DESCRIPTION

Title of Invention

BIOMARKERS FOR PREDICTING AND ASSESSING RESPONSIVENESS OF ENDOMETRIAL CANCER SUBJECTS TO LENVATINIB COMPOUNDS

Cross-Reference to Related Application

This application claims the benefit of U.S. provisional application No. 61/823,034 filed on May 14, 2013, the disclosure of which is herein incorporated by reference in its entirety.

Technical Field

The present invention relates generally to biomarkers and endometrial cancer.

Background Art

A number of kinase inhibitors have been developed as antitumor agents. For example, a group of compounds having inhibitory activity against receptor tyrosine kinases, such as vascular endothelial growth factor receptor (VEGFR), are known to inhibit angiogenesis and are regarded as a new class of antitumor agents. Lenvatinib mesylate (also known as E7080) is an oral tyrosine kinase inhibitor targeting VEGFR1-3, fibroblast growth factor receptor (FGFR) 1-4, rearranged during transfection receptor (RET), KIT, and platelet-derived growth factor receptor (PDGFR). In phase I clinical studies of lenvatinib mesylate, response to treatment was observed in various tumor types, e.g., endometrial cancers.

Unfortunately, most anti-tumor treatments are associated with undesirable side effects, such as profound nausea, vomiting, or severe fatigue. Also, while anti-tumor treatments have been successful, they do not produce significant clinical responses in all patients who receive them, resulting in undesirable side effects, delays, and costs associated with ineffective treatment. Therefore, biomarkers that can be used to predict the response of a subject to an antitumor agent prior to administration thereof are greatly needed.

WO 2012/157672 discloses that, in a sub-group of melanoma patients who either have wild type B-raf and PTEN or mutated B-raf and PTEN, high levels of Ang2, IL6, CXCR4, COL4A3, MEIS1, FGF9, FGFR1, FGFR2, FGFR3, FGFR4, or VEGFR1 and low levels of SHC1, NRP2, ARHGAP22, SCG2, or PML are predictive of responsiveness to lenvatinib compounds.

WO 2012/166899 discloses that, in subjects having thyroid or kidney cancer, low levels of Ang2, VEGFA, IFNG, or soluble KDR or high levels of IL-6, IL-13, PDGFAB, CSF3, CCL3, CCL4, FLT4, or FGF2 are predictive of responsiveness to lenvatinib

compounds. However, WO 2012/166899 does not disclose or suggest that Ang2 can be used as predictive of responsiveness to lenvatinib compounds in endometrial cancer patients.

Llovet et al. *Clin. Cancer Res.*, 18(8):2290-2300 (2012), reports that in patients with advanced hepatocellular carcinoma, while the angiogenesis biomarkers Ang2 and VEGF were predictors of survival, these biomarkers were not predictive of responsiveness to the angiogenesis inhibitor, sorafenib.

Thus, an angiogenesis biomarker like Ang2 is not expected to serve as a biomarker for responsiveness to angiogenesis inhibitors in all cancers.

Summary of Invention

The present application is based, at least in part, on the identification of biomarkers that are predictive of an endometrial cancer subject's responsiveness to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate). The expression level of certain genes (e.g., the proteins and mRNA of the genes listed in Table 1) prior to treatment is identified as a useful predictor of responsiveness (e.g., survival and/or tumor response) to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate). Thus, the biomarkers and compositions described herein are useful, for example, in identifying, stratifying, and/or selecting a patient or a subset of patients having endometrial cancer that could benefit from treatment with lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate). In addition, the methods described herein are useful, for example, in selecting appropriate treatment modalities (e.g., therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) or an alternative endometrial cancer therapy) for a subject suffering from, suspected of having, or at risk of developing an endometrial cancer.

In one aspect, the disclosure provides a method of predicting the response of a subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. The method involves assaying a biological sample obtained from the subject and determining that the concentration of Ang2 protein in the biological sample is low, as compared to a control. The subject having a low concentration of Ang2 protein in the biological sample is identified as likely to respond to the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

In addition, the disclosure provides a method of predicting the response of a subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy

comprising lenvatinib or a pharmaceutically acceptable salt thereof. The method involves assaying a biological sample obtained from the subject and determining that the concentration of HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein in the biological sample is low, as compared to a control. The subject having a low concentration of HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein in the biological sample is identified as likely to respond to the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

In a second aspect, the disclosure provides a method of predicting the response of a subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. The method involves assaying a biological sample obtained from the subject and determining that the concentration of Ang2 protein in the biological sample is high, as compared to a control. The subject having a high concentration of Ang2 protein in the biological sample is identified as unlikely to respond to the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

In addition, the disclosure provides a method of predicting the response of a subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. The method involves assaying a biological sample obtained from the subject and determining that the concentration of HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein in the biological sample is high, as compared to a control. The subject having a high concentration of HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein in the biological sample is identified as unlikely to respond to the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

In a third aspect, the disclosure provides a method of selecting a subject having, suspected of having, or at risk of developing, an endometrial cancer for a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. This method involves assaying a biological sample obtained from the human subject and determining that the concentration of Ang2 protein in the biological sample is low, as compared to a control. The method further involves selecting the human subject having a low concentration of Ang2 protein in the biological sample for the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

In addition, the disclosure provides a method of selecting a subject having,

suspected of having, or at risk of developing, an endometrial cancer for a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. This method involves assaying a biological sample obtained from the human subject and determining that the concentration of HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein in the biological sample is low, as compared to a control. The method further involves selecting the human subject having a low concentration of HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein in the biological sample for the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

In a fourth aspect, the disclosure provides a method of treating an endometrial cancer. The method involves providing a biological sample obtained from a subject that has endometrial cancer; measuring, in the biological sample, an Ang2 protein expression level that is low as compared to a control; and administering to the subject a therapeutically effective amount of lenvatinib or a pharmaceutically acceptable salt thereof.

In addition, the method involves providing a biological sample obtained from a subject that has endometrial cancer; measuring, in the biological sample, an HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein expression level that is low as compared to a control; and administering to the subject a therapeutically effective amount of lenvatinib or a pharmaceutically acceptable salt thereof.

In a fifth aspect, the disclosure provides a method of treating an endometrial cancer. The method involves administering to the subject that has an endometrial cancer a therapeutically effective amount of lenvatinib or a pharmaceutically acceptable salt thereof, wherein the subject has been identified as having an Ang2 protein expression level that is low as compared to a control. In certain embodiments, the subject has been identified as having a low concentration of Ang2 protein in a biological sample obtained from the human subject.

In addition, the method involves administering to the subject that has an endometrial cancer a therapeutically effective amount of lenvatinib or a pharmaceutically acceptable salt thereof, wherein the subject has been identified as having an HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein expression level that is low as compared to a control. In certain embodiments, the subject has been identified as having a low concentration of HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein in a biological sample obtained from the human subject.

The following embodiments are envisaged for all of the above aspects.

In one embodiment the lenvatinib or a pharmaceutically acceptable salt thereof is lenvatinib mesylate.

In one embodiment, the endometrial cancer is an advanced endometrial cancer. In another embodiment, the endometrial cancer is a recurrent endometrial cancer. In one embodiment, the endometrial cancer is a stage III endometrial cancer. In one embodiment, the endometrial cancer is a stage IV endometrial cancer. In one embodiment, the endometrial cancer is an unresectable form of stage III or stage IV endometrial cancer.

In some embodiments, the biological sample is selected from the group consisting of a blood sample, a serum sample, a plasma sample, a uterine fluid sample, a urine sample, an endometrial archived tumor sample, and an endometrial biopsy sample.

In some embodiments, the control is a pre-established cut-off value. In one embodiment, the pre-established cut-off value is an Ang2 protein concentration that is determined based on receiver operating characteristic (ROC) analysis predicting tumor response with a higher positive predictive value compared to no cut-off, and wherein a concentration of Ang2 protein equal to or below the pre-established cut-off value is a low concentration of Ang2 and a value higher than the pre-established cut-off value is a high concentration of Ang2. The tumor response is an objective response rate (ORR), a clinical benefit rate (CBR), or % of maximum tumor shrinkage. In another embodiment, the pre-established cut-off value is an Ang2 protein concentration that is determined based on simulation models predicting survival, and wherein a concentration of Ang2 protein equal to or below the pre-established cut-off value is a low concentration of Ang2 and a value higher than the pre-established cut-off value is a high concentration of Ang2. In this context, survival is progression free survival (PFS) or overall survival (OS). In a particular embodiment, the pre-established cut-off value is an Ang2 protein concentration that is within the range of 1866.5 to 6024.5 (e.g., 2082.5 pg/ml), and wherein a concentration of Ang2 protein equal to or below the pre-established cut-off value is a low concentration of Ang2 and a value higher than the pre-established cut-off value is a high concentration of Ang2.

In some embodiments, the method further includes communicating the test results to the subject's health care provider. In certain embodiments, the method further includes modifying the subject's medical record to indicate that the subject is likely or not likely to respond to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. In specific embodiments, the record is created on a computer readable medium. In certain embodiments, the method further includes prescribing a therapy comprising lenvatinib or a

pharmaceutically acceptable salt thereof for the subject if the biomarker expression profile is predictive that the subject will respond to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. In certain embodiments, the method further includes prescribing a therapy not comprising lenvatinib or a pharmaceutically acceptable salt thereof for the subject if the biomarker expression profile is predictive that the subject will not respond to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. In some embodiments, the method further includes administering to the subject a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof if the biomarker expression profile is predictive that the subject will respond to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. In some embodiments, the method further includes administering to the subject a therapy that does not comprise lenvatinib or a pharmaceutically acceptable salt thereof if the biomarker expression profile is predictive that the subject will not respond to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

In one embodiment, the concentration of the protein is measured by an immunological method. In some embodiments, the immunological method is selected from the group consisting of enzyme immunoassay, radioimmunoassay, chemiluminescent immunoassay, electrochemiluminescence immunoassay, latex turbidimetric immunoassay, latex photometric immunoassay, immuno-chromatographic assay, and western blotting. In another embodiment, the concentration of the protein is measured by mass spectrometry.

In a sixth aspect, this disclosure provides lenvatinib or a pharmaceutically acceptable salt thereof for use in treating an endometrial cancer in a human subject, wherein the human subject is identified by the methods described above as a subject that is likely to respond to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutically acceptable salt of lenvatinib is lenvatinib mesylate. In one embodiment, the endometrial cancer is an advanced endometrial cancer. In another embodiment, the endometrial cancer is a recurrent endometrial cancer. In one embodiment, the endometrial cancer is a stage III endometrial cancer. In one embodiment, the endometrial cancer is a stage IV endometrial cancer. In one embodiment, the endometrial cancer is an unresectable form of stage III or stage IV endometrial cancer.

In a seventh aspect, the disclosure provides an Ang2 protein detection agent for use in predicting the response of a human subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically

acceptable salt thereof. In one embodiment, the Ang2 protein detection agent is an anti-Ang2 antibody.

In addition, the disclosure provides an HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein detection agent for use in predicting the response of a human subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. In one embodiment, the HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein detection agent is an anti-HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA antibody.

In an eighth embodiment, the disclosure features a kit comprising an Ang2 protein detection agent for use in predicting the response of a human subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. In certain embodiments, the Ang2 protein detection agent is an anti-Ang2 antibody. In certain embodiments, the anti-Ang2 antibody is a monoclonal antibody. In other embodiments, the anti-Ang2 antibody is a polyclonal antibody. In certain embodiments, the antibody is conjugated with a detectable agent. In one embodiment, the detectable agent is horse radish peroxidase, biotin, a fluorescent moiety, a radioactive moiety, a histidine tag, or a peptide tag. In one embodiment, the detectably labeled antibody is coated on a microplate. In certain embodiments, the microplate is a 96 well microplate. In certain embodiments, the kit optionally includes one or more concentration standards, one or more buffers (e.g., wash buffers), one or more diluents (e.g., assay and/or calibration diluents), and one or more reagents that facilitate detecting whether the Ang2 protein detection agent specifically binds Ang2 in a biological sample obtained from the subject (e.g., color reagents, stop solutions).

In addition, the disclosure features a kit comprising an HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein detection agent for use in predicting the response of a human subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof. In certain embodiments, the HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA protein detection agent is an anti-HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA antibody. In certain embodiments, the antibody is a monoclonal antibody. In other embodiments, the antibody is a polyclonal antibody. In certain embodiments, the antibody is conjugated with a

detectable agent. In one embodiment, the detectable agent is horse radish peroxidase, biotin, a fluorescent moiety, a radioactive moiety, a histidine tag, or a peptide tag. In one embodiment, the detectably labeled antibody is coated on a microplate. In certain embodiments, the microplate is a 96 well microplate. In certain embodiments, the kit optionally includes one or more concentration standards, one or more buffers (e.g., wash buffers), one or more diluents (e.g., assay and/or calibration diluents), and one or more reagents that facilitate detecting whether the protein detection agent specifically binds HGF, IL-8, IP-10, MCP-1, MIP-1α, PGF, sIL-2Rα, Tie-2, TNF-α, or VEGFA in a biological sample obtained from the subject (e.g., color reagents, stop solutions).

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the exemplary methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present application, including definitions, will control. The materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of Drawings

Fig. 1 is a graphical depiction of the change in levels of blood biomarkers after E7080 treatment.

Fig. 2 is a series of graphs showing the correlation of baseline cytokine, chemokine, and angiogenic factors (CAFs) with tumor response.

Fig. 3 is a series of graphs showing that there is no significant correlation of weight, age, and histology with progression free survival (PFS) and overall survival (OS).

Fig. 4 shows the results of a multivariate analysis that does not identify potential combination factors with Ang-2 as improving predictions of clinical outcome.

Fig. 5 includes two graphs showing the median PFS and median OS of sub-groups of endometrial cancer patients stratified by baseline Ang-2 levels.

Fig. 6 includes two graphs showing the enrichment of patient populations with better objective response rate (ORR) based on baseline Ang-2 level.

Description of Embodiments

This disclosure provides methods and compositions for predicting the response of an endometrial cancer subject (such as a human patient) to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate). The disclosure provides predictive biomarkers (e.g., protein or RNA expression levels) to identify those subjects having, suspected of having, or at risk of developing, endometrial cancer (e.g., advanced or recurrent endometrial cancer) for whom administering a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) is likely to be effective or ineffective. The biomarkers, compositions, and methods described herein are useful in selecting appropriate therapeutic modalities (e.g., a lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) therapy or an alternative therapy) for subjects suffering from, suspected of having or at risk of developing endometrial cancer. Furthermore, this application provides methods of selecting patients having, suspected of having, or at risk of developing, endometrial cancer that could benefit from a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) as well as methods of treatment.

Definitions

The term "decreased/reduced expression level" means an expression level (amount) that is lower than the expression level in a control.

The term "elevated expression level" means an expression level (amount) that is higher than the expression level in a control.

The term "expression level of a gene" means expression level (amount) of the protein encoded by the gene or the RNA transcribed from the gene.

The term "low concentration" means a concentration of the substance being analyzed that is lower than the concentration of that substance in a control.

The term "high concentration" means a concentration of the substance being analyzed that is higher than the concentration of that substance in a control.

The term "lenvatinib" refers to 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide. This compound is disclosed in Example 368 (see, column 270) of U.S. Patent No. 7,253,286. U.S. Patent No. 7,253,286 is incorporated by reference in its entirety herein. The term "lenvatinib compound" refers to "lenvatinib or a pharmaceutically acceptable salt thereof." An example of a pharmaceutically acceptable salt of lenvatinib is lenvatinib mesylate. Lenvatinib mesylate is also referred to as E7080.

The term "pharmaceutically acceptable salt" is not particularly restricted as to the type of salt. Examples of such salts include, but are not limited to, inorganic acid addition salt such as hydrochloric acid salt, sulfuric acid salt, carbonic acid salt, bicarbonate salt, hydrobromic acid salt and hydriodic acid salt; organic carboxylic acid addition salt such as acetic acid salt, maleic acid salt, lactic acid salt, tartaric acid salt and trifluoroacetic acid salt; organic sulfonic acid addition salt such as methanesulfonic acid salt, hydroxymethanesulfonic acid salt, hydroxyethanesulfonic acid salt, benzenesulfonic acid salt, toluenesulfonic acid salt and taurine salt; amine addition salt such as trimethylamine salt, triethylamine salt, pyridine salt, procaine salt, picoline salt, dicyclohexylamine salt, N,N'-dibenzylethylenediamine salt, N-methylglucamine salt, diethanolamine salt, triethanolamine salt, tris(hydroxymethylamino)methane salt and phenethylbenzylamine salt; and amino acid addition salt such as arginine salt, lysine salt, serine salt, glycine salt, aspartic acid salt and glutamic acid salt. In one embodiment, the pharmaceutically acceptable salt is a methanesulfonic acid salt ("mesylate"). The methanesulfonic acid salt form (i.e., the mesylate) of 4-(3-chloro-4-(cyclopropylaminocarbonyl)aminophenoxy)-7-methoxy-6-quinolinecarboxamide is disclosed in US Patent 7,612,208, which is incorporated by reference herein in its entirety.

"Polypeptide" and "protein" are used interchangeably herein and mean any peptide-linked chain of amino acids, regardless of length or post-translational modification. Typically, a polypeptide described herein is "isolated" when it constitutes at least 60%, by weight, of the total protein in a preparation, e.g., 60% of the total protein in a sample. In some embodiments, a polypeptide described herein consists of at least 75%, at least 90%, or at least 99%, by weight, of the total protein in a preparation.

The term "responds/responsive to a therapy" means that the subject administered with the therapy shows a positive response to the therapy provided. Non-limiting examples of such a positive response are: a decrease in tumor size, a decrease in metastasis of a tumor, or an increased period of survival after treatment.

The term "subject" means a mammal, including but not limited to, a human, a chimpanzee, an orangutan, a gorilla, a baboon, a monkey, a mouse, a rat, a pig, a horse, a dog, and a cow.

Endometrial Cancer

Endometrial cancer refers to several types of malignancies that arise from the

endometrium, or lining, of the uterus. Most endometrial cancers are carcinomas (generally adenocarcinomas). In other words, they originate from the single layer of epithelial cells that line the endometrium and form the endometrial glands. Endometrial carcinomas are sometimes classified into two groups: Type I includes the cancers found in pre- and peri-menopausal women and are generally minimally invasive; Type II includes the cancers that occur in older, post-menopausal women and carry a poorer prognosis than Type I. In contrast to endometrial carcinomas, the uncommon endometrial stromal sarcomas are cancers that originate in the non-glandular connective tissue of the endometrium.

In order to choose a treatment plan for patients, doctors need to determine how the endometrial cancer has spread in a patient, or in other words "stage" the endometrial cancer. Endometrial cancer is staged based on examination of tissue removed during an operation (surgical staging). The staging system looks at how far the cancer has spread. Endometrial cancer can spread locally to the cervix and other parts of the uterus. It can also spread regionally to nearby lymph nodes. In addition, this cancer can metastasize to distant lymph nodes, the upper abdomen, the omentum, or other organs such as lung, liver, bone, and brain.

The two systems used to stage endometrial cancer are the FIGO (International Federation of Gynecology and Obstetrics) system and the American Joint Committee on Cancer (AJCC) staging system. These systems are basically the same; the difference between the AJCC system and the FIGO system is that the FIGO system does not include stage 0. Both staging systems classify endometrial cancer on the basis of three factors: the extent of the tumor (T), whether the cancer has spread to lymph nodes (N) and whether it has spread to distant sites (M). Information about the tumor, lymph nodes, and any cancer spread is then combined to assign the stage of disease, a process called stage grouping. The stages are described using the number 0 and Roman numerals from I to IV. Some stages are divided into sub-stages indicated by letters and numbers.

Stage 0: Tis, N0, M0 - This stage is also known as carcinoma *in situ*. Cancer cells are only found in the surface layer of cells of the endometrium, without growing into the layers of cells below. The cancer has not spread to nearby lymph nodes or distant sites. This is a pre-cancerous lesion. This stage is not included in the FIGO staging system.

Stage I: T1, N0, M0 - The cancer is only growing in the body of the uterus. It may also be growing into the glands of the cervix, but is not growing into the supporting connective tissue of the cervix. The cancer has not spread to lymph nodes or distant sites.

Stage IA: T1a, N0, M0 - In this earliest form of stage I, the cancer is in the

endometrium and may have grown from the endometrium less than halfway through the underlying muscle layer of the uterus (the myometrium). It has not spread to lymph nodes or distant sites.

Stage IB: T1b, N0, M0 - The cancer has grown from the endometrium into the myometrium, growing more than halfway through the myometrium. The cancer has not spread beyond the body of the uterus.

Stage II: T2, N0, M0 -The cancer has spread from the body of the uterus and is growing into the supporting connective tissue of the cervix (the cervical stroma). The cancer has not spread outside of the uterus. The cancer has not spread to lymph nodes or distant sites.

Stage III: T3, N0, M0 - Either the cancer has spread outside of the uterus or into nearby tissues in the pelvic area.

Stage IIIA: T3a, N0, M0 - The cancer has spread to the outer surface of the uterus (called the serosa) and/or to the fallopian tubes or ovaries (the adnexa). The cancer has not spread to lymph nodes or distant sites.

Stage IIIB: T3b, N0, M0 -The cancer has spread to the vagina or to the tissues around the uterus (the parametrium). The cancer has not spread to lymph nodes or distant sites.

Stage IIIC1: T1 to T3, N1, M0 - The cancer is growing in the body of the uterus. It may have spread to some nearby tissues, but is not growing into the inside of the bladder or rectum. The cancer has spread to pelvic lymph nodes but not to lymph nodes around the aorta or distant sites.

Stage IIIC2: T1 to T3, N2, M0 - The cancer is growing in the body of the uterus. It may have spread to some nearby tissues, but is not growing into the inside of the bladder or rectum. The cancer has spread to lymph nodes around the aorta (peri-aortic lymph nodes) but not to distant sites.

Stage IV: The cancer has spread to the inner surface of the urinary bladder or the rectum to lymph nodes in the groin, and/or to distant organs, such as the bones, omentum or lungs.

Stage IVA: T4, any N, M0 - The cancer has spread to the inner lining of the rectum or urinary bladder (called the mucosa). It may or may not have spread to nearby lymph nodes but has not spread to distant sites.

Stage IVB: any T, any N, M1 -The cancer has spread to distant lymph nodes, the

upper abdomen, the omentum, or to organs away from the uterus, such as the bones, omentum, or lungs. The cancer can be any size and it may or may not have spread to lymph nodes.

Methods of Predicting Responsiveness to Therapy Comprising a Lenvatinib Compound

A number of genes have been identified whose expression levels (e.g., mRNA or protein expression levels) are useful in predicting responsiveness of a subject having an endometrial cancer to a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate). These genes as identified by Gene ID, related URL, protein ID and UniProtKB Accession Nos. are listed in Table 1.

Table 1: List of Biomarkers

Official Gene Symbol	Gene ID	URL	Alternative Symbol	UniProtKB Accession No.
Ang2	285	www.ncbi.nlm.nih.gov/gene/285	Ang-2/ANG-2/ANG2 (90)/ ANG290/ ANGPT2	O15123
HGF	3082	www.ncbi.nlm.nih.gov/gene/3082	HGF (86)	P14210
IL-8	3576	www.ncbi.nlm.nih.gov/gene/3576	IL-8 (40)	P10145
IP-10	3627	www.ncbi.nlm.nih.gov/gene/3627	CXCL10	P02778
MCP-1	6347	www.ncbi.nlm.nih.gov/gene/6347	CCL2	P13500
MIP-1a	6348	www.ncbi.nlm.nih.gov/gene/6348	CCL3/MIP1a/MIP-1α	P10147
PGF	5228	www.ncbi.nlm.nih.gov/gene/5228	PGF (91)	P49763
sIL2-Ra	3559	www.ncbi.nlm.nih.gov/gene/3559	sIL2-Ra(76)	P01589
TIE-2	7010	www.ncbi.nlm.nih.gov/gene/7010	TEK/CD202B/TIE2/ VMCM/VMCM1	Q02763
TNF	7124	www.ncbi.nlm.nih.gov/gene/7124	TNFa/TNF-α	P01375
VEGFA	7422	www.ncbi.nlm.nih.gov/gene/7422	VEGF/VEGFA (100)/VEGFA100	P15692

Angiopoietins are protein growth factors that promote angiogenesis (the formation of blood vessels from pre-existing blood vessels) and maturation of tumor blood vessels. Mouse knock out studies have shown that Angiopoietin 2 (Ang2) is required for the formation of mature blood vessels. Expression of Ang2 in the endothelial cells is sufficient to recruit myeloid cells and induce inflammation even in the absence of preceding proinflammatory stimuli.

Hepatocyte growth factor (HGF) is a paracrine cellular growth, motility and morphogenic factor. It is secreted by mesenchymal cells and targets and acts primarily upon epithelial cells and endothelial cells, but also acts on haemopoietic progenitor cells. It

plays a major role in embryonic organ development, in adult organ regeneration and in wound healing. HGF regulates cell growth, cell motility, and morphogenesis by activating a tyrosine kinase signaling cascade after binding to the proto-oncogenic c-Met receptor.

Interleukin 8 (IL-8) is a chemokine produced by macrophages and other cell types such as epithelial cells and endothelial cells. Il-8 can bind several receptors including CXCR1, and CXCR2.

Interferon gamma-induced protein 10 (IP-10) is a small cytokine belonging to the CXC chemokine family. It is secreted by several cell types (e.g., monocytes, endothelial cells and fibroblasts) in response to IFN-γ. This protein has been attributed several roles, such as chemoattraction for monocytes/macrophages, T cells, NK cells, and dendritic cells, promotion of T cell adhesion to endothelial cells, antitumor activity, and inhibition of bone marrow colony formation and angiogenesis.

Monocyte chemotactic protein-1 (MCP-1) is a small cytokine that belongs to the CC chemokine family. It plays a role in recruiting monocytes, memory T cells, and dendritic cells to the sites of inflammation produced by either tissue injury or infection.

Macrophage Inflammatory Protein-1a (MIP-1a) belongs to the family of chemotactic cytokines. This protein is crucial for immune responses towards infection and inflammation. It activates granulocytes (neutrophils, eosinophils and basophils) which can lead to acute neutrophilic inflammation. In addition, it induces the synthesis and release of other pro-inflammatory cytokines such as interleukin 1 (IL-1), IL-6 and TNF-α from fibroblasts and macrophages.

Placental growth factor (PGF) is a member of the vascular endothelial growth factor sub-family. Placental growth factor-expression within human atherosclerotic lesions is associated with plaque inflammation and neovascular growth.

Soluble Interleukin-2 receptor alpha (sIL-2Ra) is the secreted extracellular domain of IL-2R alpha and is expressed by leukemia cells, lymphoma cells, a fraction of NK cells, as well as recently activated T and B cells.

Tie-2 is a cell surface receptor tyrosine kinase that binds and is regulated by the angiopoietins (Ang1, Ang2, Ang3, Ang4). This receptor is expressed mainly in endothelial cells in humans. It possesses a unique extracellular domain containing two immunoglobulin-like loops separated by three epidermal growth factor-like repeats that are connected to three fibronectin type III-like repeats. The TIE-2 signaling pathway appears to be critical for endothelial cell-smooth muscle cell communication in venous morphogenesis.

Defects in TIE-2 are associated with inherited venous malformations.

Tumor necrosis factor alpha (TNF-α) is a monocyte-derived cytotoxin that has been implicated in tumor regression, septic shock, and cachexia.

Vascular endothelial growth factor A (VEGF-A) is a glycosylated mitogen that specifically acts on endothelial cells and has various effects, including mediating increased vascular permeability, inducing angiogenesis, vasculogenesis and endothelial cell growth, promoting cell migration, and inhibiting apoptosis.

A low expression (e.g., protein or mRNA expression) level compared to a control of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11) genes listed in Table 1 is indicative/predictive that a subject will respond to a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate). For example, low concentrations (compared to a control) of Ang2 protein in a biological sample obtained from a subject prior to treatment with the therapy comprising a lenvatinib compound are predictive that the subject will respond to a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate).

In certain embodiments, a subject is determined to respond to a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate), if the subject shows a partial response following treatment with the therapy. "Partial Response" means at least 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline summed LD. In some embodiments, a subject is determined to respond to a therapy comprising a lenvatinib compound, if the subject shows tumor shrinkage post-treatment with the therapy. "% of maximum tumor shrinkage" (MTS) means percent change of sum of diameters of target lesions, taking as reference the baseline sum diameters. In other embodiments, a subject is determined to respond to a therapy comprising a lenvatinib compound, if the subject shows progression free survival. "Progression Free Survival" (PFS) refers to the period from start date of treatment to the last date before entering Progressive Disease (PD) status. PD means at least 20% increase in the sum of the LD of target lesions, taking as reference the smallest summed LD recorded since the treatment started, or the appearance of one or more new lesions. In some embodiments, a subject is determined to respond to a therapy comprising a lenvatinib compound, if the subject shows both progression free survival and tumor shrinkage.

This disclosure provides methods of identifying a subject having endometrial cancer who is likely to have both survival benefits (e.g., PFS) and tumor shrinkage following a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate). In this method, a

biological sample of the subject, obtained prior to treatment with the therapy comprising a lenvatinib compound, is assayed and the level of Ang2 protein is measured. A low concentration of the Ang2 protein compared to a control indicates that the subject will likely have both survival benefits (e.g., PFS) and tumor shrinkage following therapy comprising a lenvatinib compound. Conversely, a high concentration of the Ang2 protein compared to a control indicates that the subject will likely not have both survival benefits (e.g., PFS) and tumor shrinkage following therapy comprising a lenvatinib compound.

The methods described herein also permit identification of a subject having endometrial cancer who is likely to have survival benefits (e.g., PFS) following a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate). In this method, a biological sample of the subject, obtained prior to treatment with the therapy comprising a lenvatinib compound, is assayed and the level of at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, or one, two, three, four, five, six, seven, eight, nine, ten or eleven of Ang2, HGF, IL-8, IP-10, MCP-1, MIP-1a, PGF, sIL-2Ra, Tie-2, TNFα, and VEGFA protein is measured. A low concentration of any of these proteins (either alone or in combination with the others listed above) compared to a control indicates that the subject will likely have survival benefits (e.g., PFS) following therapy comprising a lenvatinib compound. Conversely, a high concentration of any of these proteins (either alone or in combination with the others listed above) compared to a control indicates that the subject will likely not have survival benefits (e.g., PFS) following therapy comprising a lenvatinib compound. In certain embodiments, the subject with a low concentration of one or more of the proteins listed above is likely to have a progression free survival of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty one, twenty two or twenty three months, or twenty four months.

This disclosure also provides a method of identifying a subject having endometrial cancer who is likely to have tumor shrinkage following a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate). In this method, a biological sample of the subject is assayed and the concentration of Ang2 and/or IL-8 protein is measured. A low concentration of Ang2 and/or IL-8 compared to a control indicates that the subject will likely have tumor shrinkage. Conversely, a high concentration of Ang2 and/or IL-8 compared to a control indicates that the subject will likely not show tumor shrinkage.

In one embodiment, the subject has, is suspected of having, or is at risk of

developing an endometrial cancer. In some embodiments, the endometrial cancer is advanced endometrial cancer. In other embodiments, the endometrial cancer is recurrent endometrial cancer. In certain embodiments, the endometrial cancer is stage III cancer. In some embodiments, the endometrial cancer is stage IV cancer. In certain embodiments, the endometrial cancer is unresectable stage III or stage IV cancer.

The concentration of the protein or proteins of interest can be measured using any method known in the art such as an immunological assay. Non-limiting examples of such methods include enzyme immunoassay, radioimmunoassay, chemiluminescent immunoassay, electrochemiluminescence immunoassay, latex turbidimetric immunoassay, latex photometric immunoassay, immuno-chromatographic assay, and western blotting. In certain embodiments, the concentration of the protein or proteins of interest is measured by mass spectrometry.

Controls

As described above, the methods of the present invention can involve, measuring the expression level (e.g., mRNA or protein concentration) of one or more genes (e.g., one or more genes depicted in Table 1) in a biological sample from a subject having , suspected of having or at risk of developing endometrial cancer, wherein the expression level of one or more of the genes, compared to a control, predicts the response of a subject to treatment comprising a lenvatinib compound (e.g., lenvatinib mesylate). In certain embodiments, when the concentration of a protein in Table 1 in a biological sample from a subject having, suspected of having or at risk of developing endometrial cancer is lower than the control, the subject is identified as likely to respond to a therapy comprising a lenvatinib compound. In this context, the term "control" includes a sample (from the same tissue) obtained from a subject who is known to not respond to a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate). The term "control" also includes a sample (from the same tissue) obtained in the past from a subject who is known to not respond to a therapy comprising a lenvatinib compound and used as a reference for future comparisons to test samples taken from subjects for which therapeutic responsiveness is to be predicted. The "control" expression level/concentration for a particular protein in a particular cell type or tissue may be pre-established by an analysis of protein expression in one or more (e.g., two, three, four, five, six, seven, eight, nine, 10, 15, 20, 25, 30, 35, or 40 or more) subjects, of the same species, that have not responded to treatment with a lenvatinib compound (e.g., lenvatinib mesylate). This pre-established reference value (which may be an average or median

expression level/concentration taken from multiple subjects that have not responded to the therapy) may then be used for the "control" concentration/expression level of the protein or nucleic acid in the comparison with the test sample. In such a comparison, the subject is predicted to respond to a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate) if the expression level of the gene being analyzed is lower than the pre-established reference.

The "control" concentration for a particular protein in a particular cell type or tissue may alternatively be pre-established by an analysis of gene expression in one or more subjects that have responded to treatment with a lenvatinib compound (e.g., lenvatinib mesylate). This pre-established reference value (which may be an average or median expression level taken from multiple subjects that have responded to the therapy) may then be used as the "control" expression level in the comparison with the test sample. In such a comparison, the subject is predicted to respond to a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate) if the concentration of the protein being analyzed is the same as, or comparable to (at least 85% but less than 100% of), the pre-established reference.

In certain embodiments, the "control" is a pre-determined cut-off value.

Cut-Off Values

In some embodiments, the methods described herein include determining if the concentration of a protein(s) of interest (e.g., one or more of the proteins listed in Table 1) falls above or below a predetermined cut-off value.

A cut-off value is typically a concentration of a protein above or below which is considered predictive of responsiveness of a subject to a therapy of interest. Thus, in accordance with the methods and compositions described herein, a reference concentration (e.g., of a protein of Table 1) is identified as a cut-off value, above or below of which is predictive of responsiveness to a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate). Some cut-off values are not absolute in that clinical correlations can still remain significant over a range of values on either side of the cutoff; however, it is possible to select an optimal cut-off value (e.g. varying H-scores) of concentration of proteins for a particular sample type. Cut-off values determined for use in the methods described herein can be compared with, e.g., published ranges of concentrations but can be individualized to the methodology used and patient population. It is understood that improvements in optimal cut-off values could be determined depending on the sophistication of statistical methods used and on the number and source of samples used to determine reference level values for

the different genes and sample types. Therefore, established cut-off values can be adjusted up or down, on the basis of periodic re-evaluations or changes in methodology or population distribution.

The reference concentration of one or more proteins can be determined by a variety of methods. The reference level can be determined by comparison of the concentration of a protein of interest in, e.g., populations of subjects (e.g., patients) that are responsive to a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate) or not responsive to a therapy comprising a lenvatinib compound. This can be accomplished, for example, by histogram analysis, in which an entire cohort of patients are graphically presented, wherein a first axis represents the concentration of a protein of interest and a second axis represents the number of subjects in the cohort whose sample contain one or more concentrations. Determination of the reference concentration of a protein can then be made based on an amount or concentration which best distinguishes these separate groups. The reference level can be a single number, equally applicable to every subject, or the reference level can vary, according to specific subpopulations of subjects. For example, older subjects can have a different reference level than younger subjects for the same cancer. In addition, a subject with more advanced disease (e.g., a more advanced form of endometrial cancer) can have a different reference value than one with a milder form of the disease.

The pre-established cut-off value can be a protein concentration that is determined based on receiver operating characteristic (ROC) analysis. ROC curves are used to determine a cut-off value for a clinical test. Consider the situation where there are two groups of patients and by using an established standard technique one group is known to be responsive to a lenvatinib compound, and the other is known to not respond to a lenvatinib compound. A measurement using a biological sample from all members of the two groups is used to test for responsiveness to a lenvatinib compound. The test will find some, but not all, responders to respond to a lenvatinb compound. The ratio of the responders found by the test to the total number of responders (known by the established standard technique) is the true positive rate (also known as sensitivity). The test will find some, but not all, non-responders to not respond to a lenvatinb compound. The ratio of the non-responders found by the test to the total number of non-responders (known by the established standard technique) is the true negative rate (also known as specificity). The hope is that the ROC curve analysis of the lenvatinib responsiveness test will find a cut-off value that will minimize the number of false positives and false negatives. A ROC is a graphical plot

which illustrates the performance of a binary class stratifier system as its discrimination threshold is varied. It is created by plotting the fraction of true positives out of the positives versus the fraction of false positives out of the negatives, at various threshold settings.

In one embodiment, the protein concentration is determined based on ROC analysis predicting tumor response with a positive predictive value, wherein a concentration of a protein of interest (e.g., Ang2) equal to or below the pre-established cut-off value is a low concentration of the protein of interest and a value higher than the pre-established cut-off value is a high concentration of the protein of interest. The positive predictive value is the proportion of positive test results that are true positives; it reflects the probability that a positive test reflects the underlying condition being tested for. Methods of constructing ROC curves and determining positive predictive values are well known in the art. In certain embodiments, tumor response is an objective response rate (ORR), a clinical benefit rate (CBR) or % of maximum tumor shrinkage.

In another embodiment, the pre-established cut-off value can be a protein concentration that is determined based on simulation models predicting survival, and wherein a concentration of the protein of interest (e.g., Ang2) equal to or below the pre-established cut-off value is a low concentration of the protein of interest and a value higher than the pre-established cut-off value is a high concentration of the protein of interest. In some embodiments, survival is progression free survival (PFS). In other embodiments, survival is overall survival (OS).

In certain embodiments, the pre-established cut-off value for Ang2 protein is within a concentration range of 1866.5 to 6024.5 pg/ml. In some embodiments, the pre-established cut-off value for Ang2 protein is within a concentration range of 1866.5 to 2500 pg/ml. In some embodiments, the pre-established cut-off value for Ang2 protein is within a concentration range of 1866.5 to 3000 pg/ml. In some embodiments, the pre-established cut-off value for Ang2 protein is within a concentration range of 1866.5 to 3500 pg/ml. In other embodiments, the pre-established cut-off value for Ang2 protein is within a concentration range of 2000 to 3000 pg/ml. In other embodiments, the pre-established cut-off value for Ang2 protein is within a concentration range of 2000 to 4000 pg/ml. In other embodiments, the pre-established cut-off value for Ang2 protein is within a concentration range of 2000 to 5000 pg/ml. In other embodiments, the pre-established cut-off value for Ang2 protein is within a concentration range of 3000 to 4000 pg/ml. In certain embodiments, the pre-established cut-off value for Ang2 protein is

within a concentration range of 3000 to 5000 pg/ml. In other embodiments, the pre-established cut-off value for Ang2 protein is within a concentration range of 3000 to 6000 pg/ml. In other embodiments, the pre-established cut-off value for Ang2 protein is within a concentration range of 4000 to 5000 pg/ml. In other embodiments, the pre-established cut-off value for Ang2 protein is within a concentration range of 4000 to 6000 pg/ml. In some embodiments, the pre-established cut-off value for Ang2 protein is a concentration range of 5000 to 6000 pg/ml. In a specific embodiment, the pre-established cut-off value for Ang2 protein is about 2082.5 pg/ml. In all of these embodiments, a concentration of Ang2 protein equal to or below the pre-established cut-off value is a low concentration of Ang2 and a value higher than the pre-established cut-off value is a high concentration of Ang2. In this context "about" means ±10%.

Biological Samples

Suitable biological samples for the methods described herein include any biological fluid, cell, tissue, or fraction thereof, which includes analyte biomolecules of interest such as protein or nucleic acid (e.g., DNA or mRNA). A biological sample can be, for example, a specimen obtained from a subject (e.g., a mammal such as a human) or can be derived from such a subject. For example, a sample can be a tissue section obtained by biopsy, archived tumor tissue, or cells that are placed in or adapted to tissue culture. A biological sample can also be a biological fluid such as blood, plasma, serum, urine, or such a sample absorbed onto a substrate (e.g., glass, polymer, paper). A biological sample can also include an endometrial tissue sample. In specific embodiments, the biological sample is a tumor cell(s) or a tumor tissue obtained from a region of the subject suspected of containing a tumor or a pre-cancerous lesion. For example, the biological sample may be an endometrial tumor sample. A biological sample can be further fractionated, if desired, to a fraction containing particular cell types. For example, a blood sample can be fractionated into serum or into fractions containing particular types of blood cells such as red blood cells or white blood cells (leukocytes). If desired, a sample can be a combination of samples from a subject such as a combination of a tissue and fluid sample.

The biological samples can be obtained from a subject having, suspected of having, or at risk of developing, an endometrial cancer. In certain embodiments, the subject has advanced endometrial cancer. In some embodiments, the subject has recurrent endometrial cancer. In other embodiments, the subject has a stage III endometrial cancer. In certain embodiments, the subject has a stage IV endometrial cancer. In other embodiments, the

subject has an unresectable stage III or stage IV endometrial cancer.

Any suitable methods for obtaining the biological samples can be employed, although exemplary methods include, e.g., phlebotomy, fine needle aspirate biopsy procedure. Samples can also be collected, e.g., by microdissection (e.g., laser capture microdissection (LCM) or laser microdissection (LMD)).

Methods for obtaining and/or storing samples that preserve the activity or integrity of molecules (e.g., nucleic acids or proteins) in the sample are well known to those skilled in the art. For example, a biological sample can be further contacted with one or more additional agents such as buffers and/or inhibitors, including one or more of nuclease, protease, and phosphatase inhibitors, which preserve or minimize changes in the molecules (e.g., nucleic acids or proteins) in the sample. Such inhibitors include, for example, chelators such as ethylenediamine tetraacetic acid (EDTA), ethylene glycol bis(P-aminoethyl ether) N,N,N1,Nl-tetraacetic acid (EGTA), protease inhibitors such as phenylmethylsulfonyl fluoride (PMSF), aprotinin, leupeptin, antipain, and the like, and phosphatase inhibitors such as phosphate, sodium fluoride, vanadate, and the like. Suitable buffers and conditions for isolating molecules are well known to those skilled in the art and can be varied depending, for example, on the type of molecule in the sample to be characterized (see, for example, Ausubel et al. Current Protocols in Molecular Biology (Supplement 47), John Wiley & Sons, New York (1999); Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory Press (1988); Harlow and Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Press (1999); Tietz Textbook of Clinical Chemistry, 3rd ed. Burtis and Ashwood, eds. W.B. Saunders, Philadelphia, (1999)). A sample also can be processed to eliminate or minimize the presence of interfering substances. For example, a biological sample can be fractionated or purified to remove one or more materials that are not of interest. Methods of fractionating or purifying a biological sample include, but are not limited to, chromatographic methods such as liquid chromatography, ion-exchange chromatography, size-exclusion chromatography, or affinity chromatography. For use in the methods described herein, a sample can be in a variety of physical states. For example, a sample can be a liquid or solid, can be dissolved or suspended in a liquid, can be in an emulsion or gel, or can be absorbed onto a material.

Determining Expression Levels/Concentrations of Biomarkers

Gene expression can be detected as, e.g., protein or RNA expression of a target gene. That is, the presence or expression level (amount) of a gene can be determined by

detecting and/or measuring the level of mRNA or protein expression of the gene. In some embodiments, gene expression can be detected as the activity of a protein encoded by a gene such as a gene depicted in Table 1.

In one embodiment, the expression of a gene can be determined by detecting and/or measuring expression or concentration of a protein encoded by the gene. Methods of determining protein expression/concentration are well known in the art. A generally used method involves the use of antibodies specific for the target protein of interest. For example, methods of determining protein expression include, but are not limited to, western blot or dot blot analysis, immunohistochemistry (e.g., quantitative immunohistochemistry), immunocytochemistry, enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunosorbent spot (ELISPOT; Coligan, J. E., et al., eds. (1995) Current Protocols in Immunology. Wiley, New York), radioimmunoassay, chemiluminescent immunoassay, electrochemiluminescence immunoassay, latex turbidimetric immunoassay, latex photometric immunoassay, immuno-chromatographic assay, and antibody array analysis (see, e.g., U.S. Publication Nos. 20030013208 and 2004171068, the disclosures of each of which are incorporated herein by reference in their entirety). Further description of many of the methods above and additional methods for detecting protein expression can be found in, e.g., Sambrook et al. (supra).

In one example, the presence or amount of protein expression of a gene (e.g., a gene depicted in Table 1) can be determined using a western blotting technique. For example, a lysate can be prepared from a biological sample, or the biological sample itself, can be contacted with Laemmli buffer and subjected to sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE-resolved proteins, separated by size, can then be transferred to a filter membrane (e.g., nitrocellulose) and subjected to immunoblotting techniques using a detectably-labeled antibody specific to the protein of interest. The presence or amount of bound detectably-labeled antibody indicates the presence or amount of protein in the biological sample.

In another example, an immunoassay can be used for detecting and/or measuring the protein expression of a gene (e.g., a gene depicted in Table 1). As above, for the purposes of detection, an immunoassay can be performed with an antibody that bears a detection moiety (e.g., a fluorescent agent or enzyme). Proteins from a biological sample can be conjugated directly to a solid-phase matrix (e.g., a multi-well assay plate, nitrocellulose, agarose, sepharose, encoded particles, or magnetic beads) or it can be

conjugated to a first member of a specific binding pair (e.g., biotin or streptavidin) that attaches to a solid-phase matrix upon binding to a second member of the specific binding pair (e.g., streptavidin or biotin). Such attachment to a solid-phase matrix allows the proteins to be purified away from other interfering or irrelevant components of the biological sample prior to contact with the detection antibody and also allows for subsequent washing of unbound antibody. Here as above, the presence or amount of bound detectably-labeled antibody indicates the presence or amount of protein in the biological sample.

There is no particular restriction as to the form of the antibody and the present disclosure includes polyclonal antibodies, as well as monoclonal antibodies. The antiserum obtained by immunizing animals, such as rabbits with a protein or fragment thereof of the invention (i.e., a protein or an immunological fragment thereof from Table 1) , as well polyclonal and monoclonal antibodies of all classes, human antibodies, and humanized antibodies produced by genetic recombination, are also included.

An intact protein or its partial peptide may be used as the antigen for immunization. As partial peptides of the proteins, for example, the amino (N)-terminal fragment of the protein and the carboxy (C)-terminal fragment can be given.

A gene encoding a protein of interest or a fragment thereof (e.g., an immunological fragment) is inserted into a known expression vector, and, by transforming the host cells with the vector described herein, the desired protein or a fragment thereof is recovered from outside or inside the host cells using standard methods. This protein can be used as the sensitizing antigen. Also, cells expressing the protein, cell lysates, or a chemically synthesized protein of the invention may be also used as a sensitizing antigen.

The mammal that is immunized by the sensitizing antigen is not restricted; however, it is preferable to select animals by considering the compatibility with the parent cells used in cell fusion. Generally, animals belonging to the orders rodentia, lagomorpha, or primates are used. Examples of animals belonging to the order of rodentia that may be used include, for example, mice, rats, and hamsters. Examples of animals belonging to the order of lagomorpha that may be used include, for example, rabbits. Examples of animals belonging to the order of primates that may be used include, for example, monkeys. Examples of monkeys to be used include the infraorder catarrhini (old world monkeys), for example, *Macaca fascicularis*, rhesus monkeys, sacred baboons, and chimpanzees.

Well-known methods may be used to immunize animals with the sensitizing antigen. For example, the sensitizing antigen is injected intraperitoneally or subcutaneously

into mammals. Specifically, the sensitizing antigen is suitably diluted and suspended in physiological saline, phosphate-buffered saline (PBS), and so on, and mixed with a suitable amount of general adjuvant if desired, for example, with Freund's complete adjuvant. Then, the solution is emulsified and injected into the mammal. Thereafter, the sensitizing antigen suitably mixed with Freund's incomplete adjuvant is preferably given several times every 4 to 21 days. A suitable carrier can also be used when immunizing and animal with the sensitizing antigen. After the immunization, the elevation in the level of serum antibody is detected by usual methods.

Polyclonal antibodies against the proteins of the present disclosure can be prepared as follows. After verifying that the desired serum antibody level has been reached, blood is withdrawn from the mammal sensitized with antigen. Serum is isolated from this blood using conventional methods. The serum containing the polyclonal antibody may be used as the polyclonal antibody, or according to needs, the polyclonal antibody-containing fraction may be further isolated from the serum. For example, a fraction of antibodies that specifically recognize the protein of the invention may be prepared by using an affinity column to which the protein is coupled. Then, the fraction may be further purified by using a Protein A or Protein G column in order to prepare immunoglobulin G or M.

To obtain monoclonal antibodies, after verifying that the desired serum antibody level has been reached in the mammal sensitized with the above-described antigen, immunocytes are taken from the mammal and used for cell fusion. For this purpose, splenocytes can be mentioned as preferable immunocytes. As parent cells fused with the above immunocytes, mammalian myeloma cells are preferably used. More preferably, myeloma cells that have acquired the feature, which can be used to distinguish fusion cells by agents, are used as the parent cell.

The cell fusion between the above immunocytes and myeloma cells can be conducted according to known methods, for example, the method by Galfre and Milstein (*Methods Enzymol.* 73:3-46, 1981).

The hybridoma obtained from cell fusion is selected by culturing the cells in a standard selection medium, for example, HAT culture medium (medium containing hypoxanthine, aminopterin, and thymidine). The culture in this HAT medium is continued for a period sufficient enough for cells (non-fusion cells) other than the objective hybridoma to perish, usually from a few days to a few weeks. Then, the usual limiting dilution method is carried out, and the hybridoma producing the objective antibody is screened and cloned.

Other than the above method for obtaining hybridomas, by immunizing an animal other than humans with the antigen, a hybridoma producing the objective human antibodies having the activity to bind to proteins can be obtained by the method of sensitizing human lymphocytes, for example, human lymphocytes infected with the EB virus, with proteins, protein-expressing cells, or lysates thereof *in vitro* and fusing the sensitized lymphocytes with myeloma cells derived from human, for example, U266, having a permanent cell division ability.

The monoclonal antibodies obtained by transplanting the obtained hybridomas into the abdominal cavity of a mouse and extracting ascites can be purified by, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE ion exchange chromatography, an affinity column to which the protein of the present disclosure is coupled, and so on.

Monoclonal antibodies can be also obtained as recombinant antibodies produced by using the genetic engineering technique (see, for example, Borrebaeck C.A.K. and Larrick, J.W., THERAPEUTIC MONOCLONAL ANTIBODIES, Published in the United Kingdom by MACMILLAN PUBLISHERS LTD (1990)). Recombinant antibodies are produced by cloning the encoding DNA from immunocytes, such as hybridoma or antibody-producing sensitized lymphocytes, incorporating into a suitable vector, and introducing this vector into a host to produce the antibody. The present disclosure encompasses such recombinant antibodies as well.

Antibodies or antibody fragments specific for a protein encoded by one or more biomarkers can also be generated by *in vitro* methods such as phage display.

Moreover, the antibody of the present disclosure may be an antibody fragment or modified-antibody, so long as it binds to a protein encoded by a biomarker of the invention. For instance, Fab, F (ab') 2, Fv, or single chain Fv (scFv) in which the H chain Fv and the L chain Fv are suitably linked by a linker (Huston et al., *Proc. Natl. Acad. Sci. USA*, 85:5879-5883, (1988)) can be given as antibody fragments. Specifically, antibody fragments are generated by treating antibodies with enzymes, for example, papain or pepsin. Alternatively, they may be generated by constructing a gene encoding an antibody fragment, introducing this into an expression vector, and expressing this vector in suitable host cells (see, for example, Co et al., *J. Immunol.*, 152:2968-2976, 1994; Better et al., *Methods Enzymol.*, 178:476-496, 1989; Pluckthun et al., *Methods Enzymol.*, 178:497-515, 1989; Lamoyi, *Methods Enzymol.*, 121:652-663, 1986; Rousseaux et al., *Methods Enzymol.*,

121:663-669, 1986; Bird et al., *Trends Biotechnol.*, 9:132-137, 1991).

The antibodies may be conjugated to various molecules, such as fluorescent substances, radioactive substances, and luminescent substances. Methods to attach such moieties to an antibody are already established and conventional in the field (see, e.g., US 5,057,313 and 5,156,840).

Examples of methods that assay the antigen-binding activity of the antibodies include, for example, measurement of absorbance, enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), and/or immunofluorescence. For example, when using ELISA, a protein encoded by a biomarker of the invention is added to a plate coated with the antibodies of the present disclosure, and then, the antibody sample, for example, culture supernatants of antibody-producing cells, or purified antibodies are added. Then, secondary antibody recognizing the primary antibody, which is labeled by alkaline phosphatase and such enzymes, is added, the plate is incubated and washed, and the absorbance is measured to evaluate the antigen-binding activity after adding an enzyme substrate such as p-nitrophenyl phosphate. As the protein, a protein fragment, for example, a fragment comprising a C-terminus, or a fragment comprising an N-terminus may be used. To evaluate the activity of the antibody of the invention, BIAcore (GE Healthcare) may be used.

By using these methods, the antibody of the invention and a sample presumed to contain a protein of the invention are contacted, and the protein encoded by a biomarker of the invention is detected or assayed by detecting or assaying the immune complex formed between the above-mentioned antibody and the protein.

Mass spectrometry based quantitation assay methods, for example, but not limited to, multiple reaction monitoring (MRM)-based approaches in combination with stable-isotope labeled internal standards, are an alternative to immunoassays for quantitative measurement of proteins. These approaches do not require the use of antibodies and so the analysis can be performed in a cost- and time- efficient manner (see, for example, Addona et al., *Nat. Biotechnol.*, 27:633–641, 2009; Kuzyk et al., *Mol. Cell Proteomics*, 8:1860–1877, 2009; Paulovich et al., *Proteomics Clin. Appl.*, 2:1386-1402, 2008). In addition, MRM offers superior multiplexing capabilities, allowing for the simultaneous quantification of numerous proteins in parallel. The basic theory of these methods has been well-established and widely utilized for drug metabolism and pharmacokinetics analysis of small molecules.

In another embodiment, the expression level of a gene of interest is determined by

measuring RNA levels. A variety of suitable methods can be employed to detect and/or measure the level of mRNA expression of a gene. For example, mRNA expression can be determined using Northern blot or dot blot analysis, reverse transcriptase-PCR (RT-PCR; e.g., quantitative RT-PCR), *in situ* hybridization (e.g., quantitative *in situ* hybridization) or nucleic acid array (e.g., oligonucleotide arrays or gene chips) analysis. Details of such methods are described below and in, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual Second Edition vol. 1, 2 and 3. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, USA, Nov. 1989; Gibson et al. (1999) *Genome Res.*, 6(10):995-1001; and Zhang et al. (2005) *Environ. Sci. Technol.*, 39(8):2777-2785; U.S. Publication No. 2004086915; European Patent No. 0543942; and U.S. Patent No. 7,101,663; the disclosures of each of which are incorporated herein by reference in their entirety.

In one example, the presence or amount of one or more discrete mRNA populations in a biological sample can be determined by isolating total mRNA from the biological sample (see, e.g., Sambrook et al. (supra) and U.S. Patent No. 6,812,341) and subjecting the isolated mRNA to agarose gel electrophoresis to separate the mRNA by size. The size-separated mRNAs are then transferred (e.g., by diffusion) to a solid support such as a nitrocellulose membrane. The presence or amount of one or more mRNA populations in the biological sample can then be determined using one or more detectably-labeled-polynucleotide probes, complementary to the mRNA sequence of interest, which bind to and thus render detectable their corresponding mRNA populations. Detectable-labels include, e.g., fluorescent (e.g., umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, allophycocyanin (APC), or phycoerythrin), luminescent (e.g., europium, terbium, Qdot™ nanoparticles supplied by the Quantum Dot Corporation, Palo Alto, CA), radiological (e.g., ^{125}I, ^{131}I, ^{35}S, ^{32}P, ^{33}P, or ^{3}H), and enzymatic (horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase) labels.

In another example, the presence or amount of discrete populations of mRNA (e.g., mRNA encoded by one or more genes depicted in Table 1) in a biological sample can be determined using nucleic acid (or oligonucleotide) arrays (e.g., an array described below under "Arrays"). For example, isolated mRNA from a biological sample can be amplified using RT-PCR with, e.g., random hexamer or oligo(dT)-primer mediated first strand synthesis. The amplicons can be fragmented into shorter segments. The RT-PCR step can be used to detectably-label the amplicons, or, optionally, the amplicons can be

detectably-labeled subsequent to the RT-PCR step. For example, the detectable-label can be enzymatically (e.g., by nick-translation or kinase such as T4 polynucleotide kinase) or chemically conjugated to the amplicons using any of a variety of suitable techniques (see, e.g., Sambrook et al., supra). The detectably-labeled-amplicons are then contacted with a plurality of polynucleotide probe sets, each set containing one or more of a polynucleotide (e.g., an oligonucleotide) probe specific for (and capable of binding to) a corresponding amplicon, and where the plurality contains many probe sets each corresponding to a different amplicon. Generally, the probe sets are bound to a solid support and the position of each probe set is predetermined on the solid support. The binding of a detectably-labeled amplicon to a corresponding probe of a probe set indicates the presence or amount of a target mRNA in the biological sample. Additional methods for detecting mRNA expression using nucleic acid arrays are described in, e.g., U.S. Patent Nos. 5,445,934; 6,027,880; 6,057,100; 6,156,501; 6,261,776; and 6,576,424; the disclosures of each of which are incorporated herein by reference in their entirety.

Methods of detecting and/or for quantifying a detectable label depend on the nature of the label. The products of reactions catalyzed by appropriate enzymes (where the detectable label is an enzyme; see above) can be, without limitation, fluorescent, luminescent, or radioactive or they may absorb visible or ultraviolet light. Examples of detectors suitable for detecting such detectable labels include, without limitation, x-ray film, radioactivity counters, scintillation counters, spectrophotometers, colorimeters, fluorometers, luminometers, and densitometers.

Methods for detecting or measuring gene expression (e.g., protein or mRNA expression) can optionally be performed in formats that allow for rapid preparation, processing, and analysis of multiple samples. This can be, for example, in multi-welled assay plates (e.g., 96 wells or 386 wells) or arrays (e.g., nucleic acid chips or protein chips). Stock solutions for various reagents can be provided manually or robotically, and subsequent sample preparation (e.g., RT-PCR, labeling, or cell fixation), pipetting, diluting, mixing, distribution, washing, incubating (e.g., hybridization), sample readout, data collection (optical data) and/or analysis (computer aided image analysis) can be done robotically using commercially available analysis software, robotics, and detection instrumentation capable of detecting the signal generated from the assay. Examples of such detectors include, but are not limited to, spectrophotometers, luminometers, fluorimeters, and devices that measure radioisotope decay. Exemplary high-throughput cell-based assays (e.g., detecting the

presence or level of a target protein in a cell) can utilize ArrayScan® VTI HCS Reader or KineticScan® HCS Reader technology (Cellomics Inc., Pittsburg, PA).

In some embodiments, the expression level of two genes, three genes, four genes, five genes, six genes, seven genes, eight genes, nine genes, 10 genes, 11 genes, or at least two genes, at least three genes, at least four genes, at least five genes, at least six genes, at least seven genes, at least eight genes, at least nine genes, or at least 10 genes from Table 1 can be assessed and/or measured.

To aid in detecting the presence or level of expression of one or more of the genes depicted in Table 1, any part of the nucleic acid sequence of the genes can be used, e.g., as hybridization polynucleotide probes or primers (e.g., for amplification or reverse transcription). The probes and primers can be oligonucleotides of sufficient length to provide specific hybridization to an RNA, DNA, cDNA, or fragments thereof isolated from a biological sample. Depending on the specific application, varying hybridization conditions can be employed to achieve varying degrees of selectivity of a probe or primer towards target sequence. The primers and probes can be detectably-labeled with reagents that facilitate detection (e.g., fluorescent labels, chemical labels (see, e.g., U.S. Patent Nos. 4,582,789 and 4,563,417), or modified bases).

Standard stringency conditions are described by Sambrook, et al. (supra) and Haymes, et al. Nucleic Acid Hybridization, A Practical Approach, IRL Press, Washington, D.C. (1985). In order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular hybridization conditions (e.g., solvent and salt concentrations) employed.

Hybridization can be used to assess homology between two nucleic acid sequences. A nucleic acid sequence described herein, or a fragment thereof, can be used as a hybridization probe according to standard hybridization techniques. The hybridization of a probe of interest (e.g., a probe containing a portion of a nucleotide sequence described herein or its complement) to DNA, RNA, cDNA, or fragments thereof from a test source is an indication of the presence of DNA or RNA corresponding to the probe in the test source. Hybridization conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 6.3.1-6.3.6, 1991. Moderate hybridization conditions are defined as hybridization in 2X sodium chloride/sodium citrate (SSC) at 30°C, followed by a wash in 1 X SSC, 0.1% SDS at 50°C. Highly stringent

conditions are defined as hybridization in 6X SSC at 45°C, followed by a wash in 0.2 X SSC, 0.1% SDS at 65°C.

Primers can be used in in a variety of PCR-type methods. For example, polymerase chain reaction (PCR) techniques can be used to amplify specific sequences from DNA as well as RNA, including sequences from total genomic DNA or total cellular RNA. The PCR primers are designed to flank the region that one is interested in amplifying. Primers can be located near the 5' end, the 3' end or anywhere within the nucleotide sequence that is to be amplified. The amplicon length is dictated by the experimental goals. For qPCR, the target length is closer to 100 bp and for standard PCR, it is near 500 bp. Generally, sequence information from the ends of the region of interest or beyond is employed to design oligonucleotide primers that are identical or similar in sequence to opposite strands of the template to be amplified. PCR primers can be chemically synthesized, either as a single nucleic acid molecule (e.g., using automated DNA synthesis in the 3' to 5' direction using phosphoramidite technology) or as a series of oligonucleotides. For example, one or more pairs of long oligonucleotides (e.g., >100 nucleotides) can be synthesized that contain the desired sequence, with each pair containing a short segment of complementarity (e.g., about 15 nucleotides) such that a duplex is formed when the oligonucleotide pair is annealed. DNA polymerase is used to extend the oligonucleotides, resulting in a single, double-stranded nucleic acid molecule per oligonucleotide pair.

In addition, the nucleic acid sequences or fragments thereof (e.g., oligonucleotide probes) can be used in nucleic acid arrays (such as the nucleic acid arrays described below under "Arrays") for detection and/or quantitation of gene expression.

Creating A Response Profile

The methods described herein can also be used to generate a lenvatinib compound (e.g., lenvatinib mesylate) therapy response profile for a subject having endometrial cancer. The profile can include, e.g., information that indicates the expression level of one or more genes (e.g., one or more genes depicted in Table 1) pre-and post-treatment with lenvatinib or a pharmaceutically acceptable salt thereof; and/or the histological analysis of any endometrial tumors. The response profiles described herein can contain information on the expression or expression level of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 genes listed in Table 1. The resultant information (lenvatinib therapy response profile) can be used for predicting the response of a subject (e.g., a human patient) having, suspected of having or at risk of developing

endometrial cancer to a treatment comprising a lenvatinib compound (e.g., lenvatinib mesylate).

It is understood that a lenvatinib compound (e.g., lenvatinib mesylate) response profile can be in electronic form (e.g., an electronic patient record stored on a computer or other electronic (computer-readable) media such as a DVD, CD, or floppy disk) or written form. The lenvatinib compound (e.g., lenvatinib mesylate) response profile can also include information for several (e.g., two, three, four, five, 10, 20, 30, 50, or 100 or more) subjects (e.g., human patients). Such multi-subject response profiles can be used, e.g., in analyses (e.g., statistical analyses) of particular characteristics of subject cohorts.

Responsiveness of a subject to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) can be classified in several ways and classification is dependent on the subject's disease (e.g., advanced or recurrent endometrial cancer), the severity of the disease, and the particular medicament the subject is administered. In the simplest sense, responsiveness is any decrease in the disease state as compared to pre-treatment, and non-responsiveness is the lack of any change in the disease state as compared to pre-treatment. Responsiveness of a subject (e.g., a human) with an endometrial cancer can be classified based on one or more of a number of objective clinical indicia such as, but not limited to, tumor size, Clinical Benefit (CB), Progression Free Survival (PFS), Overall Survival (OS), % of maximum tumor Shrinkage (MTS), or Objective Response Rate (ORR).

"Clinical benefit" refers to having one of the following statuses – Complete Response (CR), Partial Response (PR); or Stable Disease (SD) with 6 months or more progression free survival (PFS). "Complete Response" means complete disappearance of all target lesions. "Partial Response" means at least 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline summed LD. "Progressive Disease" (PD) means at least 20% increase in the sum of the LD of target lesions, taking as reference the smallest summed LD recorded since the treatment started, or the appearance of one or more new lesions. "Stable Disease" means neither sufficient shrinkage of the target lesions to qualify for PR nor sufficient increase to qualify for progressive disease (PD), taking as reference the smallest summed LD since the treatment started.

"Overall Survival" (OS) is defined as the time from randomization until death from any cause. "Randomization" means randomization of a patient into a test group or a control

group when therapy plan for a patient is determined.

"Progression Free Survival" (PFS) refers to the period from start date of treatment to the last date before entering PD status.

"% of Maximum Tumor shrinkage" (MTS) means percent change of sum of diameters of target lesions, taking as reference the baseline sum diameters.

"Objective Response Rate " (ORR) compares subjects with either Complete Response (CR) or Partial Response (PR) with subjects with either Stable Disease (SD) or Progressive Disease (PD).

Methods of Treatment

The methods disclosed herein enable the assessment of whether or not a subject having, suspected of having or at risk of developing endometrial cancer is likely to respond to a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate). A subject having, suspected of having or at risk of developing endometrial cancer who is likely to respond to a lenvatinib compound can be administered lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate). Conversely, a subject having, suspected of having or at risk of developing endometrial cancer who is not likely to respond to a lenvatinib compound can be administered a different therapy that is suitable for treatment of endometrial cancer.

The methods of this disclosure also enable the stratification of subjects having, suspected of having or at risk of developing endometrial cancer into groups of subjects that are more likely to benefit, and groups of subjects that are less likely to benefit, from treatment comprising a lenvatinib compound (e.g., lenvatinib mesylate). The ability to select such subjects from a pool of endometrial cancer subjects who are being considered for treatment with a lenvatinib compound is beneficial for administering an effective treatment to the subject.

Lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) shows potent anti-tumor effects, in part, by inhibiting angiogenesis. The subjects who are considered for treatment comprising a lenvatinib compound (e.g., lenvatinib mesylate) include, but are not limited to, subjects having, suspected of having, or likely to develop an endometrial cancer. In one embodiment, the subject to be treated with lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) has, is suspected of having, or is likely to develop advanced endometrial cancer. In certain embodiments, the subject to be treated with a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate) has, is suspected of having, or is likely to develop a recurrent endometrial cancer.

In other embodiments, the subject to be treated with a therapy comprising a lenvatinib compound has, is suspected of having, or is likely to develop a stage I endometrial cancer. In one embodiment, the subject to be treated with a therapy comprising a lenvatinib compound has, is suspected of having, or is likely to develop a stage II endometrial cancer. In another embodiment, the subject to be treated with a therapy comprising a lenvatinib compound has, is suspected of having, or is likely to develop a stage III endometrial cancer. In another embodiment, the subject to be treated with a therapy comprising a lenvatinib compound has, is suspected of having, or is likely to develop a stage IV endometrial cancer. In some embodiments, the endometrial cancer is a nonresectable stage III or stage IV cancer.

If the subject having an endometrial cancer is more likely to respond to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (based on concentrations of one or more of the biomarkers described above (e.g., Ang2 protein)), the subject can then be administered an effective amount of the lenvatinib compound (e.g., lenvatinib mesylate). An effective amount of the compound can suitably be determined by a health care practitioner taking into account, for example, the characteristics of the patient (age, sex, weight, race, etc.), the progression of the disease, and prior exposure to the drug. If the subject is less likely to respond to a therapy comprising a lenvatinib compound, the subject can then be optionally administered a therapy that does not comprise lenvatinib. These therapies include, but are not limited to, radioactive iodine, doxorubicin, carboplatin, cisplatin, paclitaxel, sorafenib, docetaxel, trastumab, interleukin-2, interferon, everolimus, sunitinib, pazopanib, vandetanib, and "standard of care" treatment (i.e., prevailing standard of care as determined by the health care practitioner or as specified in the clinical study) such as investigational drugs and chemotherapy.

Subjects of all ages can be affected by disorders treatable by lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate). Therefore, a biological sample used in a methods described herein can be obtained from a subject (e.g., a human) of any age, including a child, an adolescent, or an adult, such as an adult having, suspected of having, or at risk of developing an endometrial cancer.

The methods can also be applied to individuals at risk of developing an endometrial cancer treatable by lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate). Such individuals include those who have (i) a family history of (a genetic predisposition for) such disorders or (ii) one or more risk factors for developing such disorders.

After stratifying or selecting a subject based on whether the subject will be more likely or less likely to respond to lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate), a medical practitioner (e.g., a doctor) can administer the appropriate therapeutic modality to the subject. Methods of administering lenvatinib therapies are well known in the art.

It is understood that any therapy described herein (e.g., a therapy comprising a lenvatinib or a therapy that does not comprise a lenvatinib) can include one or more additional therapeutic agents. That is, any therapy described herein can be co-administered (administered in combination) with one or more additional therapeutic agents such as, but not limited to, doxorubicin, carboplatin, cisplatin, paclitaxel, docetaxel, trastumab, and everolimus. Furthermore, any therapy described herein can include one or more agents for treating, for example, pain, nausea, and/or one or more side-effects of a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate).

Combination therapies (e.g., co-administration of a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) and one or more additional therapeutic agents) can be, e.g., simultaneous or successive. For example, lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) and one or more additional therapeutic agents can be administered at the same time or a lenvatinib compound (e.g., lenvatinib mesylate) can be administered first in time and the one or more additional therapeutic agents administered second in time. In some embodiments, the one or more additional therapeutic agents can be administered first in time and lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) administered second in time.

In cases where the subject having endometrial cancer and predicted to respond to a lenvatinib compound (e.g., lenvatinib mesylate) therapy has been previously administered one or more non-lenvatinib therapies, the therapy comprising a lenvatinib compound can replace or augment a previously or currently administered therapy. For example, upon treating with the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate), administration of the one non-lenvatinib therapies can cease or diminish, e.g., be administered at lower levels. Administration of the previous therapy can be maintained while the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) is administered. In some embodiments, a previous therapy can be maintained until the level of the therapy comprising lenvatinib or a

pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) reaches a level sufficient to provide a therapeutic effect.

Arrays

Nucleic acid arrays including the nucleic acid biomarkers disclosed herein are useful in, e.g., detecting gene expression and/or measuring gene expression levels. The arrays are also useful for e.g., in predicting the response of a subject having endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate), for identifying subjects having endometrial cancer who can benefit from a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate), and for steering subjects having endometrial cancer who would not likely benefit from a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof (e.g., lenvatinib mesylate) to other cancer therapies.

An array is an orderly arrangement of samples where matching of known and unknown DNA samples is done based on base pairing rules (e.g., Adenosine pairs with Thymine or Uracil; Guanosine pairs with Cytosine). A typical microarray experiment involves the hybridization of an mRNA, a cDNA molecule, or fragments thereof, to a DNA template from which it is originated or derived. Many DNA samples are used to construct an array. DNAs from at least one, two, three, four, five, six, seven, eight, nine, ten, or eleven of the genes in Table 1 can be used to construct the array. An array experiment makes use of common assay systems such as microplates or standard blotting membranes. The sample spot sizes are typically less than 200 microns in diameter and the array usually contains thousands of spots. Thousands of spotted samples known as probes (with known identity) are immobilized on a substrate (e.g., a microscope glass slides, silicon chips, nylon membrane). The spots can be DNA, cDNA, or oligonucleotides. These are used to determine complementary binding of the unknown sequences thus allowing parallel analysis for gene expression and gene discovery. An experiment with a single DNA chip can provide information on thousands of genes simultaneously. An orderly arrangement of the probes on the support is important as the location of each spot on the array is used for the identification of a gene. The amount of mRNA bound to each site on the array indicates the expression level of the various genes that are included on the array. By using an array containing many DNA samples, one can determine, in a single experiment, the expression levels of hundreds or thousands of genes by measuring the amount of mRNA bound to each site on the array. With the aid of a computer, the amount of mRNA bound to the spots on the

microarray can be precisely measured, generating a profile of gene expression in the cell.

The two main DNA microarray platforms that are generally used are cDNA and oligonucleotide microarrays. cDNA microarrays are made with long double-stranded DNA molecules generated by enzymatic reactions such as PCR (Schena,M. et al., *Science*, 270:467–470 (1995)), while oligonucleotide microarrays employ oligonucleotide probes spotted by either robotic deposition or *in situ* synthesis on a substrate (Lockhart,D.J. et al., *Nat. Biotechnol.*, 14, 1675–1680 (1996)).

Kits

This application also provides kits. In certain embodiments, the kit can include an antibody or antibodies that can be used to detect one or more of the biomarkers listed in Table 1 or their concentration or expression levels. For example, the kit can include an antibody that specifically binds Ang2. The antibodies in the kit may be monoclonal or polyclonal and can be further conjugated with a detectable label. In some embodiments, the kit includes probes that can be used to identify or detect any of the biomarkers of Table 1. In some embodiments, the kit includes any of the nucleic acid arrays described herein. In some embodiments, the kit includes probes and antibodies that can be used to identify or detect any of the biomarkers of Table 1 or their expression or expression levels. The kits can, optionally, contain instructions for detecting and/or measuring the concentration of one or more proteins or the levels of mRNA in a biological sample.

The kits can optionally include, e.g., a control (e.g., a concentration standard for the protein being assessed) or control labeled-amplicon set containing known amounts of one or more amplicons recognized by nucleic acid probes of the array. In some instances, the control can be an insert (e.g., a paper insert or electronic medium such as a CD, DVD, or floppy disk) containing an expression level or expression level ranges of one or more proteins or RNAs predictive of a response to a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate).

In some embodiments, the kits can include one or more reagents for processing a biological sample (e.g., calibration reagents, buffers, diluents, color reagents, reagents to stop a reaction). For example, a kit can include reagents for isolating a protein from a biological sample and/or reagents for detecting the presence and/or amount of a protein in a biological sample (e.g., an antibody that binds to the protein that is the subject of the detection assay and/or an antibody that binds the antibody that binds to the protein).

In certain embodiments, the kit includes at least one microplate (e.g., a 96 well

plate; i.e., 12 strips of 8 wells). The microplate can be provided with its corresponding plate cover. The microplate can be polystyrene or of any other suitable material. The microplate can have the antibody that is used to identify the presence of a particular biomarker coated inside each well. The antibody may be conjugated to a detectable label. The kit may also include at least one adhesive strip.

In some embodiments, the kits can include a software package for analyzing the results of, e.g., expression profile or a microarray analysis.

The kits can also include one or more antibodies for detecting the protein expression of any of the genes described herein. For example, a kit can include (or in some cases consist of) one or a plurality of antibodies capable of specifically binding to one or more proteins encoded by any of the genes depicted in Table 1 and optionally, instructions for detecting and/or measuring the concentration of one or more proteins and/or a detection antibody comprising a detectably-labeled antibody that is capable of binding to at least one antibody of the plurality. In some embodiments, the kits can include antibodies that recognize one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least 10, or 11 of the proteins encoded by genes listed in Table 1.

In certain embodiments, the kit can also optionally include one or more unit doses of a lenvatinib compound (e.g., lenvatinib mesylate).

The kits described herein can also, optionally, include instructions for administering a therapy comprising a lenvatinib compound, where the concentration of one or more proteins or expression level of one or more RNAs predicts that a subject having, suspected of having or at risk of developing endometrial cancer will respond to a therapy comprising a lenvatinib compound (e.g., lenvatinib mesylate).

In a specific embodiment, the kit comprises one or more of the following:

(i) a microplate (e.g., a 96 well plate). The microplate can be coated with an anti-Ang2 antibody that is conjugated with a detectable label. The anti-Ang2 antibody may monoclonal or polyclonal. The antibody can be e.g., from mouse, rabbit, rat, or guinea pig. The detectable label can be e.g., horse radish peroxidase, biotin, a fluorescent moiety, a radioactive moiety, a histidine tag, or a peptide tag. The microplate can be provided with a cover and optionally, one or more adhesive strips.

(ii) a vial containing anti-Ang2 conjugated with a detectable label. The detectable label can be e.g., horse radish peroxidase, biotin, a fluorescent moiety, a histidine tag, a peptide tag. The vial can also include a preservative.

(iii) a vial containing an Ang2 standard of known concentration. The Ang2 can be a recombinant human Ang2.

(iv) a vial containing an assay diluent.

(v) a vial containing a calibrator diluent.

(vi) a vial containing wash buffer. The buffer may be provided as a concentrate.

(vii) one or more vials containing color reagents

(viii) a vial containing a stop solution to stop the colorimetric reaction.

The following are examples of the practice of the invention. They are not to be construed as limiting the scope of the invention in any way.

EXAMPLES

Example 1: *Identifying Predictive Biomarkers for Selecting Endometrial Cancer Patients for Treatment with E7080 (lenvatinib mesylate)*

Purpose: Angiogenesis is regulated by signaling through multiple growth factor receptors, such as VEGF receptor and FGF receptor. VEGF receptor signaling is also associated with immune cell function. E7080 is an oral angiogenesis inhibitor targeting multiple receptor tyrosine kinase; VEGFR1-3, FGFR1-4, RET, KIT, and PDGFRβ. A phase II study was performed in patients with advanced or recurrent endometrial cancer (EC) following 1 or 2 prior platinum-based treatments (Tx). The importance of angiogenesis in EC highlights the need to understand clinical mechanisms of escape from anti-angiogenic therapy. This clinical biomarker analysis was conducted to identify predictive markers of clinical benefit in EC patients upon E7080 treatment. Circulating cytokine and angiogenic factors (CAFs) can be measured in blood samples using ELISA and multiplex assay platforms. The CAFs examined in this study are listed below in Table 2. Numbers in parenthesis indicate bead region for each analyte.

Table 2: List of CAFs Examined

Ang-1	sTie-2	G-CSF(12)	IL-1β(24)	MIP-1β(66)
Ang-2	EGF(2)	GM-CSF(14)	IL-1ra(26)	PDGFAB(68)
FGF-23	ANG2(90)	HGF(86)	IL-2(28)	PDGFBB(73)
PDGF-AA	EGF(80)	IFN-γ(20)	IL-4(32)	PGF(91)
RANTES	Eotaxin(4)	IL-10(44)	IL-6(36)	sIL-2Rα(76)
sCD40L	FGF-2(6)	IL-12(p40)(46)	IL-7(38)	TGFα(78)
SDF-1α	FGF2(79)	IL-12(p70)(48)	IL-8(40)	TNFα(80)
sVEGFR1	FGF4(75)	IL-13(50)	IP-10(56)	VEGF(86)
sVEGFR2	FLT3LG(89)	IL-17(54)	MCP-1(58)	VEGFA(100)
sVEGFR3	fractalkine(10)	IL-1α(22)	MIP-1α(64)	VEGFD(78)

The purpose of this analysis was to measure cytokine, chemokine and angiogenic factors in blood samples, such as plasma and serum, obtained from patients in clinical trials at both pre- and post-treatment with E7080 and to identify blood biomarkers which can be used to predict whether patients will respond to treatment with E7080. For these analyses, the following criteria of response were employed, namely:

(a) Tumor response: objective response rate (ORR) and % of maximum tumor shrinkage (MTS); and

(b) Survival Benefits: progression free survival (PFS) /overall survival (OS).

The criteria of response are defined below.

"Complete Response" means complete disappearance of all target lesions.

"Partial Response" means at least 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline summed LD.

"Progressive Disease" (PD) means at least 20% increase in the sum of the LD of target lesions, taking as reference the smallest summed LD recorded since the treatment started, or the appearance of one or more new lesions.

"Stable Disease" means neither sufficient shrinkage of the target lesions to qualify for PR nor sufficient increase to qualify for progressive disease (PD), taking as reference the smallest summed LD since the treatment started.

"Objective Response Rate" (ORR) compares subjects with either "Complete Response (CR) or "Partial Response" (PR) with subjects to with either Stable Disease (SD) or Progressive Disease (PD).

"% of Maximum Tumor shrinkage" (MTS) means percent change of sum of diameters of target lesions, taking as reference the baseline sum diameters and correlation of

gene mutation to TS is analyzed by Pearson product-moment correlation coefficient and Spearman's rank correlation coefficient test.

"Progression Free Survival" (PFS) refers to the period from start date of treatment to the last date before entering PD status and correlation of gene mutation to PFS is analyzed by Logrank test and Cox proportional hazards model.

"Overall Survival" (OS) refers to the time from randomization until death from any cause. "Randomization" means randomization of a patient into a test group or a control group when the therapy plan for a patient is determined.

Materials and Methods: Patients who had metastatic/unresectable endometrial cancer after 1 or 2 prior platinum-based treatments received lenvatinib until disease progression or development of unmanageable toxicities. Patients received E7080 at a starting dose of 24 mg orally once daily in 28 day cycles. 133 patients were treated and evaluated for efficacy and molecular correlative analysis. This study was a part of the Phase 2 multicenter study of lenvatinib (ClinicalTrial.gov Identifier: NCT01111461). Baseline and post-treatment plasma samples were collected for molecular analyses. Plasma samples were collected at Cycle 1 Day 1 (pre-treatment) and Cycle 2 Day 1 (i.e., day 39 post-treatment). Six mL of intravenous blood was drawn into EDTA vacutainer tubes. The tubes were gently inverted 6-8 times and then centrifuged at 3000 RPM for 10 minutes to separate the plasma from the red cell layer. Plasma was collected from the tube and dispensed into a 3.5 mL cryovial using a transfer pipette, and then stored immediately at -20°C. The plasma samples were shipped on dry ice and then ultimately transferred to -80°C storage. Plasma samples from 122 patients were used for blood biomarker analysis. Serum from Cycle 1 day 1 (baseline), and cycle 2 day 1 were used in this analysis. The association of progression free survival (PFS) and overall survival (OS) were analyzed. Plasma samples were tested in batch format where all timepoints from the same subject were assayed on the same day. On the day of assay, samples were removed from -80°C and allowed to thaw and reach room temperature. The plasma samples were tested in a panel of ELISA and multiplex kit as per the manufacturer's instructions. Table 3 describes the assay kits used in the analysis. The ELISA plates were measured using a Molecular Devices UVmax kinetic microplate reader with SoftMax Pro 5.2 software. The multiplex assays were performed using the Bio-Rad Bio-Plex system with Bio-Plex Manager 4.1 software. Final protein concentrations (pg/mL) were calculated from the standard curve for each assay. Depending on the assay, plasma samples may have been diluted in assay buffer

prior to testing. In these cases, protein concentrations were multiplied by the dilution factor.

Table 3. Assay Kits

Name	Manufacturer	Catalog #	Analytes Measured
Angiopoietin-1 ELISA	R&D Systems Inc.	DANG10	Ang-1
Angiopoietin-2 ELISA	R&D Systems Inc.	DANG20	Ang-2
FGF-23 ELISA	Millipore Inc.	EZHFGF23-32k	FGF-23
SDF-1a ELISA	R&D Systems Inc.	DSA00	SDF-1 alpha
Tie-2 ELISA	R&D Systems Inc.	DTE200	Tie-2
Human cytokine multiplex	Millipore Inc.	MPXHCYTO-60k-03	sCD40L, PDGFAA, RANTES
VEGF receptor multiplex	Millipore Inc.	HSCR-32k	VEGFR1, VEGFR2, VEGFR3
Human growth factor multiplex	Origene Inc.	AM100096	PDGF-AB (68), PDGF-BB (73), FGF4 (75), VEGF-D (78), FGF2 (79), EGF (80), HGF (86), FLT3 LG (89), ANG2 (90), PGF (91), VEGF-A (100)
Human cytokine multiplex	Millipore Inc.	MPXHCYTO-60k-28	EGF, Eotaxin, FGF-2, G-CSF, GM-CSF, IFN-g, IL-1a, IL-1b, IL-1ra, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-17, IP-10, MCP-1, MIP-1a, MIP-1b, TGFa, TNFa, VEGF, Fractalkine, sIL-2Ra

Numbers in parenthesis indicate bead region for each analyte.

Results and Discussion: From 133 patient samples, samples from 122 patients were used for analyses. Significant changes in levels of 19 CAFs among 50 CAFs tested were observed at post-treatment (cycle 2 day 1) in the plasma from patients treated with E7080 compared to pre-treatment levels (cycle 1 day 1) (baseline) (**Fig.1**).

Cox proportional hazard model was performed to identify blood biomarkers that predict progression free survival by baseline levels of CAFs. Baseline levels of ANG2(90), Ang-2, HGF(86), IL-8(40), MCP-1(58), MIP-1a(58), PGF(91), sIL-2Ra(76), Tie-2, TNFa(80), and VEGFA(100) were significantly associated to longer OS, indicating that these

factors can be used as biomarkers for prognosis of disease or prediction of response to E7080 therapy (**Table 4**).

Table 4. Baseline levels of Blood Biomarkers associated with OS

param	N	N.OOR	%OOR	p.likelihood.ratio	p.wald	p.score	HRperSD	HRpersD.CI
ANG2(90)	122	0	0	<0.001	<0.001	<0.001	1.612716	1.2541-2.0738
Ang-2	122	0	0	<0.001	<0.001	<0.001	1.7951	1.4163-2.2751
HGF(86)	122	0	0	<0.001	<0.001	<0.001	1.498094	1.2195-1.8403
IL-8(40)	122	12	9.8	<0.001	<0.001	<0.001	1.873316	1.485-2.3631
MCP-1 (58)	122	0	0	0.006	0.005	0.005	1.449622	1.1178-1.8799
MIP-1a(64)	122	20	16.4	0.042	0.049	0.048	1.297641	1.0007-1.6826
PGF(91)	122	7	5.7	0.001	0.001	0.001	1.450188	1.1731-1.7927
sIL-2Ra (76)	122	16	13.1	0.001	0.001	0.001	1.611362	1.2029-2.1585
Tie-2	122	0	0	0.008	0.007	0.007	1.397993	1.094-1.7865
TNFa(80)	122	5	4.1	<0.001	<0.001	<0.001	1.7863	1.3194-2.4183
VEGFA (100)	122	0	0	0.006	0.004	0.004	1.377439	1.1069-1.714

OS: univariate Cox proportional hazard model, HRperSD:hazard ratio for 1 S.D. increase
OOR = out of range

Quartile sub-group analysis based on baseline CAF levels showed lowest baseline groups of ANG2(90), Ang-2, HGF(86), IL-8(40), MCP-1(58), MIP-1a(58), PGF(91), sIL-2Ra(76), Tie-2, and TNFa(80) had longest median OS, while highest baseline groups of Ang-2, HGF(86), IL-8(40), MCP-1(58), MIP-1a(58), PGF(91), sIL-2Ra(76), Tie-2, TNFa(80), and VEGFA(100) had shortest median OS (**Table 5**).

Table 5. Median OS with 4 subgroups based on baseline CAF

Low Expression → High Expression

	mOS (day) in each quartile			
	1st (low)	2nd	3rd	4th (high)
ANG2(90)	541	353	256	260
Ang-2	541	289	268	173
IL-8(40)	NA	315	340	127
HGF(86)	567	452	273	253
PGF(91)	NA	350	393	173
VEGFA(100)	350	541	365	260
Tie-2	541	282	291	192
MCP-1(58)	463	393	340	192
MIP-1a(64)	NA	293	353	264
sIL-2Ra(76)	562	295	387	161
TNFa(80)	654	353	393	218

Cox proportional hazard model was performed to identify blood biomarkers that predict progression free survival by baseline levels of CAFs. Low baseline levels of ANG2(90), Ang-2, HGF(86), IL-8(40), IP-10(56), MCP-1(58), MIP-1a(64), PGF(91), sIL-2Ra(76), Tie-2, TNFa(80), VEGFA(100) were significantly associated with longer PFS (**Table 6**).

Table 6. Baseline levels of Blood Biomarkers associated with PFS

PFS: univariate Cox proportional hazard model, HRperSD:hazard ratio for 1 S.D. increase

OOR = out of range

param	N	N.OOR	%OOR	p.likelihood.ratio	p.wald	p.score	HRperSD	HRpersD.CI
ANG2(90)	122	0	0.0	<0.001	<0.001	<0.001	1.644414	1.3148-2.0567
Ang-2	122	0	0.0	<0.001	<0.001	<0.001	1.906941	1.5092-2.4095
HGF(86)	122	0	0.0	0.002	0.001	0.001	1.382498	1.139-1.6781
IL-8(40)	122	12	9.8	<0.001	<0.001	<0.001	1.715626	1.3841-2.1266
IP-10(56)	122	0	0.0	0.010	0.012	0.012	1.401204	1.0772-1.8226
MCP-1(58)	122	0	0.0	0.004	0.003	0.004	1.38988	1.1144-1.7335
MIP-1a(64)	122	20	16.4	0.032	0.036	0.036	1.265194	1.0149-1.5772
PGF(91)	122	7	5.7	0.024	0.019	0.022	1.273256	1.0405-1.5581
sIL-2Ra(76)	122	16	13.1	0.006	0.009	0.008	1.392149	1.0861-1.7844
Tie-2	122	0	0.0	0.007	0.006	0.006	1.35561	1.0925-1.6821
TNFa(80)	122	5	4.1	0.000	0.001	0.001	1.529567	1.1894-1.9671
VEGFA(100)	122	0	0.0	0.038	0.033	0.033	1.255744	1.0183-1.5486

Quartile sub-group analysis based on baseline CAF levels showed lowest baseline groups of ANG2(90), Ang-2, HGF(86), IL-8(40), MCP-1(58), MIP-1a(64), sIL-2Ra(76), Tie-2, and TNFa(80) had longest median PFS, while highest baseline groups of ANG2(90), Ang-2, HGF(86), IL-8(40), IP-10(56), MCP-1(58), PGF(91), sIL-2Ra(76), Tie-2, TNFa(80), VEGFA(100) had shortest median PFS (**Table 7**).

Table 7. Median PFS with 4 Subgroups Based on Baseline CAF Expression

Low Expression → High Expression

	PFS (day)			
	0-25%	25-50%	50-75%	75-100%
ANG2(90)	225	167	145	77
Ang-2	284	167	112	77
IL-8(40)	281	222	145	71
HGF(86)	225	205	145	109
PGF(91)	170	174	218	77
VEGFA(100)	158	205	165	83
Tie-2	273	165	155	112
MCP-1(58)	227	155	167	111
MIP-1a(64)	210	155	117	166
sIL-2Ra(76)	210	161	177	72.5
TNFa(80)	282	117	166	112
IP-10(56)	167	174	220	77

It was next assessed whether baseline CAF levels were associated with tumor response (ORR and MTS). Exact Wilcox test showed median baseline levels of Ang-2 and ANG-2(90) were significantly different in patients who responded to E7080 treatment (CR or PR group) compared with the "others" group (patients with SD or PD) (**Table 8** and **Fig. 2**).

Table 8. Baseline levels of Blood Biomarkers associated with ORR

N; Number, NN(ORR+:ORR-); median difference (diff) (ORR+ : ORR-); OOR = out of range

Param	N	N.OOR	%OOR	NN	NN.OOR	p	median.diff
Ang-2	96	0	0.0	27:69	0:0	0.001	-0.1458
ANG2(90)	96	0	0.0	27:69	0:0	0.006	-0.06585

Spearman's rank correlation test showed median baseline levels of Ang-2, ANG-2,

and IL-8 were significantly associated with MTS (**Table 9** and **Fig. 2**).

Table 9. Baseline levels of Blood Biomarkers associated with MTS
MTS: Spearman's rank correlation test

Param	N	<0.0R	>=0.0R	p	R
Ang-2	100	0	0.0	0.000	0.360922
ANG2(90)	100	0	0.0	0.000	0.358635
IL-8(40)	100	12	12.0	0.034	0.212644

Cox proportional hazard model and quartile sub-group analysis based on age, weight and histology demonstrated that there were no association between these variables and survival benefits (**Fig. 3**).

Table 10 summarizes the correlative analysis of baseline CAFs with clinical outcomes for both tumor response and survival benefits. Baseline levels of 11 CAFs (Ang-2, IL-8, HGF, VEGFA, PlGF, Tie-2, MCP-1, MIP1a, sIL2Ra, TNFa, IP-10) were significantly associated with survival benefits. However, only the baseline level of angiopoietin-2 was associated with both tumor response (ORR and MTS) and survival benefits (OS and PFS). These data indicate that baseline angiopoietin-2 level predicts clinical outcomes with E7080 treatment and other baseline levels of other CAFs were associated with the prognosis in EC.

Table 10. Baseline CAF levels are associated with clinical outcomes

param	OS	PFS	ORR	%MTS
ANG2(90)	p<0.01	p<0.01	p<0.01	p<0.01
Ang-2	p<0.01	p<0.01	p<0.01	p<0.01
IL-8(40)	p<0.01	p<0.01		p<0.05
HGF(86)	p<0.01	p<0.01		
PGF(91)	p<0.01	p<0.05		
VEGFA(100)	p<0.01	p<0.05		
Tie-2	p<0.01	p<0.01		
MCP-1(58)	p<0.01	p<0.01		
MIP-1a(64)	p<0.05	p<0.05		
sIL-2Ra(76)	p<0.01	p<0.01		
TNFa(80)	p<0.01	p<0.01		
IP-10(56)		p<0.05		

OS: overall survival - Univariate Cox proportional hazard model; PFS: progression free survival - Univariate Cox proportional hazard model; ORR: objective response rate - Exact Wilcox test; %MTS: % of maximum tumor shrinkage - Spearman's rank correlation test.

Multivariate analysis was performed to examine if combining two or more factors improved predictions of clinical outcomes. All factors (i.e., weight, age, Ang-1, Ang-2, ANG2(90), FGF-2(6), FGF4(75), HGF(86), IL-8(40), PDGF-AA, PDGFAB(68), PDGFBB(73), PGF(91), Tie-2, VEGF(86), VEGFA(100), VEGFD(78)) were used as independent variables of interest, that is, as biomarker candidates. Models were identified by Cox proportional hazard model with forward selection method using variables identified by univariate analysis and biological insight. PFS and OS were used as a dependent variable, that is, as one of the clinical outcomes, in this analysis. Firstly, all factors were screened according to p-values calculated by Cox proportional hazards model with single factor. Secondly, all combinations of the screened factors were tested by Cox proportional hazards model to find significant factors in all combinations of the factors. PFS model identified Ang-2 as a single factor. OS model identified FGF-2, PGF and HGF as additional factors, although Ang-2 was selected as a much more significant factor (**Fig. 4**).

A cut-off value of Ang-2 was used to test if patients with baseline Ang-2 levels have better clinical outcomes, such as ORR, PFS, and OS. In the case of using ORR, Receiver Operating Characteristic (ROC) analysis can be applied. In given indexes in

ROC, positive prediction value (PPV) gives the performance in the sub-group having better clinical outcomes. A possible cut-off value can be selected from a range of Ang-2 defined by setting target PPV, e.g. 30% or more. Also, in the case of using PFS, separation of Kaplan-Meier curves of two groups divided by the cut-off , for example p-value from the logrank test, can be applied as a measure. A possible cut-off value can be selected from a range of Ang-2 defined by setting target p-value, e.g. P=0.001. In addition, a range of Ang-2 can be defined by combining the two methods for ORR and PFS. The first method for ORR identified the upper limit of Ang-2 as 6024.5 pg/ml and the second method for PFS identified the lower limit of Ang-2 as 1866.5 pg/ml. In the given range, ROC analysis followed by Youden and MCC index can provide optimal Ang-2 cut-off value to predict a sub-group having better ORR , as 2082.5 pg/ml (**Table 11**).

Table 11. Receiver Operating Characteristic Analysis Followed by Log-Rank test for OS

param	N	N.OOR	%OOR	AUC	ROC. correlation	ROC.youden. cutoff
Ang-2	122	0	0.0	0.724	-0.279	2082.5
	ROC.youden .OS.N	ROC.youden. OS. N high Gr	ROC.youden. OS.% high Gr	ROC.youden. OS.p.logrank	ROC.youden. OS.HR	ROC.youden. OS.HR.CI
	122	98	80.3	0.001	3.223	1.5372-6.7562

The 24 patients with low baseline Ang-2 plasma levels (i.e., <2082 pg/ml) were compared to the 98 patients with high baseline Ang-2 (i.e., > 2082 pg/ml). Log-rank test using defined cut-off values demonstrated improved mean PFS (9.5 v. 3.7 months) and mean OS (23 v. 8.9 months) in the sub-group with low baseline Ang-2 levels compared to the sub-group with high baseline Ang-2 levels (**Fig. 5**). Improved ORR (61% v. 18%) was also demonstrated in the sub-group with low baseline Ang-2 compared to the sub-group with high baseline Ang-2 (**Fig. 6**). These analyses indicate that baseline Ang-2 level identifies patients with EC that have clear benefits with E7080 treatments. In this context, it is noteworthy that ORR, mPFS and mOS without stratification based on Ang-2 levels are 21.1%, 5.4 months, and 10.6 months, respectively (**Table 12**).

Table 12. Clinical Outcomes of Low and High Ang-2 Sub-Groups of EC Patients

Clinical Outcome Category	Full Set Analysis (N = 133)	Low Ang-2 < 2082.51 (N = 24)	High Ang-2 ≥ 2082.51 (N = 98)
Objective Response (CR+PR)	**21.8%**	**60.9%**	**17.8%**
Progression-Free Survival (months)			
Median	**5.4**	**9.5**	**3.7**
Overall Survival (months)			
Median	**10.6**	**23**	**8.9**

Conclusion: Baseline levels of 11 CAFs were associated with both OS and PFS. Baseline levels of only Ang-2 were associated with both survival benefits (OS and PFS) and tumor response (ORR). Thus, low baseline levels of Ang-2 have an independent role in predicting sensitivity to E7080 treatment among endometrial cancer patients.

Example 2: *Confirming Ang2 as Specific Predictive Biomarker for Selecting Thyroid and Endometrial Cancer Patients for Treatment with E7080 (lenvatinib mesylate)*

Purpose: Predictive role of Ang2 has been demonstrated for thyroid and endometrial cancers. The predictive role for other cancer indications has not been evaluated. It is not confirmed that either Ang2 is a robust biomarker for cancers or a specific biomarker for certain cancers. The purpose of this analysis was to confirm Ang2 is Thyroid and Endometrial cancer specific biomarker for selecting patients for E7080 treatment. For this analysis, the following criteria of response were employed, namely:

(a) Tumor response: objective response rate (ORR); and

(b) Survival Benefits: overall survival (OS).

The criteria of response are defined below.

"Complete Response" means complete disappearance of all target lesions.

"Partial Response" means at least 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline summed LD.

"Progressive Disease" (PD) means at least 20% increase in the sum of the LD of target lesions, taking as reference the smallest summed LD recorded since the treatment

started, or the appearance of one or more new lesions.

"Stable Disease" means neither sufficient shrinkage of the target lesions to qualify for PR nor sufficient increase to qualify for progressive disease (PD), taking as reference the smallest summed LD since the treatment started.

"Objective Response Rate" (ORR) compares subjects with either "Complete Response (CR) or "Partial Response" (PR) with subjects to with either Stable Disease (SD) or Progressive Disease (PD).

"Overall Survival" (OS) refers to the time from randomization until death from any cause. "Randomization" means randomization of a patient into a test group or a control group when the therapy plan for a patient is determined.

Materials and Methods: Patients received lenvatinib until disease progression or development of unmanageable toxicities. This study was a part of the following Phase 2 studies of lenvatinib:

Evaluating the Safety and Efficacy of Oral E7080 in Medullary and Iodine-131 Refractory, Unresectable Differentiated Thyroid Cancers, Stratified by Histology (ClinicalTrials.gov Identifier: NCT00784303)

Study of E7080 in Patients With Advanced Hepatocellular Carcinoma (HCC) (ClinicalTrials.gov Identifier: NCT00946153)

A Study in Subjects With Recurrent Malignant Glioma (ClinicalTrials.gov Identifier: NCT01137604)

Study of E7080 in Subjects With Advanced Endometrial Cancer and Disease Progression (ClinicalTrials.gov Identifier: NCT01111461)

An Open-Label, 2-Cohort, Multicenter, Study of E7080 in Previously Treated Subjects With Unresectable Stage III or Stage IV Melanoma (ClinicalTrials.gov Identifier: NCT01136967)

Baseline and post-treatment plasma or serum samples were collected for molecular analysis and baseline samples were used in this analysis. The samples were tested in an ELISA and multiplex kit as per the manufacturer's instructions. Table 13 describes the assay kits used in the analysis. The ELISA plates were measured using a Molecular Devices UVmax kinetic microplate reader with SoftMax Pro 5.2 software. The multiplex assays were performed using the Bio-Rad Bio-Plex system with Bio-Plex Manager 4.1 software. Final Ang2 concentrations (pg/mL) were calculated from the standard curve for each assay. Depending on the assay, samples may have been diluted in assay buffer prior to testing. In

these cases, concentrations were multiplied by the dilution factor. The association between baseline Ang2 concentration with objective response rate (ORR) and overall survival (OS) were analyzed.

Table 13. Assay Kits

Name	Manufacturer	Catalog #	Analytes Measured
Angiopoietin-2 ELISA	R&D Systems Inc.	DANG20	Ang-2
Human growth factor multiplex	Origene Inc.	AM100096	ANG2 (90) or ANG2(76)

Numbers in parenthesis indicate bead region for each analyte.

Results and Discussion: From analyzed five phase 2 studies (7 cohorts), samples from 483 patients were used for analyses. Significant associations of baseline Ang2 with ORR as well as OS were observed for thyroid (both of differentiated thyroid cancer (DTC) and medullary thyroid cancer (MTC)) and endometrial cancer (**Table 14**). Ang2 level in hepatocellular carcinoma (HCC) and glioblastoma (GBM) neither showed significant associations with ORR nor OS. In melanoma (both of BRAF wildtype (wt) and mutated (mut)), Ang2 baseline level associates with not ORR but OS.

Table 14. Association of baseline levels of Ang2 with ORR and OS

	Assay	N (ORR:others)	ORR		OS	
			p-value	difference (ORR-others)	p-value	Hazard ratio per standard deviation (95% CI)
Thyroid DTC	Ang-2	53 (27:26)	0.239	-0.048	**0.001**	3.20 (1.56-6.56)
	ANG2(90)	51 (25:26)	**0.046**	-0.127	**0.044**	1.94 (1.02-3.71)
Thyroid MTC	Ang-2	55 (18:37)	**0.019**	-0.159	**<0.001**	3.16 (1.84-5.41)
	ANG2(90)	53 (18:35)	0.207	-0.089	**0.005**	2.05 (1.24-3.40)
HCC	Angiopoietin-2	46 (17:29)	0.652	-0.014	0.437	1.15 (0.81-1.64)
	ANGPT2	46 (17:29)	0.543	0.033	0.821	0.96 (0.67-1.38)
GBM	Ang-2	39 (8:31)	0.798	0.029	0.635	0.89 (0.57-1.42)
	ANG2(76)	39 (8:31)	0.209	0.090	0.857	0.97 (0.67-1.40)
Endometrial	Ang-2	122 (26:96)	**<0.001**	-0.166	**<0.001**	1.80 (1.42-2.28)
	ANG2(90)	122 (26:96)	**0.004**	-0.083	**<0.001**	1.62 (1.26-2.08)
Melanoma (BRAF wt)	Ang-2	88 (8:80)	0.112	-0.094	**0.002**	1.51 (1.17-1.97)
	ANG2(76)	87 (8:79)	0.098	-0.113	**0.001**	1.69 (1.23-2.31)
Melanoma (BRAF mut)	Ang-2	78 (8:70)	0.548	-0.030	**<0.001**	2.18 (1.61-2.94)
	ANG2(90)	80 (8:72)	0.161	-0.067	**<0.001**	2.13 (1.57-2.87)

ORR: Wilcoxon signed-rank test

OS: univariate Cox proportional hazard model

Conclusion: Ang2 baseline levels were associated with both of ORR and OS in thyroid and endometrial cancers. Other cancer types tested (HCC, GBM and Melanoma) did not show significant association with both of ORR and OS. The observation implies Ang2 is specific biomarker for certain cancers, such as thyroid and endometrial cancers.

Specific Embodiments

Specific embodiments of the invention are as follows:

[1] A method of treating an endometrial cancer, the method comprising administering to a human subject that has an endometrial cancer a therapeutically effective amount of lenvatinib or a pharmaceutically acceptable salt thereof, wherein the human subject has been identified as having an Ang2 protein expression level that is low as compared to a control.

[2] The method of [1], wherein the human subject has been identified as having a low concentration of Ang2 protein in a biological sample obtained from the human subject.

[3] A method of treating an endometrial cancer, the method comprising:

providing a biological sample obtained from a human subject that has endometrial cancer;

measuring, in the biological sample, an Ang2 protein expression level that is low as

compared to a control; and

administering to the human subject a therapeutically effective amount of lenvatinib or a pharmaceutically acceptable salt thereof.

[4] A method of predicting the response of a human subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof, the method comprising:

assaying a biological sample obtained from the human subject and determining that the concentration of Ang2 protein in the biological sample is low, as compared to a control; and

identifying the human subject having a low concentration of Ang2 protein in the biological sample as likely to respond to the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

[5] A method of predicting the response of a human subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof, the method comprising:

assaying a biological sample obtained from the human subject and determining that the concentration of Ang2 protein in the biological sample is high, as compared to a control; and

identifying the human subject having a high concentration of Ang2 protein in the biological sample as unlikely to respond to the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

[6] A method of assisting the prediction of the response of a human subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof, the method comprising:

assaying a biological sample obtained from the human subject and determining that the concentration of Ang2 protein in the biological sample is low, as compared to a control; and

identifying the human subject having a low concentration of Ang2 protein in the biological sample as likely to respond to the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

[7] A method of assisting the prediction of the response of a human subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof, the method comprising:

assaying a biological sample obtained from the human subject and determining that the concentration of Ang2 protein in the biological sample is high, as compared to a control; and

identifying the human subject having a high concentration of Ang2 protein in the biological

sample as unlikely to respond to the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

[8] The method of any one of [2] to [7], wherein the biological sample is selected from the group consisting of a blood sample, a serum sample, a plasma sample, an endometrial archived tumor sample, and an endometrial biopsy sample.

[9] The method of any one of [2] to [7], wherein the biological sample is a plasma sample.

[10] The method of any one of [1] to [9], wherein the endometrial cancer is an advanced endometrial cancer.

[11] The method of any one of [1] to [9], wherein the endometrial cancer is unresectable stage III or stage IV endometrial cancer.

[12] The method of any one of [1] to [9], wherein the endometrial cancer is a recurrent endometrial cancer.

[13] The method of any one of [1] to [12], wherein the control is a pre-established cut-off value.

[14] The method of [13], wherein the pre-established cut-off value is an Ang2 protein concentration that is determined based on receiver operating characteristic analysis predicting tumor response with a higher positive predictive value compared to no cut-off, and wherein a concentration of Ang2 protein equal to or below the pre-established cut-off value is a low concentration of Ang2 and a value higher than the pre-established cut-off value is a high concentration of Ang2.

[15] The method of [14], wherein tumor response is an objective response rate, a clinical benefit rate or % of maximum tumor shrinkage of at least 30%.

[16] The method of [13], wherein the pre-established cut-off value is an Ang2 protein concentration that is determined based on predicting survival using simulation models to separate two groups divided by the cut-off, and wherein a concentration of Ang2 protein equal to or below the pre-established cut-off value is a low concentration of Ang2 and a value higher than the pre-established cut-off value is a high concentration of Ang2.

[17] The method of [16], wherein survival is progression free survival or overall survival.

[18] The method of [13], wherein the pre-established cut-off value is an Ang2 protein concentration within the range from 1866.5 to 6024.5 pg/ml, and wherein a concentration of Ang2 protein equal to or below the pre-established cut-off value is a low concentration of

Ang2 and a value higher than the pre-established cut-off value is a high concentration of Ang2.

[19] The method of any one of [1] to [18], wherein the concentration of the protein is measured by an immunological method.

[20] The method of [19], wherein the immunological method is selected from the group consisting of enzyme immunoassay, radioimmunoassay, chemiluminescent immunoassay, electrochemiluminescence immunoassay, latex turbidimetric immunoassay, latex photometric immunoassay, immuno-chromatographic assay, and western blotting.

[21] The method of any one of [1] to [18], wherein the concentration of the protein is measured by mass spectrometry.

[22] The method of any one of [1] to [21], wherein the pharmaceutically acceptable salt of lenvatinib is lenvatinib mesylate.

[23] Lenvatinib or a pharmaceutically acceptable salt thereof for use in treating an endometrial cancer in a human subject, wherein the human subject is identified by the method of [4] as a subject that is likely to respond to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

[24] An Ang2 protein detection agent for use in predicting the response of a human subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

[25] The Ang2 protein detection agent of [24], wherein the Ang2 protein detection agent is an anti-Ang2 antibody.

[26] A pharmaceutical composition for treating an endometrial cancer in a human subject comprising lenvatinib or a pharmaceutically acceptable salt thereof, wherein the human subject is identified by the method of [4] as a subject that is likely to respond to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

Other Embodiments

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

CLAIMS

1. A method of predicting the response of a human subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof, the method comprising:

assaying a biological sample obtained from the human subject and determining that the concentration of Ang2 protein in the biological sample is low, as compared to a control; and

identifying the human subject having a low concentration of Ang2 protein in the biological sample as likely to respond to the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

2. A method of predicting the response of a human subject having, suspected of having, or at risk of developing, an endometrial cancer to a therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof, the method comprising:

assaying a biological sample obtained from the human subject and determining that the concentration of Ang2 protein in the biological sample is high, as compared to a control; and

identifying the human subject having a high concentration of Ang2 protein in the biological sample as unlikely to respond to the therapy comprising lenvatinib or a pharmaceutically acceptable salt thereof.

3. The method of claim 1 or 2, wherein the biological sample is selected from the group consisting of a blood sample, a serum sample, a plasma sample, an endometrial archived tumor sample, and an endometrial biopsy sample.

4. The method of any one of claims 1 to 3, wherein the biological sample is a plasma sample.

5. The method of any one of claims 1 to 4, wherein the endometrial cancer is an advanced endometrial cancer.

6. The method of any one of claims 1 to 4, wherein the endometrial cancer is unresectable stage III or stage IV endometrial cancer.

7. The method of any one of claims 1 to 4, wherein the endometrial cancer is a recurrent endometrial cancer.

8. The method of any one of claims 1 to 7, wherein the control is a pre-established cut-off value.

9. The method of claim 8, wherein the pre-established cut-off value is an Ang2 protein concentration that is determined based on receiver operating characteristic analysis predicting tumor response with a higher positive predictive value compared to no cut-off, and

wherein a concentration of Ang2 protein equal to or below the pre-established cut-off value is a low concentration of Ang2 and a value higher than the pre-established cut-off value is a high concentration of Ang2.

10. The method of claim 9, wherein tumor response is an objective response rate, a clinical benefit rate or % of maximum tumor shrinkage of at least 30%.

11. The method of claim 8, wherein the pre-established cut-off value is an Ang2 protein concentration that is determined based on predicting survival using simulation models to separate two groups divided by the cut-off, and wherein a concentration of Ang2 protein equal to or below the pre-established cut-off value is a low concentration of Ang2 and a value higher than the pre-established cut-off value is a high concentration of Ang2.

12. The method of claim 11, wherein survival is progression free survival or overall survival.

13. The method of claim 8, wherein the pre-established cut-off value is an Ang2 protein concentration within the range from 1866.5 to 6024.5 pg/ml, and wherein a concentration of Ang2 protein equal to or below the pre-established cut-off value is a low concentration of Ang2 and a value higher than the pre-established cut-off value is a high concentration of Ang2.

14. The method of any one of claims 1 to 13, wherein the concentration of the protein is measured by an immunological method.

15. The method of claim 14, wherein the immunological method is selected from the group consisting of enzyme immunoassay, radioimmunoassay, chemiluminescent immunoassay, electrochemiluminescence immunoassay, latex turbidimetric immunoassay, latex photometric immunoassay, immuno-chromatographic assay, and western blotting.

16. The method of any one of claims 1 to 13, wherein the concentration of the protein is measured by mass spectrometry.

17. The method of any one of claims 1 to 16, wherein the pharmaceutically acceptable salt of lenvatinib is lenvatinib mesylate.