CA). Purified antibody was sterile filtered using a 0.22µm Express™PLUS Stericups (Millipore, Billerica MA) and stored at 4°C for further testing.

[0141] Efforts were undertaken to sequence the heavy and light chains of four selected hybridomas clones (9F3-H9, 19D4-B7, 24F12-B1, and 26B3-F2). First, total RNA was isolated from each hybridoma cell line (cell pellets of 1 x 10^3 to 1 x 10^5 cells each) using the RNAqueous® kit (Ambion) according to the manufacturer's protocol. RNA was quantified using a NanoDrop™ 8000 spectrophotometer (Thermo Scientific).

[0142] Isolated RNA was then amplified via multiplex RT-PCR, performed in triplicate for each hybridoma with a Mastercycler® EP Gradient Thermocycler (Eppendorf). First, two separate gene-specific cDNA amplifications were performed for each hybridoma (≤1ug RNA/reaction) to determine which Ig heavy and light chain genes were used during Ig rearrangement. Each cocktail consisted of unique family-specific primers designed to anneal to any of the potential murine Ig V gene families (IgHv, IgKv) and Ig constant region genes ($IgHc_{Gamma}$, IgKc). cDNA generation and amplification was performed using SuperScript® III One-Step RT-PCR System with Platinum® Taq High Fidelity (Invitrogen) under the following conditions: 55°C for 30

minutes and 95°C for 2 minutes, followed by 40 cycles of 95°C for 1 minute, 55°C for 1 minute, 68°C for 1 minute, and a final 68°C for 10 minutes completion step. DNA products were electrophoresed on a 2% agarose gel. Appropriate bands were excised and gel purified using the QIAquick® Gel Extraction Kit (Qiagen) following the manufacturer's protocol. Purified DNA was submitted for sequencing (GENEWIZ, Inc., South Plainfield, NJ) to determine the germline gene segments expressed by each hybridoma.

[0143] Further RT-PCR analysis suited to the particular genes identified for each hybridoma was then performed using the same RNA source as above and gene-specific primers (in contrast to family-specific primers used in the multiplex RT-PCR mixture). To facilitate cloning, amplified Ig cDNAs were placed into an In-Fusion (IF) expression vector, each gene-specific primer also contained vector-compatible linker sequences which would enable homologous crossover. All other reagents and thermocycler conditions are the same as those used for the multiplex RT-PCR experiments, described above.

EXAMPLE 5: Characterization of Antibody Binding to FRα

[0144] Binding characteristics of the purified monoclonal antibodies to FRα were determined by surface plasmon resonance (SPR) experiments. All of the SPR experiments were performed at 25°C using a BIAcore T100 with research grade CM5 chips (GE Healthcare), as specified by the manufacturer. Initially, anti-mouse IgG provided in the mouse antibody capture kit (GE Healthcare) was immobilized by amide coupling to CM5 sensor chips. Mouse anti-FRα monoclonal antibodies (26B3, 24F12, 19D4, or 9F3) were captured on individual flow cells per binding cycle, while the fourth flow cell was used as a reference. Binding experiments were performed with HBS-P (GE Healthcare) as running buffer and at a flow rate of 30µL/min. Each monoclonal antibody sample (0.5µg/mL) was injected for 3 minutes to capture the antibody. Various concentrations of purified recombinant human FRα (rh-FRα) (1nM - 30nM) were then injected over the FRα-specific and reference surfaces for 3 minutes to record binding sensograms using a single-cycle kinetics method. The dissociation profile was monitored for 25 minutes. In between bindings, the surface was regenerated with a 30µl injection of 10mM glycine (pH 1.7). The sensograms were processed and fitted to a 1:1 Langmuir binding model using BIAcore T100 evaluation software (version 2.0.1). Some of the binding characteristics of antibodies 26B3, 24F12, 19D4, and 9F3 are provided in Table 2.

Table 2: Binding characteristics of FRα-specific antibodies.

Abbreviated Clone Name	ka (1/Ms)	kd (1/s)	KD (M)	Chi.Sq
26B3	5.24×10^{5}	1.43×10^{-5}	2.73×10^{-11}	2.48
24F12	3.93×10^{5}	3.99×10^{-5}	1.02×10^{-10}	1.08
19D4	4.27×10^{5}	2.42×10^{-4}	5.67×10^{-10}	0.656
9F3	4.34×10^{5}	3.10×10^{-4}	7.15×10^{-10}	1.89

EXAMPLE 6: Epitope Mapping of Selected FRα-specific Antibodies

[0145] FRα-specific antibodies 26B3, 24F12, and 9F3 were further assessed in epitope binding studies using Octet QK. The results showed that 26B3 and 24F12, which have high affinities to purified human FRα, compete with one another for binding to FRα. Thus, these antibodies may share a common epitope, or have epitopes that are immediately adjacent to each other. The results also indicate that the 9F3 antibody has a unique epitope, since it did not compete with other FRα-specific antibodies for binding to FRα.

[0146] Additional epitope mapping studies were carried out by ExSAR™ using hydrogen/deuterium exchange mass spectrometry and docking methods. The results of these studies for antibodies 9F3, 24F12, and 26B3 are illustrated in Figure 2. With regard to the epitope for antibody 26B3, these data suggest that it is accessible in the native, membrane-anchored structure, given the ability of 26B3 to recognize native FRα by flow cytometry. Furthermore, these data further suggest that the conformational constraints of the epitope recognized by MAb 26B3, as demonstrated by its inability to detect the protein on reduced western blots, are related to the cysteine at position 185 in the FRα protein which forms a disulfide bridge with cysteine 111.

EXAMPLE 7: Recognition of Denatured and Chemically-Preserved Forms of FRα

[0147] Experiments were conducted to determine whether any of the FRα-specific antibodies, described above, could recognize denatured forms of FRα. For these analyses CHOK1 cells stably expressing GPI-linked human FRα, β, or Δ, were lysed in 1.1% OBG buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1.1% OBG) supplemented with Complete Mini Protease Inhibitor Cocktail (Roche Diagnostics, Indianapolis, IN) and PMSF (100 nM), and placed on ice for 15 minutes. Lysates were pre-cleared by centrifugation at 13,000 rpm for 15 minutes to remove debris. For reduced and denatured samples, equal amounts of protein (20 µg) were boiled for 10 minutes in NuPAGE® LDS sample buffer (Invitrogen) containing 5% β-mercaptoethanol + 40 mM DTT. Proteins were separated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 4-12% bis-tris gel (Invitrogen) and transferred to a PVDF membrane. The membrane was blocked in PBST+ 5% non-fat milk for 1h at room temperature after which time, the membrane was washed twice with PBST. Immunoblotting was conducted using purified mouse monoclonal antibodies 9F3, 19D4, 24F12, or 26B3 (1µg/mL) specific for FRα, which were detected with a goat-anti-mouse HRP-conjugated antibody and visualized using SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL). Luminescence was visualized using the Omega 12iC molecular imaging system (Ultra-Lum, Claremont, CA) with image analysis performed using UltraQuant™ 6.0 software (Ultra-Lum).

[0148] Western blot analyses were also performed using purified folate receptor preparations. For these experiments, 0.5µg of purified human FRα, β, Γ or Δ, produced as described in

Example 1, were incubated in 1X SDS-PAGE sample loading buffer (Invitrogen) with or without 20 mM DTT, boiled for 10min, and electrophoresed on 4-12% gradient SDS-PAGE gels. Protein was transferred to PVDF membrane and blots probed as described above. Gels were run using Benchmark™ prestained protein ladder (Novex®). Gels using purified recombinant FR proteins were also visualized via silverstaining to ensure equal amounts of protein were loaded.

[0149] Western blot analyses indicate that antibodies 19D4, 9F3, 24F12, and 26B3 recognize nonreduced FRα, however, binding to reduced and denatured samples was not detected for any of these antibodies (Figure 3(A) and (B)).

[0150] Immunohistochemistry (IHC) studies were also conducted to determine whether any of these antibodies could bind to formalin fixed paraffin embedded papillary serous ovarian cancer tissue samples. Indirect IHC testing was performed for FRα using a MACH4™ Universal HRP-Polymer Detection Kit (Biocare Medical). Formalin-fixed paraffin-embedded specimens were sectioned at 5 microns on positively-charged glass slides and heated for approximately 60 minutes at 60 °C. Slides were deparaffinized in 3 sequential baths of xylene for 3 minutes each, transferred to three sequential baths of 100% alcohol for 3 minutes each, followed by three sequential baths of 95% alcohol for 3 minutes each and then rinsed for 5 minutes in deionized (DI) water. Prepared samples were then pretreated with Diva heat-induced epitope retrieval solution (Biocare Medical) diluted to 1:10 in DI water and placed inside a pressurized decloaking chamber already filled with 500 ml of DI water. The samples were incubated for 15 minutes inside the decloaking chamber, where pressurized incubation reached a maximum of 125 °C at 16 PSI for 30 seconds and then was cooled for 15 minutes down to 95 °C. Slides were then cooled at room temperature for 15 minutes. After cooling, slides were washed in 3 sequential baths of Tris Buffered Saline/0.1% Tween-20® wash buffer (TBST) for 3 minutes each. All subsequent buffer washes were also performed in this manner. Slides were then blocked in Peroxidase-1 (Biocare Medical) blocking solution for 5 minutes at room temperature, washed with TBST, and then Background Sniper (Biocare Medical) serum-free universal blocking reagent was applied for 10 minutes at room temperature. After the samples were blocked the slides were incubated with 2.5 µg/ml of 26B3 antibody diluted in Antibody Diluent (Dako) or Universal Negative Control - Mouse ready-to-use negative control antibody (Dako, for negative isotype tissue) for 60 minutes at room temperature. Slides were then washed with TBST and incubated with MACH4™ Mouse Probe Primary Antibody Enhancer (provided in the Biocare Medical MACH4™ kit) for 15 minutes at room temperature. Slides were then washed again with TBST and incubated with a Polymer-HRP reagent (provided in the Biocare Medical MACH4 kit) for 20 minutes at room temperature. Following incubation, slides were washed with TBST and incubated with a 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution (Dako) for 5 minutes at room temperature. Then slides were thoroughly rinsed with DI water 3 times for 30-60 seconds each and counterstained with hematoxylin (Dako) for 2 minutes, washed with TBST, dehydrated in 3 sequential baths each of 95% and 100% alcohol for 30 seconds each, and cleared in 3 sequential baths of xylene for 30 seconds each. Finally, coverslips were applied to the slides prior to analysis.

[0151] It is commonly thought that an antibody must be able to recognize a linear epitope of the antigen of interest to be effective for immunohistochemistry of formalin fixed paraffin embedded tissue because the antigen is devoid of tertiary structure due to the destructive nature of fixation of tissue. Thus it was surprising that antibody 26B3 was able to recognize FRα in this assay (Fig. 4), since it does not recognize reduced and denatured FRα by western blot. Furthermore, the pattern of FRα staining with antibody 26B3 observed for normal tissues was consistent with previously published literature using a variety of other antibodies and techniques, with pancreas, thyroid, lung, salivary gland, kidney, hypophysis, cervix and breast showing expression to various degrees (Table 3). As shown in Figure 5, the staining pattern in normal tissues, exemplified in normal lung (A) and normal kidney (B) sections, is highly restricted to epithelial cells and typically apical in nature.

Table 3. FRα expression in normal human tissues

Tissue Type	Staining (Number/Intensity)	Comments
Cerebrum	0/3	
Cerebellum	0/3	
Adrenal	0/3	
Ovary	0/3	
Pancreas	3/3; 2+	Limited to luminal borders of ductal and acinar cells
Thyroid	2/5; 1+ (sparse)	Cytoplasmic staining in follicular cells
Hypophysis	3/3; 1+	Predominantly cytoplasmic
Testis	0/3	
Breast	3/3; 1+/2+	Ductal cells with luminal and membrane staining
Spleen	0/3	
Tonsil	0/3	
Thymus	0/3	
Bone Marrow	0/3	
Lung	3/3; 2+	Staining in bronchial and alveolar cells
Heart	0/3	
Esophagus	0/3	
Stomach	0/2	
Small Intestine	0/3	
Colon	0/3	
Liver	0/3	
Salivary Gland	3/3; 3+	Ductal and acinar cells

Tissue Type	Staining (Number/Intensity)	Comments
Kidney	3/3; 3+	Luminal staining of proximal tubular cells
Prostate	0/3	
Endometrium	0/3	
Cervix	1/3; 1+	Endocervical cells
Skeletal Muscle	0/3	
Skin	0/3	
Nerve	0/3	
Mesothelium (pleura and lung)	3/3; 2+	Alveolar cells

EXAMPLE 8: Recognition of Native forms of FRα

[0152] Flow cytometry studies were conducted to assess the ability of selected FRα-specific antibodies to bind to the native protein. For these studies Chinese hamster ovary (CHO) cells expressing FRα, were harvested, washed, and re-suspended in ice-cold growth media (RPMI supplemented with 10% FBS). Cells were incubated for 1 hour on ice with 9F3, 19D4, 24F12, or 26B3 (1µg/mL), washed and then incubated with FITC-conjugated secondary antibodies [dilution 1:100] (Southern Biotech, Birmingham, AL). Prior to analysis, cells were labeled with 7-amino-actinomycin D (7-AAD) (BD Biosciences, Franklin Lakes, NJ) for the exclusion of nonviable cells. CHO cells not expressing FRα were also subjected to the same experimental procedures, as a negative control. Cells were analyzed on an EasyCyte™ Flow Cytometer (Guava® Technologies, Hayward, CA). The data provided in Table 4 indicate that all four antibodies are capable of binding native FRα.

Table 4: FRα-specific antibodies recognize FRα expressed on the cell surface

Target	Geometric Mean Observed for Antibody:			
	9F3	26B3	24F12	19D4
Cells only	2.7	2.7	2.7	2.0
CHOK1	5.9	5.7	5.9	---
FRα	759.5	853.7	777.0	1130.5
FRP	6.1	5.9	6.6	---
FRΔ	5.6	5.4	5.9	---

EXAMPLE 9: Detection of FRα in the Serum of Subjects known to have Ovarian Cancer

[0153] Electrochemiluminescence studies were conducted to determine whether the FRα-specific antibodies described herein could detect FRα in the serum of patients known to have ovarian cancer. For these experiments MAb 26B3 was used as the capture MAb and added to ECL plates at a concentration of 75µg/mL. Plates were washed and 50 µL of sample serum was added to each well and incubated for 2 hours. Serum samples were obtained from normal healthy females (negative control) and from ovarian cancer patients. Samples were diluted 1:4 in PBST (phosphate buffered saline, pH7.4, containing 0.01% Tween®20). Following incubation, samples were washed with PBST and 25 µL/well of MAb 19D4 (1µg/mL), labeled with Ru at a ratio of approximately 13 labels/IgG molecule, was added to each well to detect bound sample. After a 2-hour incubation period, the plates were washed with PBST and read with 2X MSD Buffer T. The results in Table 5 show that FRα in serum can be captured and detected using monoclonal antibodies 26B3 and 19D4.

Table 5: Relative serum levels of FRα

Category (n)	Mean FRA pg/mL	Standard Deviation
Normal (15)	223	74
Ovarian Cancer (15)	1815	3896

EXAMPLE 10: Detection of FRα in the Serum and Urine of Subjects known to have Ovarian Cancer

[0154] Electrochemiluminescence studies were then conducted to determine whether the FRα-specific antibodies described herein could detect FRα in the serum and urine of patients known to have ovarian cancer. For these experiments MAb 26B3 was used as the capture MAb and added to ECL plates at a concentration of 75µg/mL. Plates were washed and 50 µL of sample serum was added to each well and incubated for 2 hours. Matched serum and urine samples were obtained from normal healthy females (negative control) and from ovarian cancer patients. Samples (serum or urine) were diluted 1:4 in PBST (phosphate buffered saline, pH7.4, containing 0.01% Tween®20). Following incubation, samples were washed with PBST and 25 µL/well of MAb 19D4 (1µg/mL), labeled with Ru at a ratio of approximately 13 labels/IgG molecule, was added to each well to detect bound sample. After a 2-hour incubation period, the plates were washed with PBST and read with 2X MSD Buffer T. The results in Table 6 show that FRα in serum and urine can be captured and detected using monoclonal antibodies 26B3 and 19D4.

Table 6: Relative serum and urine levels of FRα

Patient Designation	Serum FRalpha pg/mL	Urine FRalpha pg/mL
Normal 1	398	3080
Normal 2	236	11508
Normal 3	315	7704
Normal 4	320	13198

Patient Designation	Serum FRalpha pg/mL	Urine FRalpha pg/mL
Ovarian Cancer 1	19479	368066
Ovarian Cancer 2	4144	23738
Ovarian Cancer 3	986	165826
Ovarian Cancer 4	719	414187

EXAMPLE 11: M-Score as a Metric for Immunohistochemistry Results

[0155] A metric for staining (M-score) of each sample was developed and can be defined as follows:

$$M_i = \frac{\Sigma_{j=1}^{3} w_j \cdot x_{ij}}{\Sigma_{j=1}^{3} w_j} = \frac{\Sigma_{j=1}^{3} w_j \cdot x_{ij}}{6}$$

In the equation, x_{ij} is the percentage of tumor stained at intensity j for patient i and w_j is the absolute value of the intensity (ranging from 0 to 3+). The metric has a theoretical range from zero (no positive staining) to fifty (100% of cells staining at 3+ intensity). As such, the M-score is a weighted score for FRα IHC tumor cell membrane staining that captures both the proportion of FRα positive cells and staining intensity. M-scores for each patient were averaged over multiple tissue microarray (TMA) samples, where appropriate. If a sample was void of results, *i. e.* no tumor present or necrotic tissue, the M-score was assigned to the non-void determinations.

[0156] A practical application of the above equation is presented below:

3+	2+	1+	0	M Score
x = 40	y = 30	z = 10		M = (3x + 2y +z)/6
3 x 40 = 120	2 x 30 = 60	1x10=10		(120+60+10)/6=31.67

Here, x = % of tumor stained with intensity 3+; y = % of tumor stained with intensity 2+; z = % of tumor stained with intensity 1+.

[0157] The positivity rate for FRα expression within a given histology was calculated as the proportion of samples that were stained positive according to the definition of a positive result (≥ 5% of the total tumor cells staining). Exact binomial confidence intervals were determined using established methods (Clopper C.J. and Pearson R.C., Biometrika. 26:404-13 (1934)). Summary statistics are presented herein for all demographic variables and for the M-score. Differences for mean values were determined using one-way ANOVA with *post-hoc* tests controlling for overall type I error. Differences in mean values were statistically different if the p-value associated with the test was less than the Bonferroni adjusted type I error for that test (maximum Type I error=0.05).

EXAMPLE 12: Comparative Staining of Lung Carcinoma Cells with Antibody 26B3 and Antibody BN3.2

[0158] There is significant variation in the literature with respect to the percent of various carcinomas that express FRα as determined by IHC, in part due to the use of a variety of antibodies, most of which are not commercially available. One FRα specific MAb that is commercially available and has been demonstrated to detect FRα on FFPE sections by IHC is antibody BN3.2 (Leica Microsystems, Buffalo Grove, IL). Therefore studies were conducted to compare antibody BN3.2 to antibody 26B3 for both specificity and sensitivity for the detection of FRα using a commercial TMA containing various histological types of lung cancer. Both antibodies were highly specific for adenocarcinoma as compared with other histologic subtypes, particularly squamous cell carcinoma. However, antibody 26B3 was significantly more sensitive than BN3.2, identifying 26/36 (72%; M-score mean ± SD = 19.84 ± 18.64) and 22/36 (61%; M-score mean ± SD = 11.38 ± 14.25) adenocarcinoma samples, respectively ($p<0.0001$). These data demonstrate that antibody BN3.2 is significantly less sensitive than antibody 26B3 for detecting FRα expression on FFPE tissue samples and, as shown in Figure 6, the relationship in observed M-scores on lung adenocarcinoma samples for these two antibodies is non-linear.

EXAMPLE 13: Detection of FRα in Subjects known to have Adenocarcinoma of the Lung

[0159] Experiments were conducted to determine whether the presence of FRα positive histology, as detected by antibody 26B3, was associated with particular forms of lung cancer. A tissue microarray having duplicate samples of normal and cancerous, stage I, stage II, stage III, and stage IV, lung tissue specimens was assessed for FRα expression via IHC staining using antibody 26B3, as described in Example 7. As can be seen from the data in Table 6, FRα is associated with adenocarcinomas relative to squamous cell carcinomas, which exhibited limited positive staining.

Table 7: Histological evaluation of cancerous tissue samples

Membrane Staining			Membrane Positive		Total
			Negative	Positive	
Histology	Adenocarcinoma	Count	11	27	38
		% within Histology Groups	28.9%	71.1%	100.0%
	Squamous	Count	28	3	31
		% within Histology Groups	90.3%	9.7%	100.0%

Membrane Staining			Membrane Positive		Total
			Negative	Positive	
Groups	Other Carcinomas	Count	17	4	21
		% within Histology Groups	81.0%	19.0%	100.0%
	Normal	Count	2	8	10
		% within Histology Groups	20.0%	80.0%	100.0%
Total		Count	58	42	100
		% within Histology Groups	58.0%	42.0%	100.0%

[0160] Further analyses were performed on 89 of the histological samples in the tissue microarray where 36 (40%) were adenocarcinoma, 32 (36%) were squamous cell carcinoma, 2 (2%) were adenosquamous carcinomas, and the remaining 19 (21%) represented a variety of histologies (Table 8). The overall rates of FRα positivity varied substantially for each of the histologic subtypes. A significantly higher proportion of adenocarcinoma tumors were positive for FRα when compared to squamous cell carcinomas (72% versus 13%, $p<0.0001$). Of the 4 positive squamous cell carcinoma samples, only 1 showed 3+ staining on both samples; 1 had intermediate (2+) staining on both samples and the other 2 were very weakly positive in a single sample (5-10% of tumor cells at 1+). Furthermore, the two adenosquamous carcinoma samples were also shown to be positive for FRα, with staining restricted to the adenocarcinoma portion of these samples (Figure 7).

Table 8: Distribution of FRα Expression Across NSCLC Type#

Variable	***FRα negative N (%)***	***FRα positive N (%)***	***Total***	***P value****
Tumor Histology				
Normal	1 (10%)	9 (90%)	10	
Squamous cell carcinoma	28 (87%)	4 (14%)	32	<0.0001
Large cell carcinoma	3 (60%)	2 (40%)	5	
Small cell carcinoma	7 (87%)	1 (13%)	8	
Neuroendocrine carcinoma	4 (67%)	2 (33%)	6	
Adenocarcinoma**	10 (16%)	28 (74%)	38	
Tumor Grade				
Grade 1	1 (20%)	4 (80%)	5	
Grade 2	5 (22%)	18 (78%)	23	

Variable	*FRα negative N (%)*	*FRα positive N (%)*	*Total*	*P value**
Grade 3	4 (40%)	6 (60%)	10	0.517
Tumor Stage				
Stage I	4 (29%)	11 (71%)	15	
Stage II	2 (17%)	10 (83%)	12	
Stage III + IV***	4 (36)	7 (64)	11	0.563
Gender				
Female	3 (18%)	14 (82%)	17	
Male	7 (33%)	14 (67%)	21	0.46

#US Biomax Lung Cancer TMA (catalog # BC041114; 90 cases, duplicate cores)
* P values determined using Fisher's exact test or chi-square test: squamous cell carcinoma *versus* adenocarcinoma p<0.0001; males *versus* female, p=0.46; stage, p=0.563; grade, p=0.517
** Includes 2 adenosquamous cases, both positive for FRα in the adenocarcinoma portion only
*** Only 1 stage IV case

[0161] M-score analyses of duplicate adenocarcinoma histology samples showed little variation in staining by antibody 26B3 (Figure 8), a reflection of the robustness of antibody 26B3 staining. Also, an examination of M-scores by stage and grade within the adenocarcinoma histologic subtype indicated that neither stage nor grade of disease was associated with the degree of staining as defined by the M-scores (data not shown).

[0162] The M-score distribution for FRα staining of lung adenocarcinoma and squamous cell carcinoma samples is shown in Figure 9. The mean (± SD) M-scores for adenocarcinoma and squamous cell carcinoma samples stained with antibody 26B3 were 19.84 (± 18.64) and 1.39 (± 5.54), respectively (p<0.0001). The M-score for adenocarcinoma was also significantly higher when compared against all other lung cancer histologic types. In addition, a Tree Analysis was performed to determine the odds for the histology of the cancer being adenocarcinoma. An M-score >21.7 resulted in an odds ratio (OR) of 16, further demonstrating that FRα is predominately expressed in the adenocarcinoma histology (analysis not shown).

[0163] Formalin-fixed, paraffin-embedded (FFPE) tissue blocks are rarely available from patients diagnosed with late stage lung cancer, as surgical resections are not typically performed. Therefore, studies were performed to determine the suitability of fine needle aspirate (FNA) specimens for FRα IHC using MAb 26B3, as late stage lung cancer is most frequently diagnosed via small biopsy or cytology material. For these studies, samples were obtained from nine late-stage adenocarcinoma patients diagnosed by cytological evaluation of a thoracic lymph node aspirate (Figure 10) and demonstrated that the rate of FRα positivity (63%) was comparable to that seen for the histological specimens assessed on the lung cancer TMA. Although only a small sample size, these data suggest that cytologic specimens

may be a suitable tissue source for determining FRα expression in late stage adenocarcinoma patients.

EXAMPLE 14 - FRα is Expressed by CK+/CD45- Cells, but not CK-/CD45+ Cells, Isolated from the Blood of Patients known to have Non-Small Cell Lung Carcinoma

[0164] Studies were conducted to determine the expression profile of FRα on circulating tumor cells (CTCs) of Patients known to have Non-Small Cell Lung Carcinoma. For these studies, blood samples were obtained from 15 healthy donors and 5 stage IV lung cancer patients and then enriched for CTCs using ApoCell's ApoSteam™ system. After enrichment, each sample was stained for cytokeratin (CK), CD45 (protein tyrosine phosphatase receptor type C), nuclei, and FRα. FRα staining was performed using antibody 26B3 as the primary antibody, which was then detected using a mouse-specific, secondary antibody conjugated to DyLight® 649. As shown in Table 9, FRα expression was observed by CK+/CD45- CTCs, but not CK-/CD45+ CTCs.

Table 9 - Expression of FRα by circulating tumor cells of patients known to have non-small cell lung carcinoma

Patient ID	CK-/CD45+ count	CK-/CD45+/FRa+ %	FRα MFI (CK-/CD45+)
Patient 1	2,270	0.0	NA
Patient 2	24,462	0.0	NA
Patient 3	26,503	0.0	NA
Patent 1	16,540	0.0	NA
Patient 5	2,652	0.0	NA
Patient ID	**CK+/CD45- cell count in 7.5 mL of blood**	**CK+/CD45-/FRα+ %**	**FRα MFI(CK+/CD45-)**
Patent 1	55	15.6	82,195
Patent 2	105	32.8	172,669
Patent 3	216	9.3	146,521
Patient 4	57	15.7	179,027
Patient 5	47	8.1	277,335

EXAMPLE 15: 5-year Survivorship of Subjects with and without FRα-expressing Adenocarcinoma of the Lung

[0165] Experiments were conducted to determine whether the presence of FRα positive

histology, as detected by antibody 26B3, could be associated with either improved or diminished 5-year survivorship. Normal and cancerous, stage I or stage II adenocarcinoma, lung tissue specimens were subjected to IHC staining, as described in Example 7, and then read. The percentage of 3+, 2+, 1+ and 0 intensity of the stain on the tumor was recorded. There were 177 slides that were interpretable as duplicate or triplicate of a patient. When combined with the evaluable clinical and histological data 53 evaluable cases were identified. The analyses were performed in view of data relating to demographic, clinical and survival status at 5 years past diagnosis of non-small cell adenocarcinoma of the lung.

[0166] To determine an optimal cut-point for the M-score a receiver operating characteristic (ROC) analysis was performed. Diagnostic accuracy was of no importance in this analysis; however, the ratio of the diagnostic likelihood ratio of the positive test to the diagnostic likelihood ratio of the negative test was important. These ratios are defined as described in Pepe MS, The statistical evaluation of medical tests for classification and prediction, New York: Oxford University Press (2003). At a cut-point of 10 the odds ratio achieved a maximum of 6.62. This value of M was chosen to determine the positivity of a stained slide.

[0167] Kaplan-Maier survival functions were produced with FRα association as the prognostic factor. A log rank test indicated that being positive for FRα was beneficial for non-fatal events (Chi-sq=7.34, df=1 p=0.007). Figure 11 illustrates the survival functions for stage I and stage II adenocarcinoma groups deemed to be FRα positive and FRα negative by 26B3 detection. At 5 years the hazard ratio is 2.42. This indicates that subjects having tumors that are negative (M<10) for FRα are 2.5 times more likely to die within five years of diagnosis than subjects with FRα-positive tumors (M≥10).

EXAMPLE 16: Folate Receptor Alpha Expression is Associated with Triple-Negative Forms of Breast Cancer

[0168] Studies were conducted to assess the expression of FRα by breast cancer tissue samples. Analyses were conducted using tissue microarray (TMA) samples stained with antibody 26B3 as described in Example 7 and FFPE histology samples prepared and stained with antibody 26B3 as described in Example 7.

[0169] The distribution of histologies present on the breast cancer TMA (U.S. BioMAX catalog # BR1503a; 72 cases, duplicate cores) are shown in Table 10, the majority of the cases represented being identified as invasive ductal carcinoma (IDC). The TMA included 2 normal breast samples, which were positive for FRα expression as determined by MAb 26B3. Staining in normal breast was restricted to ductal cells with luminal and membrane staining. Two of three fibroadenoma cases (67%), 0/2 cystosarcoma cases (0%) and 1/6 ductal carcinoma in situ cases (17%) were positive for FRα. The single invasive lobular carcinoma (ILC) was negative for FRα staining. Of the 59 IDC samples 18 (31%) were positive for FRα (Figure 12). Given the small number of positive cases on this TMA a valid analysis of FRα expression relative to stage or grade was not possible; however, it should be noted that the majority of

samples were either T1 or T2. FRα expression was shown to associate with ER/PR negative tumors relative to ER/PR positive tumors (p=0.012) and with triple negative breast cancers (TNBC) (ER/PR+ or Her2+ versus ER/PR/Her2-, p<0.0001).

[0170] Of the 18 FRα positive IDC cases, only 2 (11 %) were Her2 positive meaning that the vast majority (89%) were Her2 negative. These data suggest that FRα positivity tracks more closely with Her2 negativity. Further, of the 18 FRα positive IDC cases, 3 were estrogen receptor (ER) positive and 4 were progesterone receptor (PR) positive, but all ER/PR positive/FRα positive cases were Her2 negative. Of the FRα positive IDC cases 12/18 (67%) were triple negative breast cancers (TNBC), suggesting that FRα may be a marker and target for very poor prognosis TNBC molecular subtype. Looking at the TMA as a whole, only 2/13 (15%) of all Her2 positive cases were also positive for FRα while 16/46 (35%) of the Her2 negative cases were also FRα positive, supporting the suggestion that FRα expression correlates negatively with Her2 expression. A representation of the distribution of M-scores for this TMA relative to molecular subtype (her-2 (+) and her-2 (-)) is shown in Figure 13.

[0171] The TMA described above was composed primarily of early stage breast cancers: stage I, 6/60 (10%); stage II, 44/60 (73%); stage III, 10/60 (17%). Therefore, to confirm and extend the results obtained on the TMA, 61 FFPE tissue blocks from stage IV(T4) Her2 negative breast cancers with known ER/PR expression ranging from 0-100% positive were assessed (FFPE tissue blocks were obtained from the archives of Genzyme Genetics). All 61 of these samples were from metastases, not the primary tumor. The results of this study are summarized in Table 11.

Table 10: Distribution of FRα positivity across histology types – TMA data

Tumor Histology	*FRα positive* *N (%)*	*FRα negative* *N (%)*	*Total*	*P value**
Normal	2 (100%)	0 (0%)	2	
Fibroadenoma	2 (67%)	1 (33%)	3	
Cystosarcoma	0 (0%)	2 (100%)	2	
DCIS – Ductal carcinoma in situ	1 (17%)	5 (83%)	6	
ILC – Invasive lobular carcinoma	0 (0%)	1 (100%)	1	
IDC – Invasive ductal carcinoma	18 (31%)	41 (69%)	59	
Total carcinomas:	**21 (30%)**	**50 (70%)**	**71**	
IDC Molecular subtype analysis:				
ER/PR+	4 (14%)	24 (86%)	28	
ER/PR-	14 (45%)	17 (55%)	31	**0.012**
Her2+	2 (15%)	11 (85%)	13	
Her2-	16 (35%)	30 (65%)	46	0.307
ER/PR/Her2-	12 (67%)	6 (33%)	18	**<0.0001** (ER/PR+ or Her2+ *versus* ER/PR/Her2-)
T1	3 (43%)	4 (57%)	7	
T2	10 (26%)	29 (74%)	39	
T3	5 (63%)	3 (37%)	8	

T4	0 (0%)	5 (100%)	5	
N0	18 (35%)	33 (65%)	51	
N1/N2**	0 (0%)	8 (100%)	8	0.092
Grade 1	1 (14%)	6 (86%)	7	
Grade 2	12 (36%)	21 (64%)	33	0.393
Grade 3	5 (26%)	14 (74%)	19	0.6465

* P values calculated via 2X2 contingency table analysis using Fisher's exact test.
** 4/8 (50%) of N1/N2 samples were Her2+

[0172] FRα expression (Figure 14) was found in 22/61 (36%) of these patients, demonstrating that the percent of FRα positive specimens/tumors determined in early stage disease is retained in late stage metastatic disease in a Her2 negative population (TMA positivity = 35%; stage IV metastatic disease = 36%). Of the 22 FRα positive stage IV metastatic patients, only 3 (14%) showed any positivity for ER/PR with such positivity trending in the low range (up to 30%). As such, 19/22 (86%) FRα positive patients were of the triple negative molecular subtype. Again, these data compare favorably with the data obtained in early stage disease on the TMA where 67% of all FRα positive patients were of the triple negative subtype.

Table 11: Distribution of FRα positivity in molecular subtypes of metastatic breast cancer samples

	FRα positive	*FRα negative*		
Tumor Molecular subtype	*N (%)*	*N (%)*	*Total*	*P value**
Total Samples:	**22 (36%)**	**39 (64%)**	**61**	
ER/PR+	3 (14%)	20 (86%)	23	
ER/PR/Her2-	19 (50%)	19 (50%)	38	0.0054 (ER/PR+ *versus* ER/PR/Her2-)
Grade 1	3 (30%)	7 (70%)	10	
Grade 2	11 (28%)	28 (72%)	39	1.0 (Grade 1 *versus* Grade 2)
Grade 3	8 (67%)	4 (33%)	12	0.037 (Grade 1 or 2 *versus* Grade 3)
* P values calculated via 2X2 contingency table analysis using Fisher's exact test.				

[0173] Additionally, samples from stage IV metastatic disease were obtained from a number of metastatic sites including lymph node, bone, skin and liver as well as fluid and fine needle aspirate (FNA) samples obtained primarily from pleura and paracentesis. Several of these 'fluid biopsies' stained positive for FRα (Figure 15) suggesting the general applicability of the described IHC methodology to multiple samples types.

EXAMPLE 17: Evaluation of Histological Gynecologic Cancer Samples for Expression of

Folate Receptor Alpha

[0174] Immunohistochemical studies were conducted to evaluate FRα expression in gynecologic malignancies involving ovary, endometrium and fallopian tube. Analyses were conducted using tissue microarray (TMA) samples stained with antibody 26B3 as described in Example 7 and FFPE histology samples prepared and stained with antibody 26B3 as described in Example 7. Commercial tissue microarrays were obtained from US Biomax, Inc. (Rockville, MD) for ovarian carcinomas (catalog # OV1921; 96 cases, duplicate cores); endometrial carcinomas (catalog # EMC1021; 102 cases, single cores); and fallopian tube carcinomas (catalog # UTE601; 30 cases, duplicate cores).

[0175] A sample was considered positive for FRα expression if the percentage of the tumor cells positive for membranous staining was greater than or equal to 5% at any intensity. A sample was rejected and therefore not included in the analyses if the gynecologic pathologist determined it was either missing entirely or was composed of necrotic tissue with an insufficient number of viable cells for evaluation. Of the endometrial samples, six contained only atypical complex hyperplasia without adenocarcinoma. Histologic classification of cell type and grade were based the WHO Classification of Breast and Female Genital Organs (Tavassoli and Devilee). Clinical stage based on FIGO and TNM system was provided by the manufacturer of the TMA (US Biomax).

[0176] The positivity rate for FRα expression within a given tumor type was calculated as the proportion of tumors that were stained positive according to the definition of a positive result (± 5% tumor cell membrane staining). Differences in FRα positivity between groups, e.g. histologies, stage or grade, were assessed using 2x2 contingency tables and Fisher's exact test. Differences in mean values were statistically different if the p-value associated with the test was less than the Bonferroni adjusted type I error for that test (maximum Type I error=0.05).

[0177] Membrane and cytoplasmic staining intensity was scored as 0, no staining; 1+, weak; 2+, moderate and 3+, strong. The percent of cells for each intensity in the sample was also determined. Tissue was analyzed under 4x, 10x, 20x and 40x objectives. Strong membrane staining (3+) was readily visualized under 4x and confirmed at 10x. Moderate membrane staining (2+) was visible at 10x and confirmed at 20x. Weak staining (1+) required 20x or 40x (Figure 16). In the presence of 3+ staining, the membrane was thick occurring at apical and lateral cell borders. In tangential sections, a complete circumferential pattern was evident (Figure 16 (A) and (B)). 2+ membrane staining was weaker in intensity and thinner than 3+, usually localized on the apical luminal borders and occasionally on lateral cell borders. 1+ weak membrane was generally limited to the luminal borders. The accompanying cytoplasmic staining was variable, depending on the type of tumors.

[0178] Of the 94 evaluable samples on the ovarian tumor TMA, 70 (74%) were of the serous

type, 10 (11%) were mucinous, 4 (4%) endometrioid, 3 (3%) clear cell type and the remaining 7 (8%) were miscellaneous rare tumors. Of the 87 samples of ovarian carcinomas, the FRα positive rate for each cell type was as follows: 100% (70/70) in serous type, 80% (8/10) mucinous type, 75% (3/4) endometrioid type, and 67% (2/3) clear cell type. The difference between serous and mucinous type is significant with p value at 0.014 by Fisher's exact test (Table 12). FRα status was not significant for histologic grade or clinical stage. Co-existing cytoplasmic staining was usually 2+ or 3+ in serous type and weaker and less frequent in other tumor types.

Table 12: Distribution of FRα positivity in relation to histology type, clinical stage and histologic grade in ovarian carcinomas

*Tumor Histology**	*FRα negative N (%)*	*FRα positive N (%)*	*Total*	*P value***
Serous carcinoma	0 (0%)	70 (100%)	70	
Mucinous carcinoma	2 (20%)	8 (80%)	10	0.014
Endometrioid carcinoma	1 (25%)	3 (75%)	4	
Clear cell carcinoma	1 (33%)	2 (67%)	3	
Total	4 (5%)	83 (95%)	87	
Stage II	4 (9%)	41(91%)	45	
Stage III	0 (0%)	29 (100%)	29	0.15
Stage IV	0 (0%)	13 (100%)	13	
Grade 1	2 (15%)	11 (85%)	13	NS***
Grade 2	1 (3%)	31 (97%)	32	NS
Grade 3	1 (3%)	39 (97%)	40	NS

* 1 transitional cell carcinoma, 1 squamous carcinoma, 1 Embryonal carcinoma, 2 yolk sac tumors and 2 granulosa cell tumors not included in analysis
** P values determined using Fisher's exact test or chi-square test: serous carcinoma *versus* mucinous carcinoma p=0.014
*** NS=Not Significant

[0179] In the endometrial samples, FRα was expressed in 80% (4/5) of normal (Figure 17(A)), 100% (6/6) of atypical complex hyperplasia (Figure 17(B)), and 89% (80/90) of adenocarcinomas, including 88 endometrioid type and 1 clear cell type (Figures 18 and 19). Eight endometrioid adenocarcinomas contained areas of squamous metaplasia. In the normal endometrium, membrane staining was weak and limited to the apical luminal borders (Figure 17(A)). In atypical complex hyperplasia and carcinomas, staining was predominantly luminal

with additional staining on lateral cell borders in some cases (Figure 17(B)). In the presence of 3+ membrane staining, cytoplasmic staining varied in intensity from strong (Figure 18(A)) to weak (Figures 18(B) and (C)). Tumor cells with 1+ or 2+ membrane staining rarely expressed cytoplasmic staining. The majority of metaplastic squamous cells and clear cells exhibited moderate to strong membrane staining. (Figures 19(A) and (B)).

[0180] FRα expression was positive in 100% of grade 1, 96% of grade 2 and 74% of grade 3 tumors (grade 1 vs. grade 3, p value=0.0029; grade 2 vs. grade 3, p=0.034). FRα status was not significant in relation to T1 vs. T2/3; N0 vs. N1, and stage I vs. stage II/III.

[0181] Seventeen cores of normal fallopian tubes, 16 samples of chronic salpingitis and 20 tubal serous carcinomas were all strongly positive for membrane and cytoplasmic staining (Figures 20 (A) to (C)).

EXAMPLE 18: Evaluation of Histological Colorectal Samples for Expression of Folate Receptor Alpha

[0182] Immunohistochemical studies were conducted to evaluate FRα expression in colorectal tissue samples. Analyses were conducted using tissue microarray (TMA) samples obtained from US Biomax (catalog # BC051111). The TMA contained 90 duplicate samples of tissues obtained from subjects known to have colorectal cancer and 10 normal colorectal samples. The samples were stained with antibody 26B3 as described in Example 7. Of the 90 samples obtained from subjects known to have colorectal cancer, 18 (20%) were positive for FRα expression, while none of the normal samples were positive. In addition, positive stating was generally medium to weak and no apparent relationship to stage of disease was discernible.

EXAMPLE 19: Evaluation of Histological Thryoid Samples for Expression of Folate Receptor Alpha

[0183] Immunohistochemical studies were conducted to evaluate FRα expression in thyroid tissue samples. Analyses were conducted using tissue microarray (TMA) samples obtained from US Biomax (catalog # TH802a). The samples were stained with antibody 26B3 as described in Example 7. Thyroid papillary carcinoma was strongly positive for FRα membrane expression (26/28, 93%) and was distinguishable from medullary carcinoma, where all 5 samples were negative for FRα staining, in agreement with previous reports. Interestingly, follicular adenomas were separable into macrofollicular type and microfollicular type with 3/13 (23%) and 18/22 (82%) showing positivity for FRα expression, respectively. Some positivity was also seen in the small number of Hurthle cell tumors (2/3, 67%) and follicular carcinoma (3/7, 43%) samples on this TMA. These results are summarized in Table 13.

Table 13 - Expression of FRα in thyroid tissue samples

Thyroid Cancer Hisotologic Subtype (N = 78)	FRα positive N (%)	FRα negative N (%)
Papillary carcinoma, 28 (36%)	26 (93%)	2 (7%)
Medullary carcinoma, 5 (6%)	0 (0%)	5 (100%)
Follicular Adenoma, macrofollicular type, 13 (17%)	3 (23%)	10 (77%)
Follicular Adenoma, microfollicular type, 22 (28%)	18 (82%)	4 (18%)
Hurthle cell tumor, 3 (4%)	2 (67%)	1 (33%)
Follicular carcinoma, 7 (9%)	3 (43%)	4 (57%)

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                85                  90                  95

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    50                  55                  60

Tyr Ser Leu Thr Ile Ser Ser Val Glu Ala Glu Asp Ala Ala Thr Tyr
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Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala
        35                  40                  45

Ser Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser
    50                  55                  60

Arg Thr Asp Phe Thr Leu Thr Ile Asp Pro Val Glu Ala Asp Asp Ala
65                  70                  75                  80
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        35                  40                  45

Gly Asn Thr Lys Tyr Asp Pro Lys Phe Gln Gly Lys Ala Thr Ile Thr
    50                  55                  60

Ala Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr
65                  70                  75                  80

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Pro Ala Ser Leu Ser Ala Ser Val Gly Glu Thr Val Thr Ile Thr Cys
1 5 10 15

Arg Thr Ser Glu Asn Ile Phe Ser Tyr Leu Ala Trp Tyr Gln Gln Lys
20 25 30

Gln Gly Ile Ser Pro Gln Leu Leu Val Tyr Asn Ala Lys Thr Leu Ala
35 40 45

Glu Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Phe
50 55 60

Ser Leu Lys Ile Asn Ser Leu Gln Pro Glu Asp Phe Gly Ser Tyr Tyr
65 70 75 80

Cys Gln His His Tyr Ala Phe Pro Trp Thr Phe Gly Gly Gly Ser Lys
85 90 95

Leu Glu Ile Lys Arg Ala Asp Ala Ala Pro
100 105

<210> 30
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 30
Gly Tyr Phe Met Asn
1 5

<210> 31
<211> 17

<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 31
Arg Ile Phe Pro Tyr Asn Gly Asp Thr Phe Tyr Asn Gln Lys Phe Lys
1 5 10 15

Gly

<210> 32
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 32
Gly Thr His Tyr Phe Asp Tyr
1 5

<210> 33
<211> 128
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 33
Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys
1 5 10 15

Ala Ser Asp Tyr Ser Phe Thr Gly Tyr Phe Met Asn Trp Val Met Gln
20 25 30

Ser His Gly Lys Ser Leu Glu Trp Ile Gly Arg Ile Phe Pro Tyr Asn

35 40 45

Gly Asp Thr Phe Tyr Asn Gln Lys Phe Lys Gly Arg Ala Thr Leu Thr
50 55 60

Val Asp Lys Ser Ser Ser Thr Ala His Met Glu Leu Arg Ser Leu Ala
65 70 75 80

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Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Gly Thr His Tyr Phe
                85                  90                  95

Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr
            100                 105                 110

Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr
        115                 120                 125
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<210> 34
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 34
agggccagct caactgtaag ttacagttac ttgcac 36

<210> 35
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 35
ggcacatcca acttggcttc t 21

<210> 36
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 36
cagcagtaca gtggttaccc actcacg 27

<210> 37
<211> 323
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 37
ccagcaatca tgtctgcatc tccaggggaa aaggtcacca tgacctgcag ggccagctca 60

actgtaagtt acagttactt gcactggtac cagcagaagt caggtgcctc cccccaactc 120

tggatttatg gcacatccaa cttggcttct ggagtccctg ctcgcttcag tggcagtggg 180

tctgggacct cttactctct cacaatcagc agtgtggagg ctgaagatgc tgccacttat 240

tactgccagc agtacagtgg ttacccactc acgttcggtg ctgggaccaa gctggagctg 300

aaacgggctg atgctgcacc aac 323

<210> 38
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 38
agtggttatt actggaac 18

<210> 39
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 39
tacataaagt ccgacggtag caataattac aacccatctc tcaaaaat 48

<210> 40
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 40

gagtggaagg ctatggacta c 21

<210> 41
<211> 389
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 41
gagtcaggac ctggcctcgt gagaccttct cagtctctgt ctctcacctg ctctgtcact 60

ggctactcca tcaccagtgg ttattactgg aactggatcc ggcagtttcc aggaagcaga 120

ctggaatgga tgggctacat aaagtccgac ggtagcaata attacaaccc atctctcaaa 180

aatcgaatct ccatcactcg tgacacatct aagaaccagt ttttcctgaa gttgaattct 240

gtgactactg aggacacagc tacatatttc tgtacaaggg agtggaaggc tatggactac 300

tggggtcagg gaacctcagt caccgtctcc tcagccaaaa caacaccccc atcagtctat 360

ccactggccc ctgggtgtgg agatacaac 389

<210> 42
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 42
agagccagtg aaagtgttga tacttatggc aataatttta tacac 45

<210> 43
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 43
cttgcatcca acctagaatc t 21

<210> 44
<211> 27

<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 44
cagcaaaata atggggatcc gtggacg 27

<210> 45
<211> 331
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 45
ccagcttctt tggctgtgtc tctagggcag agggccacca tatcctgcag agccagtgaa 60

agtgttgata cttatggcaa taattttata cactggtacc agcagaaacc aggacagcca 120

cccaaactcc tcatttatct tgcatccaac ctagaatctg gggtccctgc caggttcagt 180

ggcagtgggt ctaggacaga cttcaccctc accattgatc ctgtggaggc tgatgatgct 240

gcaacctatt actgtcagca aaataatggg gatccgtgga cgttcggtgg aggcaccaag 300

ctggagatca aacgggctga tgctgcacca a 331

<210> 46
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 46
caccctata tgcac 15

<210> 47
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 47
aggattgatc ctgcgaatgg taatactaaa tatgacccga agttccaggg c 51

<210> 48
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 48
gaggaggtgg cggactatac tatggactac 30

<210> 49
<211> 380
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 49
ggggcagagc ttgtgaagcc aggggcctca gtcaagttgt cctgcacagc ttctggcttc 60

aacattaaac acccctatat gcactgggtg aagcagaggc ctgaccaggg cctggagtgg 120

attggaagga ttgatcctgc gaatggtaat actaaatatg acccgaagtt ccagggcaag 180

gccactataa cagcagacac atcctccaac acagcctacc tacagctcag cagcctgaca 240

tctgaggaca ctgccgtcta ttactgtggt agagaggagg tggcggacta tactatggac 300

tactggggtc aaggaacctc agtcaccgtc tcctcagcca aaacaacagc cccatcggtc 360

tatccactgg cccctgtgtg 380

<210> 50
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 50
agtgcaagtc agggcattaa caatttttta aac 33

<210> 51

<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 51
tacacatcaa gtttacactc a 21

<210> 52
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 52
cagcacttta gtaagcttcc gtggacg 27

<210> 53
<211> 320
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 53
acatcctccc tgtctgcctc tctgggagac agagtcacca tcagttgcag tgcaagtcag 60

ggcattaaca atttttaaa ctggtatcag cagaaaccag atggcactgt taaactcctg 120

atctattaca catcaagttt acactcagga gtcccatcaa ggttcagtgg cagtgggtct 180

gggacagatt attctctcac catcagcaac ctggaacctg aagatattgc catatactat 240

tgtcagcact ttagtaagct tccgtggacg ttcggtggag gcaccaagct ggaaatcaaa 300

cgggctgatg ctgcaccaac 320

<210> 54
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 54
agctatgcca tgtct 15

<210> 55
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 55
gaaattggta gtggtggtag ttacacctac tatccagaca ctgtgacggg c 51

<210> 56
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 56
gaaactacgg cgggctactt tgactac 27

<210> 57
<211> 336
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 57
tctgggggag gcttagtgag gcctggaggg tccctgaaac tctcctgtgc agcctctgga 60

ttcactttca gtagctatgc catgtcttgg gttcgccagt ctccagagaa gaggctggag 120

tgggtcgcag aaattggtag tggtggtagt tacacctact atccagacac tgtgacgggc 180

cgattcacca tctccagaga caatgccaag agcaccctgt acctggaaat gagcagtctg 240

aggtctgagg acacggccat ctattactgt gcaagggaaa ctacggcggg ctactttgac 300

tactggggcc aaggcaccac tctcacagtc tcctca 336

<210> 58

<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 58
cgaacaagtg agaatatttt cagttattta gca 33

<210> 59
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 59
aatgcaaaaa ccttagcaga g 21

<210> 60
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 60
caacatcatt atgcttttcc gtggacg 27

<210> 61
<211> 320
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 61
ccagcctccc tatctgcatc tgtgggagaa actgtcacca tcacatgtcg aacaagtgag 60

aatattttca gttatttagc atggtatcag cagaaacagg gaatatctcc tcagctcctg 120

gtctataatg caaaaacctt agcagagggt gtgccatcaa ggttcagtgg cagtggatca 180

ggcacacagt tttctctgaa gatcaacagc ctgcagcctg aagattttgg gagttattac 240

tgtcaacatc attatgcttt tccgtggacg ttcggtggag gctccaagct ggaaatcaaa 300

cgggctgatg ctgcaccaac 320

<210> 62
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 62
ggctacttta tgaac 15

<210> 63
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 63
cgtatttttc cttacaatgg tgatactttc tacaaccaga agttcaaggg c 51

<210> 64
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 64
gggactcatt actttgacta c 21

<210> 65
<211> 386
<212> DNA
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 65

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ggacctgagc tggtgaagcc tggggcttca gtgaagatat cctgcaaggc ttctgattac      60

tcttttactg gctactttat gaactgggtg atgcagagcc atggaaagag ccttgagtgg     120

attggacgta tttttcctta caatggtgat actttctaca accagaagtt caagggcagg     180

gccacattga ctgtagacaa atcctctagc acagcccaca tggagctccg gagcctggca     240

tctgaggact ctgcagtcta tttttgtgca agagggactc attactttga ctactggggc     300

caaggcacca ctctcactgt ctcctcagcc aaaacgacac ccccatctgt ctatccactg     360

gcccctggat ctgctgccca aactaa                                          386
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<210> 66
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic 6xHis tag"

<400> 66

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His His His His His His
1               5
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REFERENCES CITED IN THE DESCRIPTION

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PATENTKRAV

1. Et isoleret antistof, eller antigenbindende fragment deraf, specifikt for folatreceptoralfa (FRα), der omfatter en let kæde CDR1, der har aminosyresekvensen SEQ ID NO: 26, en let kæde CDR2, der har aminosyresekvensen SEQ ID NO: 27, en let kæde CDR3, der har aminosyresekvensen SEQ ID NO: 28, en tung kæde CDR1, der har aminosyresekvensen SEQ ID NO: 30, en tung kæde CDR2, der har aminosyresekvensen SEQ ID NO: 31, og en tung kæde CDR3, der har aminosyresekvensen SEQ ID NO: 32.

2. Det isolerede antistof eller antigenbindende fragment ifølge krav 1, hvori antistoffet er et murint antistof, et kimerisk antistof eller et humaniseret antistof.

3. Det isolerede antistof eller antigenbindende fragment ifølge krav 1, der har en let kædevariabel region, der omfatter aminosyresekvensen SEQ ID NO: 29 og/eller en tung kædevariabel region, der omfatter aminosyresekvensen SEQ ID NO: 33.

4. Et isoleret polynukleotid, der koder et antistof, eller antigenbindende fragment deraf, specifikt for folatreceptoralfa (FRα), hvori den lette kæde CDR1 af det kodede antistof omfatter aminosyresekvensen SEQ ID NO: 26, den lette kæde CDR2 af det kodede antistof omfatter aminosyresekvensen SEQ ID NO: 27, den lette kæde CDR3 af det kodede antistof omfatter aminosyresekvensen SEQ ID NO: 28, den tunge kæde CDR1 af det kodede antistof omfatter aminosyresekvensen SEQ ID NO: 30, den tunge kæde CDR2 af det kodede antistof omfatter aminosyresekvensen SEQ ID NO: 31, og den tunge kæde CDR3 af det kodede antistof omfatter aminosyresekvensen SEQ ID NO: 32.

5. Polynukleotidet ifølge krav 4, der omfatter nukleotidsekvenserne SEQ ID NO: 61 og 65.

6. Det isolerede polynukleotid ifølge krav 4, der omfatter nukleotidsekvenserne SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 63, og SEQ ID NO: 64.

7. En vektor, der omfatter det isolerede polynukleotid ifølge ethvert af kravene 5 til 6.

8. En rekombinant celle, der omfatter vektoren ifølge krav 7.

9. Den rekombinante celle ifølge krav 8, hvori cellen er en eukaryotisk celle, en plantecelle eller et bakterium.

10. Et isoleret antistof specifikt for folatreceptoralfa (FRα), der produceres af cellerækken, der aflejres med ATCC, der har registreringsnummer PTA-11885.

11. En metode til detektering af folatreceptoralfa (FRα) eller FRα-udtrykkende cancer i en biologisk prøve, der omfatter eksponering af prøven for antistoffet ifølge 1 eller 10, eller det antigenbindende fragment deraf, og detektering af folatreceptoralfa (FRα).

12. Metoden ifølge krav 11, hvor den biologiske prøve er afledt fra et menneske, en gnaver, ikke-human primat, kanin eller hund.

13. En metode til diagnosticering af en folatreceptoralfa-udtrykkende cancer hos en person, der omfatter:

a. eksponering af en biologisk prøve fra personen for antistoffet ifølge krav 1 eller krav 10, eller et antigenbindende fragment deraf.

b. kvantificering af mængden af folatreceptoralfa (FRα), der er til stede i prøven.

c. sammenligning af mængden af folatreceptoralfa (FRα), der er til stede i prøven, med en kendt standard, og

d. bestemmelse af, om personens niveauer af folatreceptoralfa (FRα) falder inden for niveauerne af folatreceptoralfa (FRα), der er forbundet med cancer.

14. Metoden ifølge krav 13, hvor et fund af, at personens adenocarcinomceller udtrykker folatreceptoralfa, angiver, at personen har en større sandsynlighed for en forbedret 5-årig overlevelsesrate, end hvis adenocarcinomcellerne ikke udtrykker folatreceptoralfa.

15. En metode til overvågning af en folatreceptoralfa-udtrykkende cancer hos en person, der omfatter:

a. eksponering af en biologisk prøve fra personen for antistoffet ifølge krav 1 eller krav 10, eller et antigenbindende fragment deraf.

b. kvantificering af mængden af folatreceptoralfa (FRα), der er til stede i prøven, som er bundet af antistoffet eller det antigenbindende fragment deraf.

c. sammenligning af mængden af folatreceptoralfa (FRα), der er til stede i prøven, med enten

i. en kendt standard, eller

ii. en biologisk prøve, der er taget fra personen på et tidligere tidspunkt, og

d. bestemmelse af, om personens niveauer af folatreceptoralfa (FRα) tyder på cancerprogression, -regression eller stabil sygdom.

16. Metoden ifølge ethvert af kravene 11 til 15, hvori den biologiske prøve er afledt af urin, blod, serum, plasma, slim, ascites, cirkulerende celler, cirkulerende tumorceller, celler, der ikke er vævsforbundne, væv, kirurgisk reseceret tumorvæv, biopsier, prøver fra finnålsaspiration eller histologiske præparater.

17. Metoden ifølge krav 13, hvor canceren er brystcancer, thyroideacancer, kolorektal cancer, endometriecancer, tubacancer eller ovariecancer.

18. Metoden ifølge krav 17, hvor canceren er lungecancer.

19. Metoden ifølge krav 18, hvor lungecanceren er adenocarcionom.

20. Metoden ifølge krav 13, hvor den kendte standard omfatter

a. niveauer af folatreceptoralfa (FRα) afledt af personer, der identificeres som værende fri for cancer, eller et præparatfolatreceptoralfa-protein ved en kendt koncentration.

b. FRα-niveauer afledt fra personer, der identificeres som havende folatreceptoralfa-udtrykkende cancer i tidligt stadie.

c. FRα-niveauer afledt fra personer, der identificeres som havende folatreceptoralfa-udtrykkende cancer i mellemstadie, eller

d. FRα-niveauer afledt fra personer, der identificeres som havende folatreceptoralfa-udtrykkende cancer i sent stadie.

21. Metoden ifølge krav 20, hvor canceren er brystcancer, thyroideacancer, kolorektal cancer, endometriecancer, tubacancer, ovariecancer eller lungecancer.

22. Metoden ifølge ethvert af kravene 13 til 21, der desuden omfatter følgende udtalte eksponerende trin, der eksponerer personens biologiske prøve for et andet antistof, eller antigenbindende fragment deraf, udvalgt fra:

i. antistoffet ifølge krav 1.

ii. antistoffet ifølge krav 10.

iii. et isoleret antistof, eller antigenbindende fragment deraf, specifikt for folatreceptoralfa (FRα), der omfatter en let kæde CDR1, der har aminosyresekvensen SEQ ID NO: 10, en let kæde CDR2, der har aminosyresekvensen SEQ ID NO: 11, en let kæde CDR3, der har aminosyresekvensen SEQ ID NO: 12, en tung kæde CDR1, der har aminosyresekvensen SEQ ID NO: 14, en tung kæde CDR2, der har aminosyresekvensen SEQ ID NO: 15, og en tung kæde CDR3, der har aminosyresekvensen SEQ ID NO: 16.

iv. et isoleret antistof specifikt for folatreceptoralfa (FRα), der produceres af cellerækken, der aflejres med ATCC, der har registreringsnummer PTA-11884.

v. et isoleret antistof, eller antigenbindende fragment deraf, specifikt for folatreceptoralfa (FRα), der omfatter en let kæde CDR1, der har aminosyresekvensen SEQ ID NO: 18, en let kæde CDR2, der har aminosyresekvensen SEQ ID NO: 19, en let kæde CDR3, der har aminosyresekvensen SEQ ID NO: 20, en tung kæde CDR1, der har aminosyresekvensen SEQ ID NO: 22, en tung kæde CDR2, der har aminosyresekvensen SEQ ID NO: 23, og en tung kæde CDR3, der har aminosyresekvensen SEQ ID NO: 24.

vi. et isoleret antistof specifikt for folatreceptoralfa (FRα), der produceres af cellerækken, der aflejres med ATCC, der har registreringsnummer PTA-11886.

vii. et isoleret antistof, eller antigenbindende fragment deraf, specifikt for folatreceptoralfa (FRα), der omfatter en let kæde CDR1, der har aminosyresekvensen SEQ ID NO: 2, en let kæde CDR2, der har aminosyresekvensen SEQ ID NO: 3, en let kæde CDR3, der har aminosyresekvensen SEQ ID NO: 4, en tung kæde CDR1, der har aminosyresekvensen SEQ ID NO: 6, en tung kæde CDR2, der har aminosyresekvensen SEQ ID NO: 7, og en tung kæde CDR3, der har aminosyresekvensen SEQ ID NO: 8, og

viii. et isoleret antistof specifikt for folatreceptoralfa (FRα), der produceres af celleraekken, der aflejres med ATCC, der har registreringsnummer PTA-11887, hvor det andet antistof er forskelligt fra det første antistof.

23. Metoden ifølge krav 22, hvor en biologisk prøve fra personen eksponeres for antistoffet ifølge krav 1 eller krav 10, eller et antigenbindende fragment deraf, og derefter eksponeres for:

a. et isoleret antistof, eller antigenbindende fragment deraf, specifikt for folatreceptoralfa (FRα), der omfatter en let kæde CDR1, der har aminosyresekvensen SEQ ID NO: 10, en let kæde CDR2, der har aminosyresekvensen SEQ ID NO: 11, en let kæde CDR3, der har aminosyresekvensen SEQ ID NO: 12, en tung kæde CDR1, der har aminosyresekvensen SEQ ID NO: 14, en tung kæde CDR2, der har aminosyresekvensen SEQ ID NO: 15, og en tung kæde CDR3, der har aminosyresekvensen SEQ ID NO: 16.

b. et isoleret antistof specifikt for folatreceptoralfa (FRα), der produceres af celleraekken, der aflejres med ATCC, der har registreringsnummer PTA-11884.

c. et isoleret antistof, eller antigenbindende fragment deraf, specifikt for folatreceptoralfa (FRα), der omfatter en let kæde CDR1, der har aminosyresekvensen SEQ ID NO: 18, en let kæde CDR2, der har aminosyresekvensen SEQ ID NO: 19, en let kæde CDR3, der har aminosyresekvensen SEQ ID NO: 20, en tung kæde CDR1, der har aminosyresekvensen SEQ ID NO: 22, en tung kæde CDR2, der har aminosyresekvensen SEQ ID NO: 23, og en tung kæde CDR3, der har aminosyresekvensen SEQ ID NO: 24.

d. et isoleret antistof specifikt for folatreceptoralfa (FRα), der produceres af celleraekken, der aflejres med ATCC, der har registreringsnummer PTA-11886.

e. et isoleret antistof, eller antigenbindende fragment deraf, specifikt for folatreceptoralfa (FRα), der omfatter en let kæde CDR1, der har aminosyresekvensen SEQ ID NO: 2, en let kæde CDR2, der har aminosyresekvensen SEQ ID NO: 3, en let kæde CDR3, der har aminosyresekvensen SEQ ID NO: 4, en tung kæde CDR1, der har aminosyresekvensen SEQ ID NO: 6, en tung kæde CDR2, der har aminosyresekvensen SEQ ID NO: 7, og en tung kæde CDR3, der har aminosyresekvensen SEQ ID NO: 8.

f. et isoleret antistof specifikt for folatreceptoralfa (FRα), der produceres af celleraekken, der aflejres med ATCC, der har registreringsnummer PTA-11887.

24. Metoden ifølge ethvert af kravene 15 til 21, hvor metoden udføres efter behandling af personen for cancer.

25. Metoden ifølge ethvert af kravene 11 til 24, hvor antistoffet, eller antistoffragmentet, mærkes.

26. Metoden ifølge krav 25, hvor mærket er et radioaktivt mærke, et fluorescerende mærke, et epitoptag, biotin, et kromofor-mærke, et ECL-mærke eller et enzym.

27. Metoden ifølge ethvert af kravene 11 til 24, hvor den eksponerede folatreceptoralfa (FRα) er eller ikke er bundet til en celle.

28. Metoden ifølge ethvert af kravene 11 til 24, hvor den eksponerede folatreceptoralfa (FRα) i prøven detekteres ved hjælp af Western Blot, immunohistokemi, flowcytometri, radioimmunoassay, immunoprecipitation, elektrokemiluminescensimmunoassay (ECLIA) eller ELISA.

29. Et kit til detektering af tilstedeværelsen af folatreceptoralfa (FRα) i en biologisk prøve, der omfatter mindst ét antistof ifølge krav 1 eller krav 10, eller et antigenbindende fragment deraf.

30. Et kit til detektering af tilstedeværelsen af folatreceptoralfa (FRα) i en biologisk prøve, der omfatter:

mindst ét antistof ifølge krav 1 eller krav 10, eller et antigenbindende fragment deraf.

hvor det inkluderede antistof, eller antigenbindende fragment deraf, er fastgjort til en solid støtte.

31. Et kit til detektering af tilstedeværelsen af folatreceptoralfa (FRα) i en biologisk prøve, der omfatter:

mindst ét antistof ifølge krav 1 eller krav 10, eller et antigenbindende fragment deraf.

hvor det inkluderede antistof, eller antigenbindende fragment deraf, er detekterbart mærket.

32. Metoden ifølge krav 13 eller krav 14, hvor personens niveauer af folatreceptoralfa (FRα) tyder på den type cancer, personen lider af.

33. Metoden ifølge krav 32, hvor canceren er lungeadenocarcinom eller pladecelle-lungecarcinom.

34. Det isolerede antistof ifølge ethvert af kravene 1 til 3 eller 10, hvor antistoffet er detekterbart mærket.

35. Antistoffet ifølge krav 34, hvor det detekterbare mærke er et radioaktivt mærke, et fluorescerende mærke, et epitoptag, biotin, et kromofor-mærke, et ECL-mærke eller et enzym.

36. Antistoffet ifølge krav 35, hvor mærket er ruthenium, ^{111}In-DOTA, ^{111}Indiethylentriaminpentaeddikesyre (DTPA), peberrodsperoxidase, alkalisk phosphatase og beta-galaktosidase eller poly-histidin.

37. Metoden ifølge ethvert af kravene 11 til 21, hvor antistoffet, eller det antigenbindende fragment, er fastgjort til en solid støtte.

38. Metoden ifølge krav 13 eller krav 14, der desuden omfatter forudsigelse af et gunstigt resultat for en person, som har adenocarcinom, der udtrykker folatreceptoralfa (FRα), hvor et gunstigt resultat defineres som havende en forhøjet 5-årig overlevelsesrate.

DRAWINGS

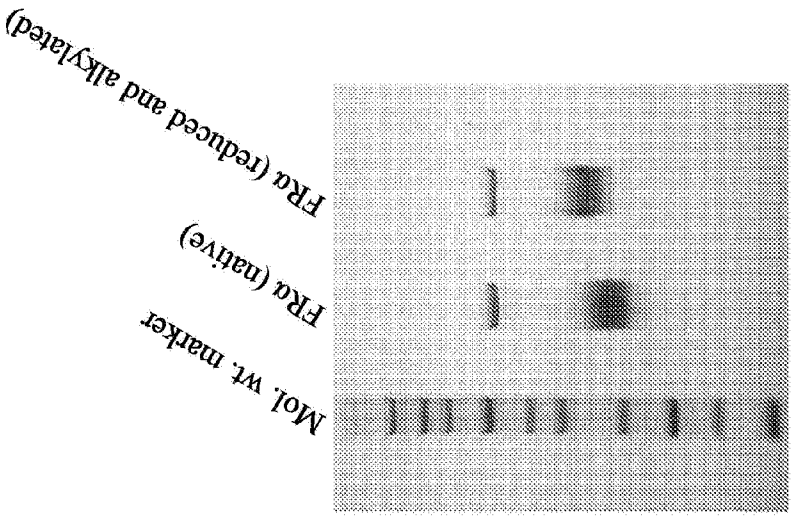
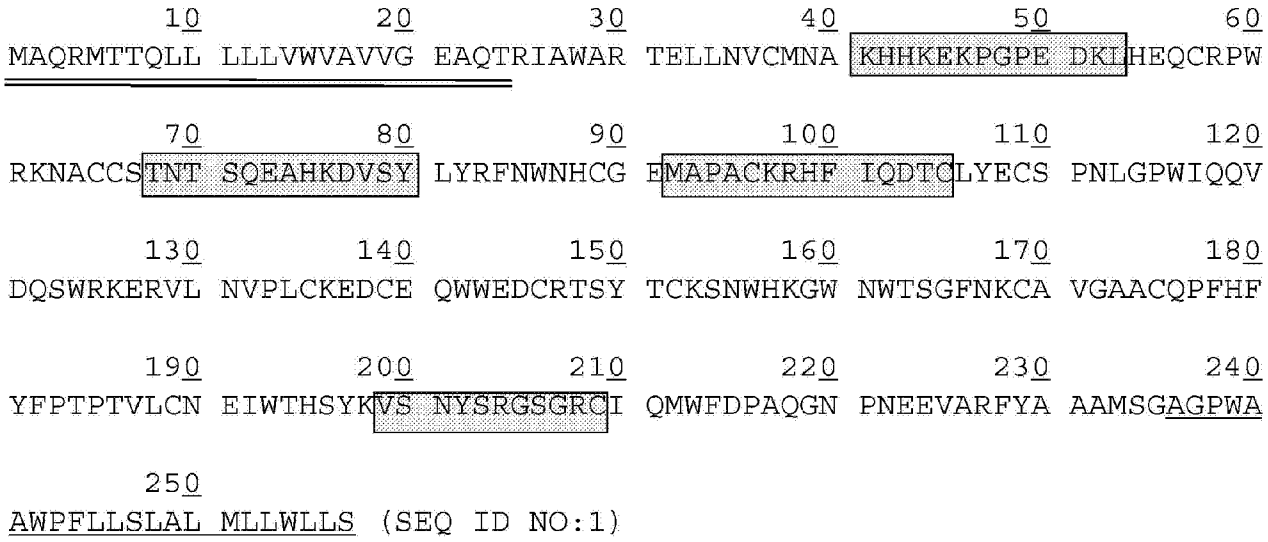


Figure 1



Legend

Residues 1-24 (double underscore) = Signal Sequence (not present in rh-FRα)
Residues 41-53 (shaded) = MAb 9F3 (PTA-11887) epitope
Residues 68-80 and 92-105 (shaded) = MAb 24F12 (PTA-11886) epitope
Residues 199-209 (shaded) = MAb 26B3 (PTA-11885) epitope
Residues 236-257 (underscore) = GPI-anchor Sequence (not present in rh-FRα)

Figure 2

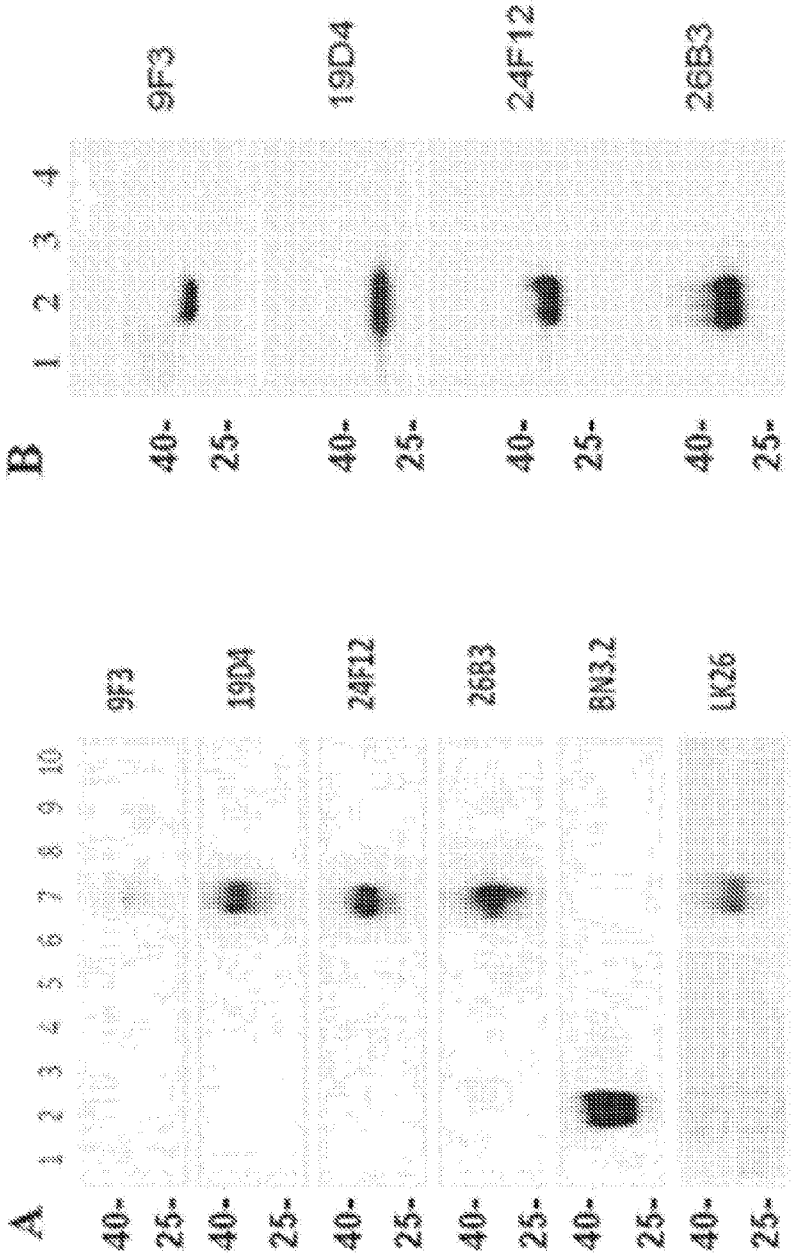

A
1 2 3 4 5 6 7 8 9 10
9F3
19D4
24F12
26B3
BW3.2
LK26
40-
25-
B
1 2 3 4
9F3
19D4
24F12
26B3
40-
25-

Figure 3

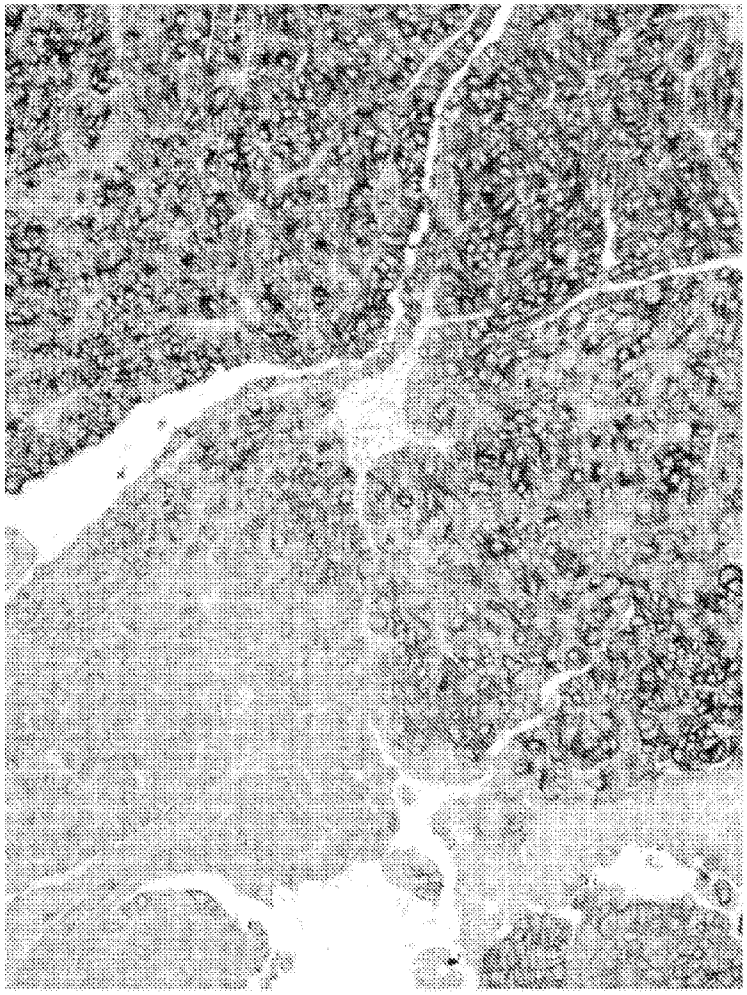


Figure 4

Figure 5

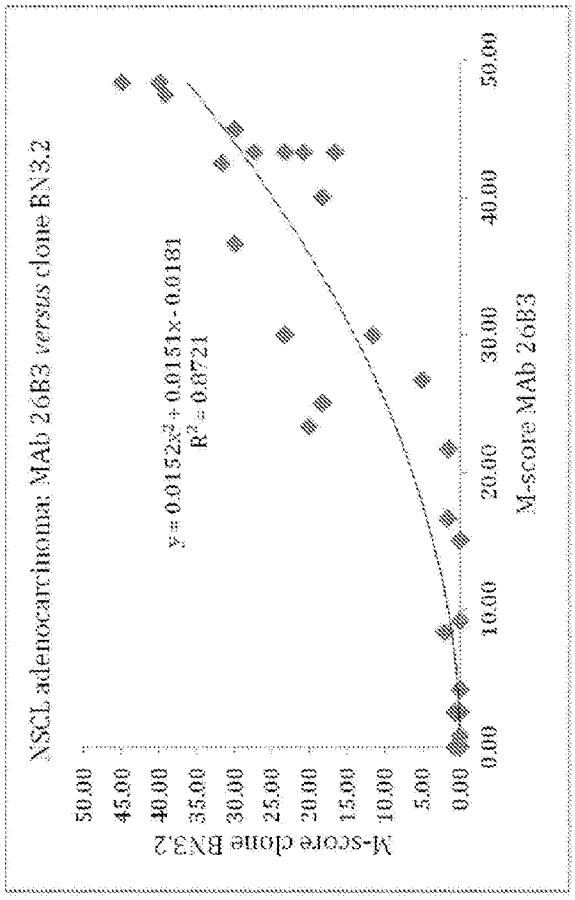

NSCL adenocarcinoma: MAb 26B3 versus clone BN3.2
y = 0.0152x² + 0.0151x - 0.0181
R² = 0.8721
M-score clone BN3.2
M-score MAb 26B3
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
0.00
10.00
20.00
30.00
40.00
50.00

Figure 6

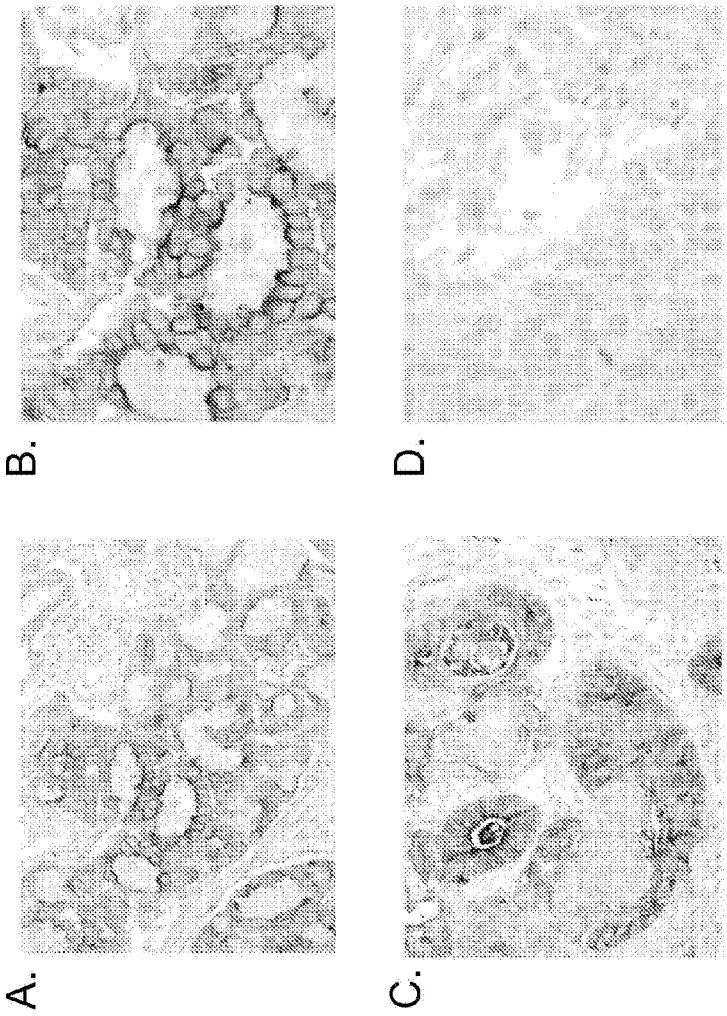


Figure 7

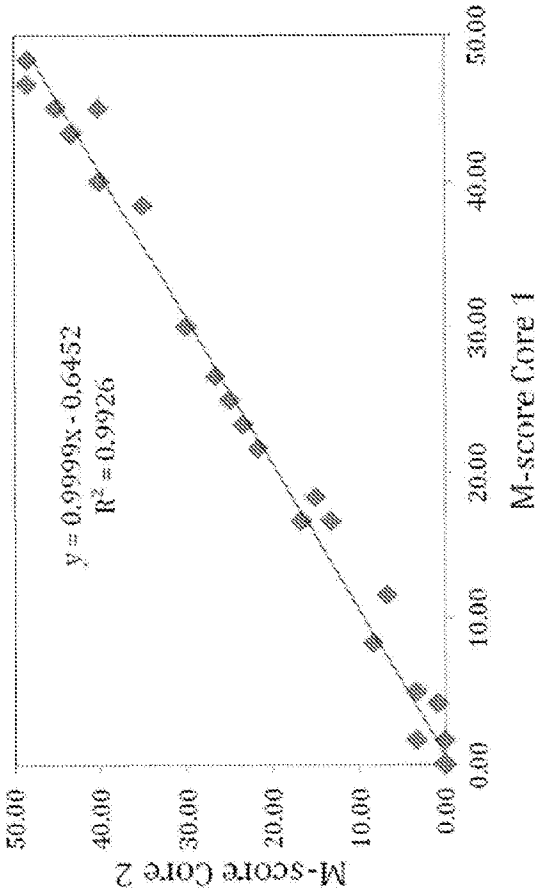

y = 0,9999x - 0,6452
R² = 0,9926
M-score Core 2
M-score Core 1
0.00
10.00
20.00
30.00
40.00
50.00

Figure 8

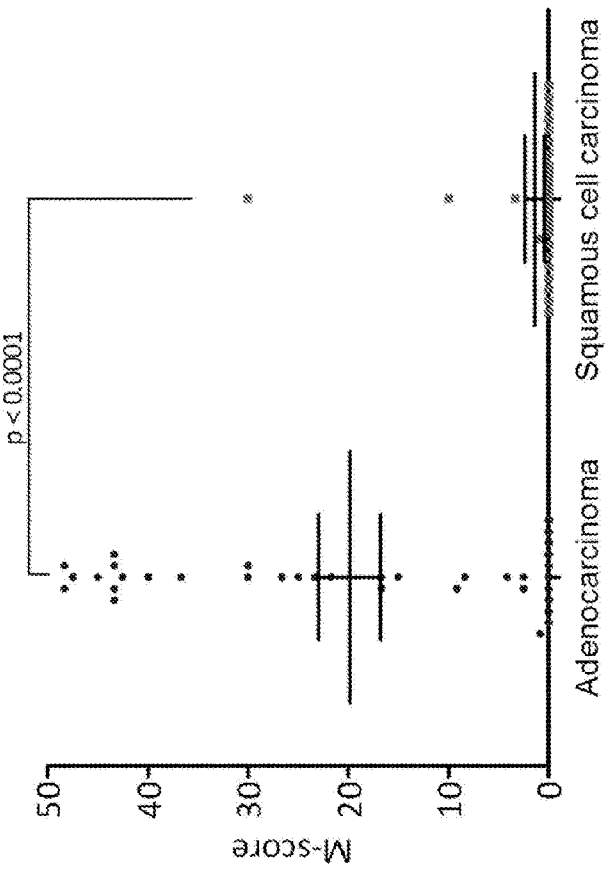

p < 0.0001
M-score
50
40
30
20
10
0
Adenocarcinoma
Squamous cell carcinoma
Lung Histology

Figure 9

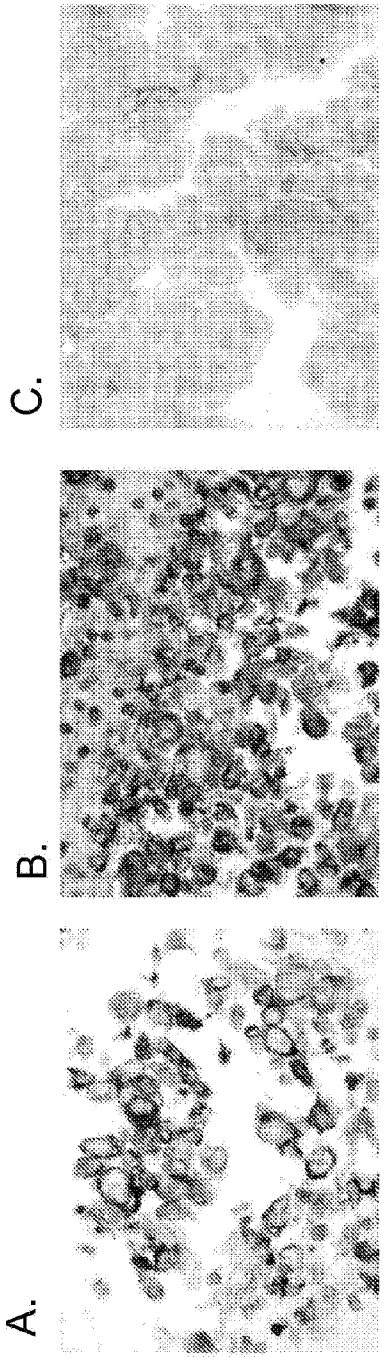


Figure 10

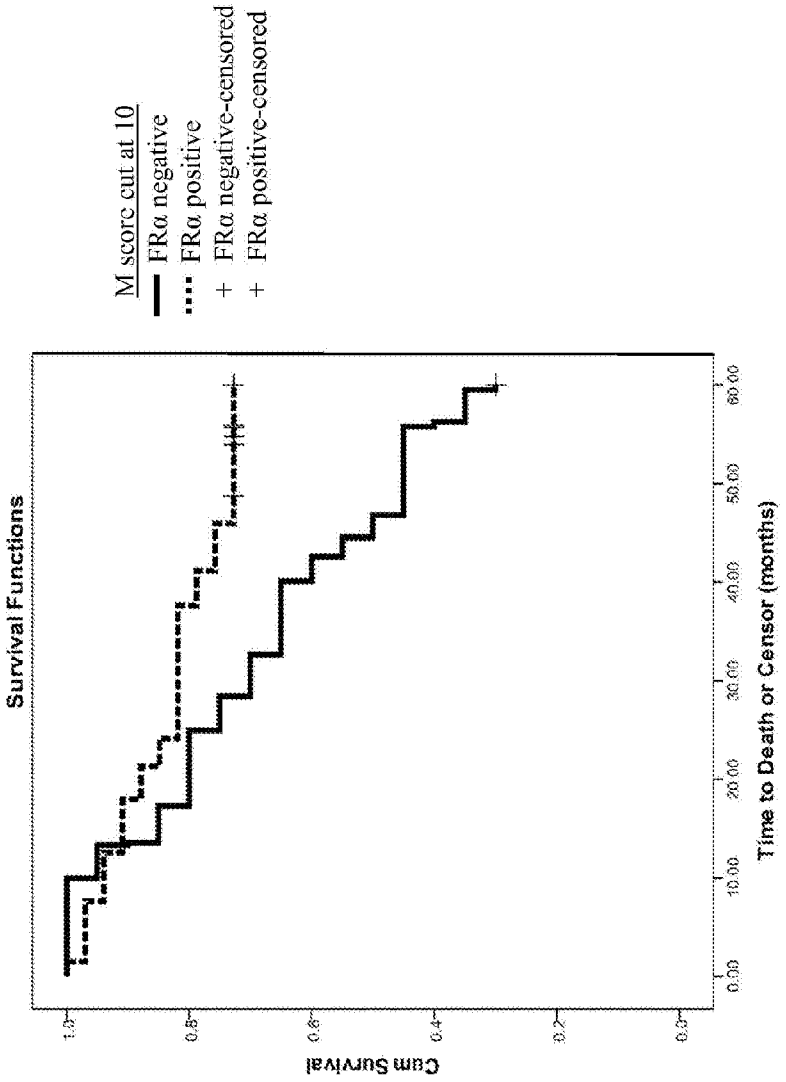

M score cut at 10
FRα negative
FRα positive
FRα negative-censored
FRα positive-censored
Survival Functions
Cum Survival
1.0
0.8
0.6
0.4
0.2
0.0
0.00
10.00
20.00
30.00
40.00
50.00
60.00
Time to Death or Censor (months)

Figure 11

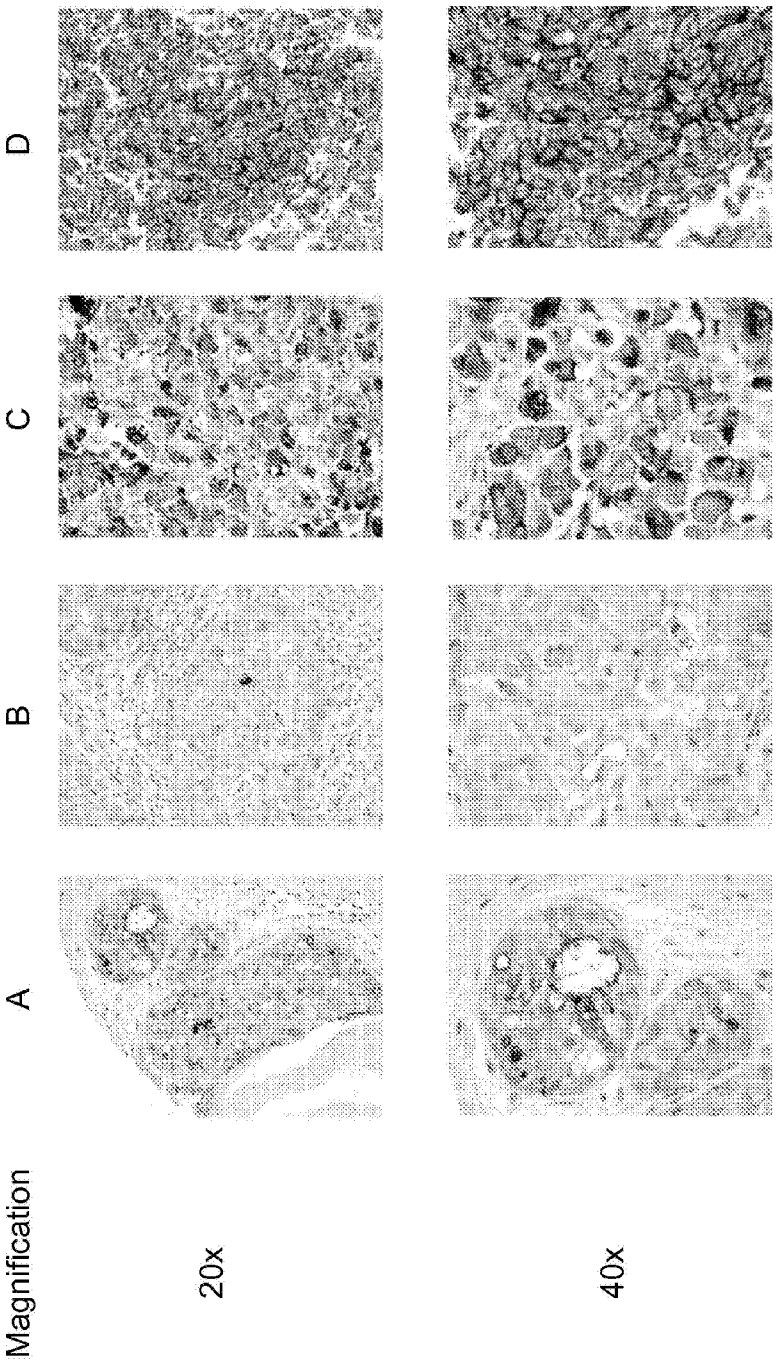


Figure 12

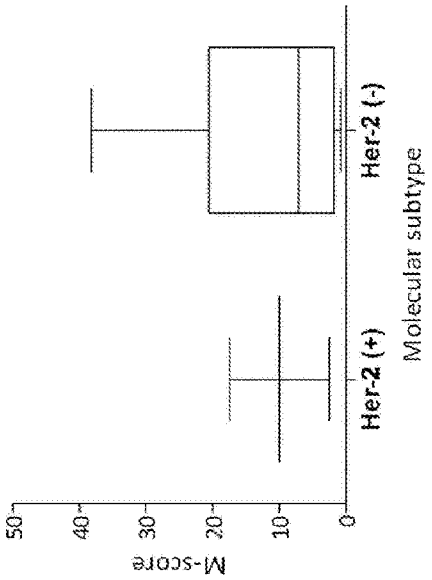


Figure 13

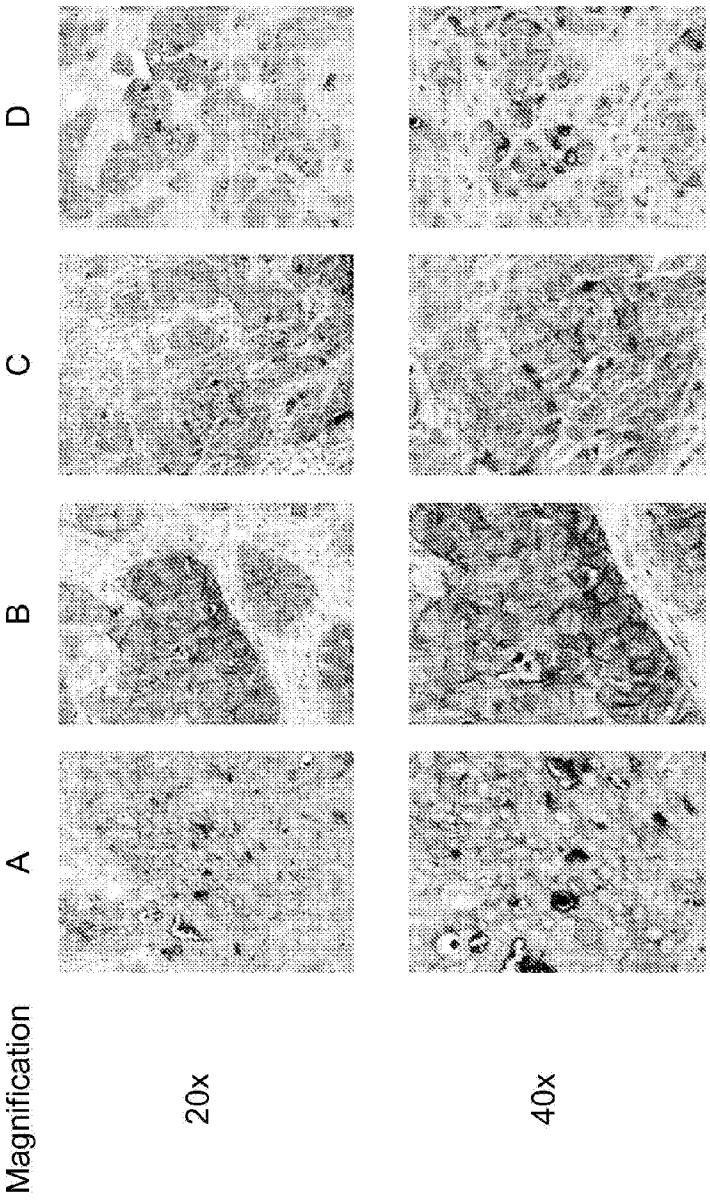


Figure 14

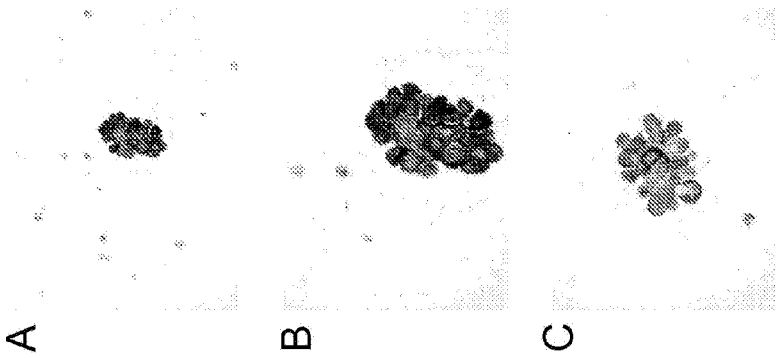


Figure 15

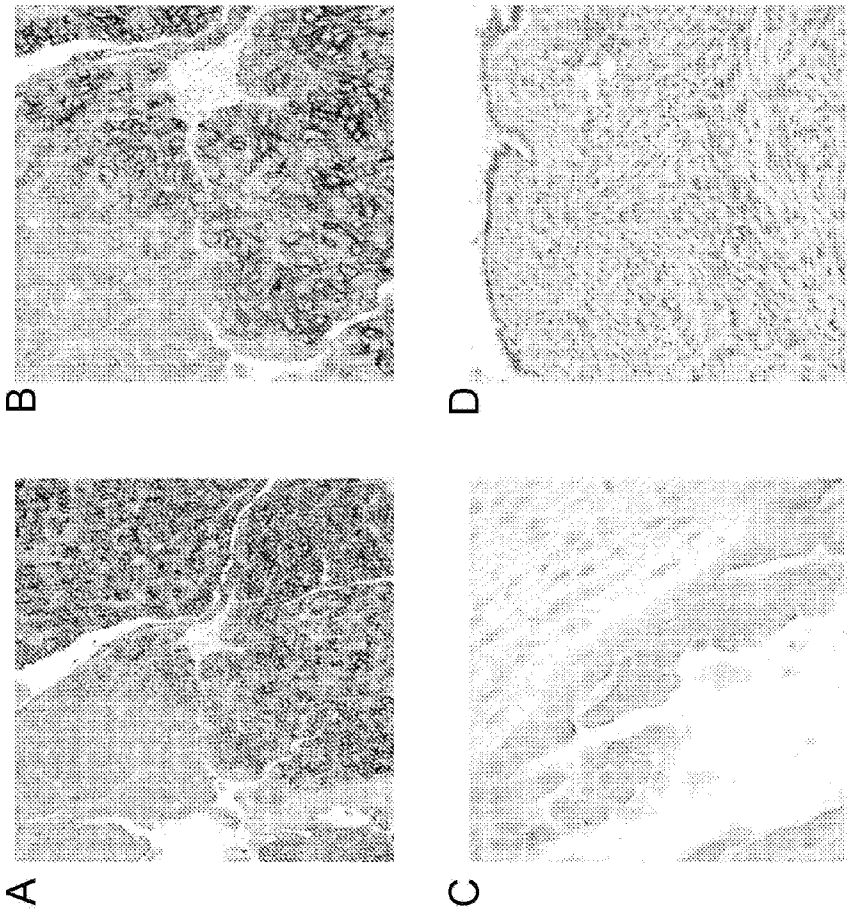
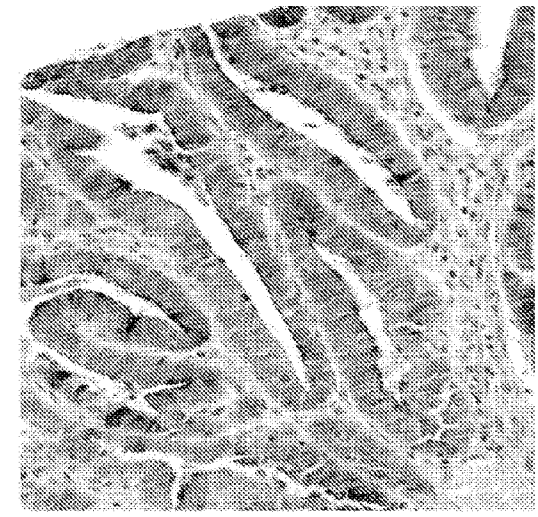


Figure 16



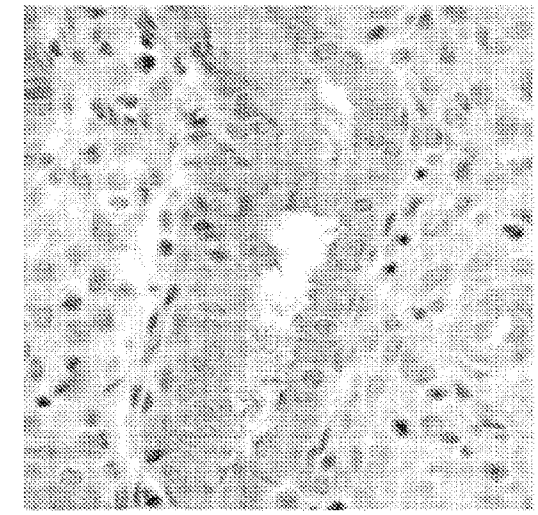


Figure 17

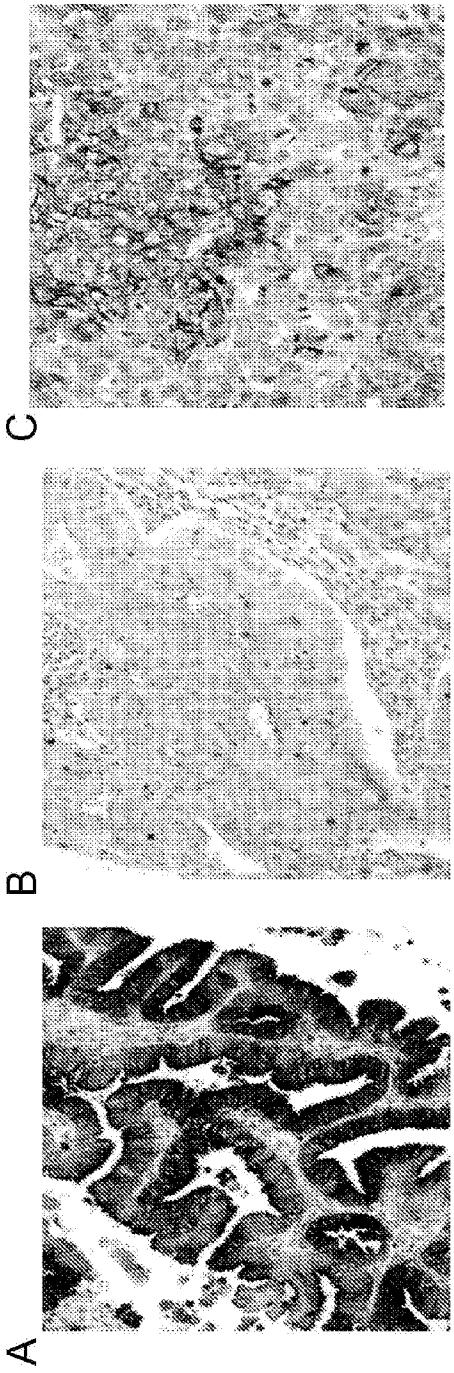
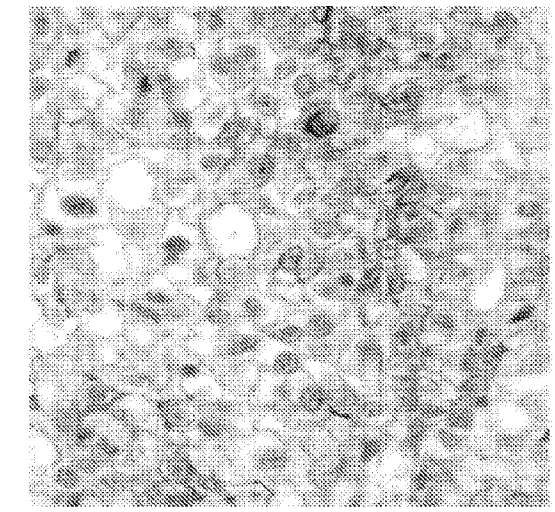


Figure 18



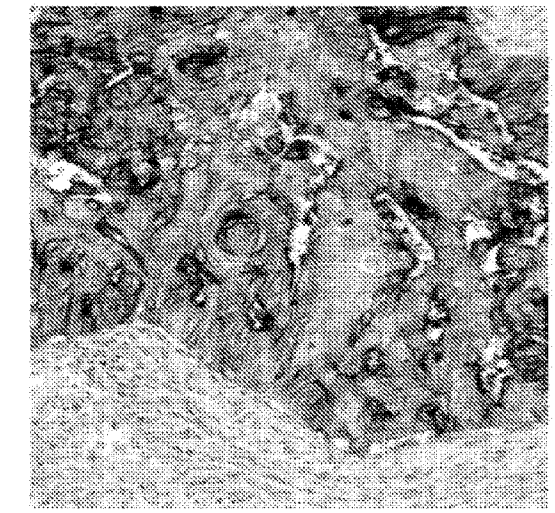


Figure 19

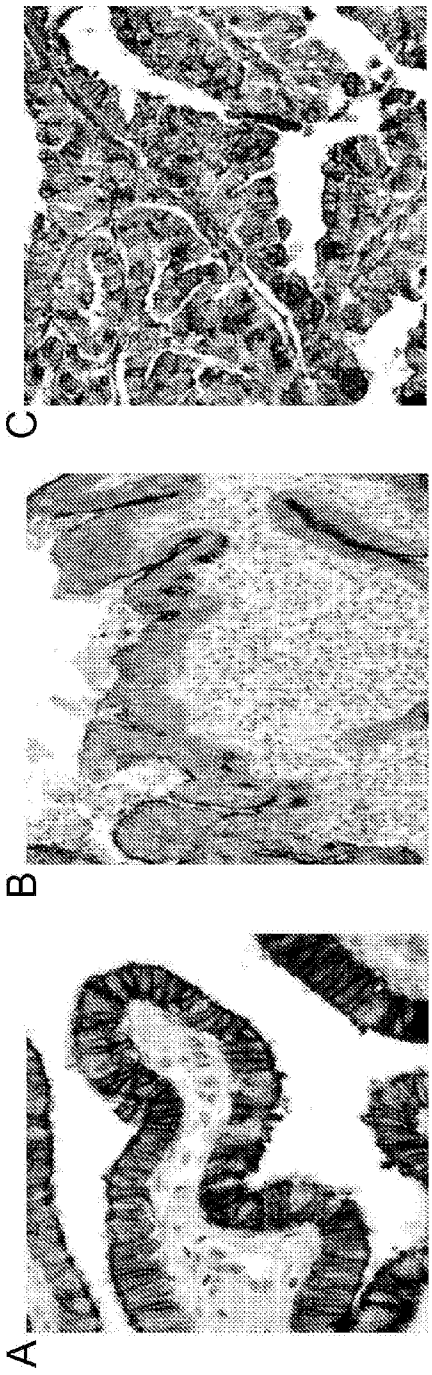


Figure 20

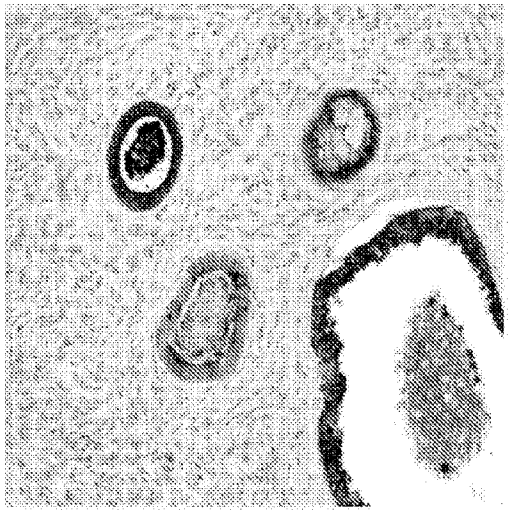


Figure 21