



HU000032693T2

(19) **HU**(11) Lajstromszám: **E 032 693**(13) **T2****MAGYARORSZÁG****Szellemi Tulajdon Nemzeti Hivatala**

EURÓPAI SZABADALOM SZÖVEGÉNEK FORDÍTÁSA

(21) Magyar ügyszám: **E 13 189711**(22) A bejelentés napja: **2009. 11. 20.**

(96) Az európai bejelentés bejelentési száma:

EP 20090189711

(97) Az európai bejelentés közzétételi adatai:

EP 2752189 A1 **2014. 07. 09.**

(97) Az európai szabadalom megadásának meghirdetési adatai:

EP 2752189 B1 **2016. 10. 26.**(51) Int. Cl.: **A61K 31/337** (2006.01)**A61K 31/513** (2006.01)**A61K 31/675** (2006.01)**A61K 31/704** (2006.01)**A61K 39/395** (2006.01)**A61K 45/06** (2006.01)**A61P 35/04** (2006.01)

(30) Elsőbbségi adatok:

117102 P **2008. 11. 22.** **US****178009 P** **2009. 05. 13.** **US****179307 P** **2009. 05. 18.** **US**

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(54)

Anti-vegf antitest alkalmazása kemoterápiával kombinációban emlőrák kezelésére

Az európai szabadalom ellen, megadásának az Európai Szabadalmi Közlönyben való meghirdetésétől számított kilenc hónapon belül, felszólalást lehet benyújtani az Európai Szabadalmi Hivatalnál. (Európai Szabadalmi Egyezmény 99. cikk(1))

A fordítást a szabadalmat az 1995. évi XXXIII. törvény 84/H. §-a szerint nyújtotta be. A fordítás tartalmi helyességét a Szellemi Tulajdon Nemzeti Hivatala nem vizsgálta.

(19)



(11)

EP 2 752 189 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:

26.10.2016 Bulletin 2016/43

(51) Int Cl.:

A61K 31/337 ^(2006.01) **A61K 39/395** ^(2006.01)
A61P 35/04 ^(2006.01) **A61K 31/513** ^(2006.01)
A61K 31/675 ^(2006.01) **A61K 31/704** ^(2006.01)
A61K 45/06 ^(2006.01)

(21) Application number: **13189711.8**

(22) Date of filing: **20.11.2009**

(54) **USE OF ANTI-VEGF ANTIBODY IN COMBINATION WITH CHEMOTHERAPY FOR TREATING BREAST CANCER**

VERWENDUNG VON ANTI-VEGF ANTIKÖRPER IN KOMBINATION MIT CHEMOTHERAPIE ZUR BEHANDLUNG VON BRUSTKREBS

UTILISATION D'ANTICORPS ANTI-VEGF COMBINÉS À LA CHIMIOTHÉRAPIE POUR LE TRAITEMENT DU CANCER DU SEIN

(84) Designated Contracting States:
**AT BE BG CH CY CZ DE DK EE ES FI FR GR HR
HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT
RO SE SI SK SM TR**

(30) Priority: **22.11.2008 US 117102 P**
13.05.2009 US 178009 P
18.05.2009 US 179307 P

(43) Date of publication of application:
09.07.2014 Bulletin 2014/28

(60) Divisional application:
16188246.9

(62) Document number(s) of the earlier application(s) in
accordance with Art. 76 EPC:
09764134.4 / 2 361 085

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Description**FIELD OF THE INVENTION**

[0001] This invention relates in general to treatment of human diseases and pathological conditions. More specifically, the invention relates to anti-angiogenesis therapy, either alone or in combination with other anti-cancer therapies, for the treatment of breast cancer.

BACKGROUND

[0002] Cancer remains to be one of the most deadly threats to human health. In the U.S., cancer affects nearly 1.3 million new patients each year, and is the second leading cause of death after heart disease, accounting for approximately 1 in 4 deaths. Breast cancer is the second most common form of cancer and the second leading cancer killer among American women. It is also predicted that cancer may surpass cardiovascular diseases as the number one cause of death within 5 years. Solid tumors are responsible for most of those deaths. Although there have been significant advances in the medical treatment of certain cancers, the overall 5-year survival rate for all cancers has improved only by about 10% in the past 20 years. Cancers, or malignant tumors, metastasize and grow rapidly in an uncontrolled manner, making timely detection and treatment extremely difficult.

[0003] Breast cancer is a disease that kills many women each year in the United States. According to the American Cancer Society, approximately 40,000 will die from the disease in 2008. Over 180,000 new cases of breast cancer are diagnosed annually, and it is estimated that one in eight women will develop breast cancer. These numbers indicate that breast cancer is one of the most dangerous diseases facing women today.

[0004] Metastatic breast cancer is generally incurable with only a few patients achieving long-term survival after standard chemotherapy. Greenberg et al., J. Clin. Oncol. 14:2197-2205 (1996).

[0005] Knowledge of the basic biology of breast cancer has expanded exponentially over the last three decades with some having an impact on therapy. A multinational, open-label phase II trial of 222 women with HER2 overexpressing metastatic breast cancer found a response rate of 15% with six confirmed complete responses using a recombinant humanized monoclonal antibody (trastuzumab, also known as Herceptin®, Genentech, South San Francisco) directed against HER2 (Cobleigh et al., Proc. Am. Soc. Clin. Oncol. 17:97 (1998)). A randomized phase III trial evaluated the safety and efficacy of adding Herceptin to first-line chemotherapy with either paclitaxel or the combination of doxorubicin plus cyclophosphamide. Overall response rate and time to progression significantly improved with the addition of Herceptin to chemotherapy compared to chemotherapy alone (Slamon et al., Proc. Am. Soc. Clin. Oncol. 17:98 (1998)). More importantly, the addition of Herceptin prolonged overall survival (Norton et al., Proc. Am. Soc. Clin. Oncol. 18:127a (1999)).

[0006] Though trastuzumab is the first novel, biologically-based therapeutic agent approved for the treatment of a subpopulation of breast cancer patients having HER2 overexpressing cancers, several other approaches have shown promise and have entered the clinic. There are estimates that 75 percent of women will newly diagnosed metastatic breast cancer are HER2-negative. Compounds which inhibit angiogenesis have generated particular interest for reaching additional breast cancer populations and have been and are the subject of clinical trials both in the US and abroad.

[0007] Angiogenesis is an important cellular event in which vascular endothelial cells proliferate, prune and reorganize to form new vessels from preexisting vascular network. There is compelling evidence that the development of a vascular supply is essential for normal and pathological proliferative processes (Folkman and Klagsbrun Science 235:442-447(1987)). Delivery of oxygen and nutrients, as well as the removal of catabolic products, represent rate-limiting steps in the majority of growth processes occurring in multicellular organisms.

[0008] While induction of new blood vessels is considered to be the predominant mode of tumor angiogenesis, recent data have indicated that some tumors may grow by co-opting existing host blood vessels. The co-opted vasculature then regresses, leading to tumor regression that is eventually reversed by hypoxia-induced angiogenesis at the tumor margin. Holash et al. Science 284:1994-1998 (1999).

[0009] One of the key positive regulators of both normal and abnormal angiogenesis is vascular endothelial growth factor (VEGF)-A. VEGF-A is part of a gene family including VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and PlGF. VEGF-A primarily binds to two high affinity receptor tyrosine kinases, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR), the latter being the major transmitter of vascular endothelial cell mitogenic signals of VEGF-A. Additionally, neuropilin-1 has been identified as a receptor for heparin-binding VEGF-A isoforms, and may play a role in vascular development.

[0010] In addition to being an angiogenic factor in angiogenesis and vasculogenesis, VEGF, as a pleiotropic growth factor, exhibits multiple biological effects in other physiological processes, such as endothelial cell survival, vessel permeability and vasodilation, monocyte chemotaxis and calcium influx. Ferrara and Davis-Smyth (1997), *supra*. Moreover, studies have reported mitogenic effects of VEGF on a few non-endothelial cell types, such as retinal pigment epithelial cells, pancreatic duct cells and Schwann cells. Guerrin et al. J. Cell Physiol. 164:385-394 (1995); Oberg-Welsh

et al. Mol. Cell. Endocrinol. 126:125-132 (1997); Sondell et al. J. Neurosci. 19:5731-5740 (1999).

[0011] The recognition of VEGF as a primary regulator of angiogenesis in pathological conditions has led to numerous attempts to block VEGF activities in conditions that involve pathological angiogenesis. VEGF expression is upregulated in a majority of malignancies and the overexpression of VEGF correlates with a more advanced stage or with a poorer prognosis in many solid tumors. Therefore, molecules that inhibit VEGF signaling pathways have been used for the treatment of relatively advanced solid tumors in which pathological angiogenesis is noted.

[0012] Heinemann V, EJC SUPPLEMENTS, vol.6, no.8 (2008), pages 13-18; Cameron D et al., EJC SUPPLEMENTS, vol.6, no.6 (2008), pages 40-50; ClinicalTrials.gov, trial number NCT00262067; and O'Shaughnessy and Brufsky, (2008), Clinical Breast Cancer, 8(4): 370-373 describe the RIBBON 1 clinical trial protocol but contain no results. Additionally, Heinemann and Sledge et al., Journal of Clinical Oncology, vol.25, no. 18S (2007), page 1013 refer to a single-arm XCalibr trial investigating bevacizumab (15 mg/kg IV on day 1) plus capecitabine (1000mg/m² twice daily on days 1-15 of every 21-day cycle) as first-line treatment for HER2-negative metastatic breast cancer patients but no results with respect to the extension of progression free survival (PFS) are provided.

[0013] Since cancer is still one of the most deadly threats, additional cancer treatments for patients are needed. Specifically, treatments for patients with MBC are needed to improve control of disease to prevent symptoms, while minimizing toxicity. The invention addresses these and other needs, as will be apparent upon review of the following disclosure.

SUMMARY

[0014] The invention concerns uses of anti-VEGF antibody for effectively treating breast cancer patients for previously untreated metastatic breast cancer. In particular, the invention provides data from a randomized phase III clinical trial of bevacizumab (AVASTIN®) in combination with chemotherapy regimes in subjects with previously untreated metastatic breast cancer in human subjects. Such chemotherapy regimes include capecitabine therapy. In some embodiments, the treatment is used as first line therapy for locally recurrent or previously untreated metastatic breast cancer. The success of the trial shows that adding anti-VEGF antibody to a standard chemotherapy provides statistically significant and clinically meaningful benefits to breast cancer patients. In addition, safety was consistent with results of prior bevacizumab trials.

[0015] The results obtained in clinical studies of the use of bevacizumab in human subjects with metastatic breast cancer show that the efficacy, as evaluated by progression free survival (PFS) was positive especially when compared to PFS data for chemotherapeutic agents alone. Subjects in the clinical trials who received bevacizumab in combination with taxane therapy (e.g., docetaxel or paclitaxel protein-bound particles (e.g., Abraxane®))/anthracycline therapy (e.g., doxorubicin, epirubicin or combinations thereof) had an increase in progression free survival compared to subjects treated with the taxane therapy (e.g., docetaxel or paclitaxel protein-bound particles (e.g., Abraxane®))/anthracycline therapy (e.g., doxorubicin, epirubicin or combinations thereof) alone. Subjects in the clinical trials who received bevacizumab in combination with capecitabine therapy as described below, had an increase in progression free survival compared to subjects treated with capecitabine therapy alone. The difference was significantly significant.

[0016] Accordingly, described herein are methods of treating a subject diagnosed with previously untreated metastatic breast cancer, comprising administering to the subject a treatment regimen comprising an effective amount of at least one chemotherapy and an anti-VEGF antibody, wherein said subject has not received any chemotherapy for locally recurrent or metastatic breast cancer. Optionally, the subject is HER2-negative. In some embodiments, the subject is HER2 positive. Optionally, the subject has not received prior adjuvant chemotherapy in recurrence less than or equal to 12 months since last dose. The treatment regimen combining the chemotherapy and the administration of the anti-VEGF effectively extends the progression free survival (PFS) of the subject. In certain embodiments, the treatment regimen combining the chemotherapy and the anti-VEGF antibody has a safety profile that is consistent with results of prior bevacizumab trials.

[0017] Further described herein are uses of an anti-VEGF antibody with at least one chemotherapeutic agent in the manufacture of a medicament for treating previously untreated metastatic breast cancer in a subject, wherein said subject has not received any chemotherapy for locally recurrent or metastatic breast cancer. Optionally, the subject is HER2-negative. In some embodiments, the subject is HER2 positive. Optionally, the subject has not received prior adjuvant chemotherapy in recurrence less than or equal to 12 months since last dose. The use of the anti-VEGF and the chemotherapeutic agent effectively extends the progression free survival (PFS) of the subject. In certain embodiments, the use of the chemotherapy and the anti-VEGF antibody has a safety profile that is consistent with results of prior bevacizumab trials.

[0018] Provided also herein are anti-VEGF antibodies for use in a method of treating locally recurrent or metastatic breast cancer in a subject, the method comprising administering to the subject a treatment regimen comprising an effective amount of a chemotherapy and an anti-VEGF antibody, wherein said subject has not received any chemotherapy for locally recurrent or metastatic breast cancer. Optionally, the subject is HER2-negative. In some embodiments, the

subject is HER2 positive. Optionally, the subject has not received prior adjuvant chemotherapy in recurrence less than or equal to 12 months since last dose. The treatment regimen combining the chemotherapy and the administration of the anti-VEGF effectively extends the progression free survival (PFS) of the subject. In certain embodiments, the treatment regimen combining the chemotherapy and the anti-VEGF antibody has a safety profile that is consistent with results of prior bevacizumab trials.

[0019] In certain embodiments of any of the methods, uses and compositions described or provided herein, the PFS is extended about 1 month, 1.2 months, 2 months, 2.4 months, 2.9 months, 3 months, 3.5 months, 4, months, 6 months, 7 months, 8 months, 9 months, 1 year, about 2 years, about 3 years, etc. In one embodiment, the PFS is extended about 2.9 months to 3.5 months (e.g., with capecitabine). In one embodiment, the PFS is extended about 1.2 months to about 2.4 months (e.g., with taxane/anthracycline).

[0020] Any chemotherapeutic agent exhibiting anticancer activity can be used according to any of the methods, uses and compositions provided herein. In certain embodiments, the chemotherapeutic agent is selected from the group consisting of alkylating agents, antimetabolites, folic acid analogs, pyrimidine analogs, purine analogs and related inhibitors, vinca alkaloids, epipodopyllotoxins, antibiotics, L-Asparaginase, topoisomerase inhibitor, interferons, platinum coordination complexes, anthracenedione substituted urea, methyl hydrazine derivatives, adrenocortical suppressant, adrenocorticosteroides, progestins, estrogens, antiestrogen, androgens, antiandrogen, and gonadotropin-releasing hormone analog. In certain embodiments, the chemotherapeutic agent is for example, capecitabine, taxane, anthracycline, paclitaxel, docetaxel, paclitaxel protein-bound particles (e.g., Abraxane®), doxorubicin, epirubicin, 5-fluorouracil, cyclophosphamide or combinations thereof. Two or more chemotherapeutic agents can be used (e.g., in a cocktail) to be administered in combination with administration of the anti-VEGF antibody.

[0021] Clinical benefits of the any of the methods, uses and compositions described herein can be measured by, for example, duration of progression free survival (PFS), time to treatment failure, objective response rate and duration of response.

[0022] Accordingly, the disclosure includes a method of instructing a human subject with, e.g., breast, cancer by providing instructions to receive treatment with an anti-VEGF antibody so as to increase progression free survival of the subject, to decrease the subject's risk of cancer recurrence or to increase the subject's likelihood of survival. In some embodiments the method further comprises providing instructions to receive treatment with at least one chemotherapeutic agent. The treatment with the anti-VEGF antibody may be concurrent with or sequential to the treatment with the chemotherapeutic agent. In certain embodiments the subject is treated as instructed by the method of instructing.

[0023] The disclosure also includes a promotional method, comprising promoting the administration of an anti-VEGF antibody for treatment of, e.g., breast, cancer in a human subject. In some embodiments the method further comprises promoting the administration of at least one chemotherapeutic agent. Administration of the anti-VEGF antibody may be concurrent with or sequential to administration of the chemotherapeutic agent. Promotion may be conducted by any means available. In some embodiments the promotion is by a package insert accompanying a commercial formulation of the anti-VEGF antibody. The promotion may also be by a package insert accompanying a commercial formulation of the chemotherapeutic agent. Promotion may be by written or oral communication to a physician or health care provider. In some embodiments the promotion is by a package insert where the package inset provides instructions to receive therapy with anti-VEGF antibody. In some embodiments the promotion is followed by the treatment of the subject with the anti-VEGF antibody with or without the chemotherapeutic agent.

[0024] The disclosure includes a business method, comprising marketing an anti-VEGF antibody for treatment of, e.g., breast, cancer in a human subject so as to increase progression free survival, or decrease the subject's likelihood of cancer recurrence or increase the subject's likelihood of survival. In some embodiments the method further comprises marketing a chemotherapeutic agent for use in combination with the anti-VEGF antibody. In some embodiments the marketing is followed by treatment of the subject with the anti-VEGF antibody with or without the chemotherapeutic agent.

[0025] Also described is a business method, comprising marketing a chemotherapeutic agent in combination with an anti-VEGF antibody for treatment of, e.g., breast, cancer in a human subject so as to increase progression free survival, or decrease the subject's likelihood of cancer recurrence or increase the subject's likelihood of survival. In some embodiments, the marketing is followed by treatment of the subject with the combination of the chemotherapeutic agent and the anti-VEGF antibody.

[0026] In any of the methods, uses and compositions provided herein, the anti-VEGF antibody may be substituted with a VEGF specific antagonist, e.g., a VEGF receptor molecule or chimeric VEGF receptor molecule as described herein. In certain embodiments of the methods, uses and compositions provided herein, the anti-VEGF antibody is bevacizumab. The anti-VEGF antibody, or antigen-binding fragment thereof, can be a monoclonal antibody, a chimeric antibody, a fully human antibody, or a humanized antibody. Exemplary antibodies useful in the methods of the invention include bevacizumab (AVASTIN®), a G6 antibody, a B20 antibody, and fragments thereof. In certain embodiments, the anti-VEGF antibody has a heavy chain variable region comprising the following amino acid sequence:

EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWRQA PGKGLEWVGW

INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP

HYYGSSHWYF DVWGQGTLLV VSS (SEQ ID No. 1)

and a light chain variable region comprising the following amino acid sequence:

DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIIYF

TSSLHSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ

GTKVEIKR (SEQ ID No. 2).

[0027] The antibody, or antigen-binding fragment thereof, can also be an antibody that lacks an Fc portion, an F(ab')₂, an Fab, or an Fv structure.

[0028] In one embodiment of the methods, uses and compositions provided herein, the treatment is a combination of a VEGF-specific antagonist, e.g., anti-VEGF antibody, and at least one chemotherapeutic agent. In other embodiments of the methods, uses and compositions provided herein, the VEGF-specific antagonist is a monotherapy.

[0029] Each of any of the methods, uses and compositions provided herein may be practiced in relation to the treatment of cancers including, but not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include breast cancer, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, renal cancer, vulval cancer, thyroid cancer, hepatic carcinoma, gastric cancer, melanoma, and various types of head and neck cancer. In some embodiments of the methods of the invention the subject has metastatic breast cancer. In some embodiments of the methods, uses and compositions provided herein the subject has previously untreated metastatic breast cancer. In some embodiments the subject has HER2-negative metastatic breast cancer.

[0030] Each of the above aspects can further include monitoring the subject for recurrence of the cancer. Monitoring can be accomplished, for example, by evaluating progression free survival (PFS) or overall survival (OS) or objective response rate (ORR). In one embodiment, the PFS or the OS or the ORR is evaluated after initiation of treatment.

[0031] Depending on the type and severity of the disease, preferred dosages for the anti-VEGF antibody, e.g., bevacizumab, are described herein and can range from about 1 µg/kg to about 50 mg/kg, most preferably from about 5 mg/kg to about 15 mg/kg, including but not limited to 5 mg/kg, 7.5 mg/kg, 10 mg/kg or 15 mg/kg. The frequency of administration will vary depending on the type and severity of the disease. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until the cancer is treated or the desired therapeutic effect is achieved, as measured by the methods described herein or known in the art. In one example, the anti-VEGF antibody is administered once every week, every two weeks, or every three weeks, at a dose range from about 5 mg/kg to about 15 mg/kg, including but not limited to 5 mg/kg, 7.5 mg/kg, 10 mg/kg or 15 mg/kg. However, other dosage regimens may be useful. The progress of the therapy of the invention is easily monitored by conventional techniques and assays.

[0032] In additional embodiments of each of the above aspects, the VEGF-specific antagonist, e.g., anti-VEGF antibody is administered locally or systemically (e.g., orally or intravenously). In other embodiments, one aspect of the treatment is with the VEGF-specific antagonist in a monotherapy or a monotherapy for the duration of the VEGF-specific antagonist treatment period, e.g., in extended treatment phase or maintenance therapy, as assessed by the clinician or described herein.

[0033] In other embodiments of the methods, uses and compositions provided herein, treatment, use or composition with the VEGF-specific antagonist is in combination with an additional anti-cancer therapy, including but not limited to, surgery, radiation therapy, chemotherapy, differentiating therapy, biotherapy, immune therapy, an angiogenesis inhibitor, a cytotoxic agent and/or an anti-proliferative compound. Treatment, use and composition with the VEGF-specific antagonist can also include any combination of the above types of therapeutic regimens. In some embodiments, the chemotherapeutic agent and the VEGF-specific antagonist are administered concurrently.

[0034] In the embodiments of the methods, uses and compositions provided herein which include an additional anti-

cancer therapy, the subject can be further treated with the additional anti-cancer therapy before, during (e.g., simultaneously), or after administration of the VEGF-specific antagonist. In one embodiment, the VEGF-specific antagonist, administered either alone or with an anti-cancer therapy, can be administered as maintenance therapy.

[0035] Other features and advantages of the invention will be apparent from the following Detailed Description, the drawings, and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036]

Figure 1 depicts the study design for the metastatic breast cancer trial using bevacizumab (BV) or placebo (PL) with various chemotherapies.

Figure 2 depicts progression free survival (PFS) curves for capecitabine arm of the trial. INV (investigator) is PFS assessed by investigator and IRC is PFS assessed by independent review committee (IRC), where placebo is PL and bevacizumab is BV.

Figure 3 depicts PFS curves for taxane/anthracycline arm of the trial. INV is PFS assessed by investigator and IRC is PFS assessed by independent review committee (IRC), where placebo is PL and bevacizumab is BV.

Figure 4 depicts a subgroup analyses of PFS in the capecitabine and taxane/anthracycline groups of the trial.

Figure 5 depicts the objective response rate for capecitabine (Cape) and taxane/anthracycline (T/Antra) groups.

Figure 6 depicts a subgroup analysis of PFS for taxane/anthracycline (T/Anthra) cohorts.

DETAILED DESCRIPTION

I. DEFINITIONS

[0037] The term "VEGF" or "VEGF-A" is used to refer to the 165-amino acid human vascular endothelial cell growth factor and related 121-, 145-, 189-, and 206- amino acid human vascular endothelial cell growth factors, as described by, e.g., Leung et al. Science, 246:1306 (1989), and Houck et al. Mol. Endocrin., 5:1806 (1991), together with the naturally occurring allelic and processed forms thereof. VEGF-A is part of a gene family including VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and PIGF. VEGF-A primarily binds to two high affinity receptor tyrosine kinases, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR), the latter being the major transmitter of vascular endothelial cell mitogenic signals of VEGF-A. Additionally, neuropilin-1 has been identified as a receptor for heparin-binding VEGF-A isoforms, and may play a role in vascular development. The term "VEGF" or "VEGF-A" also refers to VEGFs from non-human species such as mouse, rat, or primate. Sometimes the VEGF from a specific species is indicated by terms such as hVEGF for human VEGF or mVEGF for murine VEGF. Typically, VEGF refers to human VEGF. The term "VEGF" is also used to refer to truncated forms or fragments of the polypeptide comprising amino acids 8 to 109 or 1 to 109 of the 165-amino acid human vascular endothelial cell growth factor. Reference to any such forms of VEGF may be identified in the application, e.g., by "VEGF (8-109)," "VEGF (1-109)" or "VEGF165." The amino acid positions for a "truncated" native VEGF are numbered as indicated in the native VEGF sequence. For example, amino acid position 17 (methionine) in truncated native VEGF is also position 17 (methionine) in native VEGF. The truncated native VEGF has binding affinity for the KDR and Flt-1 receptors comparable to native VEGF.

[0038] An "anti-VEGF antibody" is an antibody that binds to VEGF with sufficient affinity and specificity. The antibody selected will normally have a binding affinity for VEGF, for example, the antibody may bind hVEGF with a K_d value of between 100 nM-1 pM. Antibody affinities may be determined by a surface plasmon resonance based assay (such as the BIAcore assay as described in PCT Application Publication No. WO2005/012359); enzyme-linked immunoabsorbent assay (ELISA); and competition assays (e.g. RIA's), for example. In certain embodiments, the anti-VEGF antibody of the invention can be used as a therapeutic agent in targeting and interfering with diseases or conditions wherein the VEGF activity is involved. Also, the antibody may be subjected to other biological activity assays, e.g., in order to evaluate its effectiveness as a therapeutic. Such assays are known in the art and depend on the target antigen and intended use for the antibody. Examples include the HUVEC inhibition assay; tumor cell growth inhibition assays (as described in WO 89/06692, for example); antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytotoxicity (CDC) assays (US Patent 5,500,362); and agonistic activity or hematopoiesis assays (see WO 95/27062). An anti-VEGF antibody will usually not bind to other VEGF homologues such as VEGF-B or VEGF-C, nor other growth factors such as PIGF, PDGF or bFGF.

[0039] A "VEGF antagonist" refers to a molecule capable of neutralizing, blocking, inhibiting, abrogating, reducing or interfering with VEGF activities including its binding to one or more VEGF receptors. VEGF antagonists include anti-VEGF antibodies and antigen-binding fragments thereof, receptor molecules and derivatives which bind specifically to VEGF thereby sequestering its binding to one or more receptors, anti-VEGF receptor antibodies and VEGF receptor

antagonists such as small molecule inhibitors of the VEGFR tyrosine kinases.

[0040] A "native sequence" polypeptide comprises a polypeptide having the same amino acid sequence as a polypeptide derived from nature. Thus, a native sequence polypeptide can have the amino acid sequence of naturally-occurring polypeptide from any mammal. Such native sequence polypeptide can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence" polypeptide specifically encompasses naturally-occurring truncated or secreted forms of the polypeptide (e.g., an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide.

[0041] A polypeptide "variant" means a biologically active polypeptide having at least about 80% amino acid sequence identity with the native sequence polypeptide. Such variants include, for instance, polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the polypeptide. Ordinarily, a variant will have at least about 80% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity, and even more preferably at least about 95% amino acid sequence identity with the native sequence polypeptide.

[0042] The term "antibody" is used in the broadest sense and includes monoclonal antibodies (including full length or intact monoclonal antibodies), polyclonal antibodies, multivalent antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments (see below) so long as they exhibit the desired biological activity.

[0043] Throughout the present specification and claims, the numbering of the residues in an immunoglobulin heavy chain is that of the EU index as in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

[0044] The "Kd" or "Kd value" according to this invention is in one embodiment measured by a radiolabeled VEGF binding assay (RIA) performed with the Fab version of the antibody and a VEGF molecule as described by the following assay that measures solution binding affinity of Fabs for VEGF by equilibrating Fab with a minimal concentration of (¹²⁵I)-labeled VEGF(109) in the presence of a titration series of unlabeled VEGF, then capturing bound VEGF with an anti-Fab antibody-coated plate (Chen, et al., (1999) *J. Mol Biol* 293:865-881). In one example, to establish conditions for the assay, microtiter plates (Dynex) are coated overnight with 5 ug/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbant plate (Nunc #269620), 100 pM or 26 pM [¹²⁵I] VEGF(109) are mixed with serial dilutions of a Fab of interest, e.g., Fab-12 (Presta et al., (1997) *Cancer Res.* 57:4593-4599). The Fab of interest is then incubated overnight; however, the incubation may continue for 65 hours to insure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature for one hour. The solution is then removed and the plate washed eight times with 0.1% Tween-20 in PBS. When the plates had dried, 150 ul/well of scintillant (MicroScint-20; Packard) is added, and the plates are counted on a Topcount gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays. According to another embodiment the Kd or Kd value is measured by using surface plasmon resonance assays using a BIAcore™-2000 or a BIAcore™-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized hVEGF (8-109) CM5 chips at ~ 10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore Inc.) are activated with *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Human VEGF is diluted with 10mM sodium acetate, pH 4.8, into 5ug/ml (~0.2uM) before injection at a flow rate of 5ul/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of human VEGF, 1M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% Tween 20 (PBST) at 25°C at a flow rate of approximately 25ul/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIAcore Evaluation Software version 3.2) by simultaneous fitting the association and dissociation sensorgram. The equilibrium dissociation constant (Kd) was calculated as the ratio k_{off}/k_{on} . See, e.g., Chen, Y., et al., (1999) *J. Mol Biol* 293:865-881. If the on-rate exceeds $10^6 \text{ M}^{-1} \text{ S}^{-1}$ by the surface plasmon resonance assay above, then the on-rate is can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20nM anti-VEGF antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of human VEGF short form (8-109) or mouse VEGF as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-Aminco spectrophotometer (ThermoSpectronic) with a stirred cuvette.

[0045] A "blocking" antibody or an antibody "antagonist" is one which inhibits or reduces biological activity of the antigen it binds. For example, a VEGF-specific antagonist antibody binds VEGF and inhibits the ability of VEGF to induce angiogenesis, to induce vascular endothelial cell proliferation or to induce vascular permeability. In certain embodiments, blocking antibodies or antagonist antibodies completely inhibit the biological activity of the antigen.

[0046] Unless indicated otherwise, the expression "multivalent antibody" is used throughout this specification to denote an antibody comprising three or more antigen binding sites. For example, the multivalent antibody is engineered to have the three or more antigen binding sites and is generally not a native sequence IgM or IgA antibody.

[0047] "Antibody fragments" comprise only a portion of an intact antibody, generally including an antigen binding site of the intact antibody and thus retaining the ability to bind antigen. Examples of antibody fragments encompassed by the present definition include: (i) the Fab fragment, having VL, CL, VH and CH1 domains; (ii) the Fab' fragment, which is a Fab fragment having one or more cysteine residues at the C-terminus of the CH1 domain; (iii) the Fd fragment having VH and CH1 domains; (iv) the Fd' fragment having VH and CH1 domains and one or more cysteine residues at the C-terminus of the CH1 domain; (v) the Fv fragment having the VL and VH domains of a single arm of an antibody; (vi) the dAb fragment (Ward et al., Nature 341, 544-546 (1989)) which consists of a VH domain; (vii) isolated CDR regions; (viii) F(ab')₂ fragments, a bivalent fragment including two Fab' fragments linked by a disulphide bridge at the hinge region; (ix) single chain antibody molecules (e.g. single chain Fv; scFv) (Bird et al., Science 242:423-426 (1988); and Huston et al., PNAS (USA) 85:5879-5883 (1988)); (x) "diabodies" with two antigen binding sites, comprising a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (see, e.g., EP 404,097; WO 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)); (xi) "linear antibodies" comprising a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata et al. Protein Eng. 8(10):1057-1062 (1995); and US Patent No. 5,641,870).

[0048] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the invention may be made by the hybridoma method first described by Kohler et al., Nature 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., Nature 352:624-628 (1991) or Marks et al., J. Mol. Biol. 222:581-597 (1991), for example.

[0049] An "Fv" fragment is an antibody fragment which contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in tight association, which can be covalent in nature, for example in scFv. It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs or a subset thereof confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although usually at a lower affinity than the entire binding site.

[0050] As used herein, "antibody variable domain" refers to the portions of the light and heavy chains of antibody molecules that include amino acid sequences of Complementarity Determining Regions (CDRs; i.e., CDR1, CDR2, and CDR3), and Framework Regions (FRs). V_H refers to the variable domain of the heavy chain. V_L refers to the variable domain of the light chain. According to the methods used in this invention, the amino acid positions assigned to CDRs and FRs may be defined according to Kabat (Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md., 1987 and 1991)). Amino acid numbering of antibodies or antigen binding fragments is also according to that of Kabat.

[0051] As used herein, the term "Complementarity Determining Regions" (CDRs; i.e., CDR1, CDR2, and CDR3) refers to the amino acid residues of an antibody variable domain the presence of which are necessary for antigen binding. Each variable domain typically has three CDR regions identified as CDR1, CDR2 and CDR3. Each complementarity determining region may comprise amino acid residues from a "complementarity determining region" as defined by Kabat (i.e. about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (i.e. about residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). In some instances, a complementarity determining region can include amino acids from both a CDR region defined according to Kabat and a hypervariable loop. For example, the CDRH1 of the heavy chain of antibody 4D5 includes amino acids 26 to 35.

[0052] "Framework regions" (hereinafter FR) are those variable domain residues other than the CDR residues. Each variable domain typically has four FRs identified as FR1, FR2, FR3 and FR4. If the CDRs are defined according to Kabat, the light chain FR residues are positioned at about residues 1-23 (LCFR1), 35-49 (LCFR2), 57-88 (LCFR3), and 98-107 (LCFR4) and the heavy chain FR residues are positioned about at residues 1-30 (HCFR1), 36-49 (HCFR2), 66-94 (HCFR3), and 103-113 (HCFR4) in the heavy chain residues. If the CDRs comprise amino acid residues from hypervariable loops, the light chain FR residues are positioned about at residues 1-25 (LCFR1), 33-49 (LCFR2), 53-90 (LCFR3),

and 97-107 (LCFR4) in the light chain and the heavy chain FR residues are positioned about at residues 1-25 (HCFR1), 33-52 (HCFR2), 56-95 (HCFR3), and 102-113 (HCFR4) in the heavy chain residues. In some instances, when the CDR comprises amino acids from both a CDR as defined by Kabat and those of a hypervariable loop, the FR residues will be adjusted accordingly. For example, when CDRH1 includes amino acids H26-H35, the heavy chain FR1 residues are at positions 1-25 and the FR2 residues are at positions 36-49.

[0053] The "Fab" fragment contains a variable and constant domain of the light chain and a variable domain and the first constant domain (CH1) of the heavy chain. F(ab')₂ antibody fragments comprise a pair of Fab fragments which are generally covalently linked near their carboxy termini by hinge cysteines between them. Other chemical couplings of antibody fragments are also known in the art.

[0054] "Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains, which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, Vol 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

[0055] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H and V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

[0056] The expression "linear antibodies" refers to the antibodies described in Zapata et al., *Protein Eng.*, 8(10):1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (V_H-C_H1-V_H-C_H1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[0057] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)).

[0058] "Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

[0059] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al. *Nature Biotechnology* 14:309-314 (1996); Sheets et al. *Proc. Natl. Acad. Sci.* 95:6157-6162 (1998)); Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991)). Human antibodies can also be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., *Biotechnology* 10: 779-783 (1992); Lonberg et al., *Nature* 368: 856-859 (1994); Morrison, *Nature* 368:812-13 (1994); Fishwild et al., *Nature Biotechnology* 14: 845-51 (1996); Neuberger, *Nature Biotechnology* 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995). Alternatively, the human antibody may be prepared via immortalization of human B lymphocytes producing an antibody

directed against a target antigen (such B lymphocytes may be recovered from an individual or may have been immunized in vitro). See, e.g., Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner et al., *J. Immunol.*, 147 (1):86-95 (1991); and U.S. Pat. No. 5,750,373.

[0060] An "affinity matured" antibody is one with one or more alterations in one or more CDRs thereof which result an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al. *Bio/Technology* 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas et al. *Proc Nat. Acad. Sci, USA* 91:3809-3813 (1994); Schier et al. *Gene* 169:147-155 (1995); Yelton et al. *J. Immunol.* 155:1994-2004 (1995); Jackson et al., *J. Immunol.* 154(7):3310-9 (1995); and Hawkins et al., *J. Mol. Biol.* 226:889-896 (1992).

[0061] A "functional antigen binding site" of an antibody is one which is capable of binding a target antigen. The antigen binding affinity of the antigen binding site is not necessarily as strong as the parent antibody from which the antigen binding site is derived, but the ability to bind antigen must be measurable using any one of a variety of methods known for evaluating antibody binding to an antigen. Moreover, the antigen binding affinity of each of the antigen binding sites of a multivalent antibody herein need not be quantitatively the same. For the multimeric antibodies herein, the number of functional antigen binding sites can be evaluated using ultracentrifugation analysis as described in Example 2 of U.S. Patent Application Publication No. 20050186208. According to this method of analysis, different ratios of target antigen to multimeric antibody are combined and the average molecular weight of the complexes is calculated assuming differing numbers of functional binding sites. These theoretical values are compared to the actual experimental values obtained in order to evaluate the number of functional binding sites.

[0062] An antibody having a "biological characteristic" of a designated antibody is one which possesses one or more of the biological characteristics of that antibody which distinguish it from other antibodies that bind to the same antigen.

[0063] In order to screen for antibodies which bind to an epitope on an antigen bound by an antibody of interest, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed.

[0064] A "species-dependent antibody" is one which has a stronger binding affinity for an antigen from a first mammalian species than it has for a homologue of that antigen from a second mammalian species. Normally, the species-dependent antibody "binds specifically" to a human antigen (*i.e.* has a binding affinity (K_d) value of no more than about 1×10^{-7} M, preferably no more than about 1×10^{-8} M and most preferably no more than about 1×10^{-9} M) but has a binding affinity for a homologue of the antigen from a second nonhuman mammalian species which is at least about 50 fold, or at least about 500 fold, or at least about 1000 fold, weaker than its binding affinity for the human antigen. The species-dependent antibody can be any of the various types of antibodies as defined above, but typically is a humanized or human antibody.

[0065] As used herein, "antibody mutant" or "antibody variant" refers to an amino acid sequence variant of the species-dependent antibody wherein one or more of the amino acid residues of the species-dependent antibody have been modified. Such mutants necessarily have less than 100% sequence identity or similarity with the species-dependent antibody. In one embodiment, the antibody mutant will have an amino acid sequence having at least 75% amino acid sequence identity or similarity with the amino acid sequence of either the heavy or light chain variable domain of the species-dependent antibody, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%. Identity or similarity with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical (*i.e.* same residue) or similar (*i.e.* amino acid residue from the same group based on common side-chain properties, see below) with the species-dependent antibody residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody sequence outside of the variable domain shall be construed as affecting sequence identity or similarity.

[0066] To increase the half-life of the antibodies or polypeptide containing the amino acid sequences of this invention, one can attach a salvage receptor binding epitope to the antibody (especially an antibody fragment), as described, e.g., in US Patent 5,739,277. For example, a nucleic acid molecule encoding the salvage receptor binding epitope can be linked in frame to a nucleic acid encoding a polypeptide sequence of this invention so that the fusion protein expressed by the engineered nucleic acid molecule comprises the salvage receptor binding epitope and a polypeptide sequence of this invention. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG₁, IgG₂, IgG₃, or IgG₄) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule (*e.g.*, Ghetie et al., *Ann. Rev. Immunol.* 18:739-766 (2000), Table 1). Antibodies with substitutions in an Fc region thereof and increased serum half-lives are also described in WO00/42072, WO 02/060919; Shields et al., *J. Biol. Chem.* 276:6591-6604 (2001); Hinton, *J. Biol. Chem.* 279:6213-6216 (2004)). In another embodiment, the serum half-life can also be increased, for example, by attaching other polypeptide sequences. For example, antibodies or other polypeptides useful in the methods of the invention can be attached to serum albumin or a portion of serum albumin that binds to the FcRn receptor or a serum albumin binding peptide so that serum albumin binds to the antibody or

polypeptide, e.g., such polypeptide sequences are disclosed in WO0145746. In one embodiment, the serum albumin peptide to be attached comprises an amino acid sequence of DICLP RWGCLW. In another embodiment, the half-life of a Fab is increased by these methods. See also, Dennis et al. J. Biol. Chem. 277:35035-35043 (2002) for serum albumin binding peptide sequences.

[0067] A "chimeric VEGF receptor protein" is a VEGF receptor molecule having amino acid sequences derived from at least two different proteins, at least one of which is a VEGF receptor protein. In certain embodiments, the chimeric VEGF receptor protein is capable of binding to and inhibiting the biological activity of VEGF.

[0068] An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In certain embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0069] By "fragment" is meant a portion of a polypeptide or nucleic acid molecule that contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, or more nucleotides or 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 190, 200 amino acids or more.

[0070] An "anti-angiogenesis agent" or "angiogenesis inhibitor" refers to a small molecular weight substance, a polynucleotide, a polypeptide, an isolated protein, a recombinant protein, an antibody, or conjugates or fusion proteins thereof, that inhibits angiogenesis, vasculogenesis, or undesirable vascular permeability, either directly or indirectly. It should be understood that the anti-angiogenesis agent includes those agents that bind and block the angiogenic activity of the angiogenic factor or its receptor. For example, an anti-angiogenesis agent is an antibody or other antagonist to an angiogenic agent as defined throughout the specification or known in the art, e.g., but are not limited to, antibodies to VEGF-A or to the VEGF-A receptor (e.g., KDR receptor or Flt-1 receptor), VEGF-trap, anti-PDGFR inhibitors such as Gleevec™ (Imatinib Mesylate). Anti-angiogenesis agents also include native angiogenesis inhibitors, e.g., angiostatin, endostatin, etc. See, e.g., Klagsbrun and D'Amore, *Annu. Rev. Physiol.*, 53:217-39 (1991); Streit and Detmar, *Oncogene*, 22:3172-3179 (2003) (e.g., Table 3 listing anti-angiogenic therapy in malignant melanoma); Ferrara & Alitalo, *Nature Medicine* 5:1359-1364 (1999); Tonini et al., *Oncogene*, 22:6549-6556 (2003) (e.g., Table 2 listing known antiangiogenic factors); and Sato, *Int. J. Clin. Oncol.*, 8:200-206 (2003) (e.g., Table 1 lists anti-angiogenic agents used in clinical trials).

[0071] A "maintenance" dose herein refers to one or more doses of a therapeutic agent administered to the subject over or after a treatment period. Usually, the maintenance doses are administered at spaced treatment intervals, such as approximately every week, approximately every 2 weeks, approximately every 3 weeks, or approximately every 4 weeks.

[0072] "Survival" refers to the subject remaining alive, and includes progression free survival (PFS) and overall survival (OS). Survival can be estimated by the Kaplan-Meier method, and any differences in survival are computed using the stratified log-rank test.

[0073] "Progression free survival (PFS)" refers to the time from treatment (or randomization) to first disease progression or death. For example it is the time that the subject remains alive, without return of the cancer, e.g., for a defined period of time such as about 1 month, 1.2 months, 2 months, 2.4 months, 2.9 months, 3 months, 3.5 months, 4, months, 6 months, 7 months, 8 months, 9 months, 1 year, about 2 years, about 3 years, etc., from initiation of treatment or from initial diagnosis. In one embodiment, the PFS is extended about 2.9 months to 3.5 months (e.g., with capecitabine). In one embodiment, the PFS is extended about 1.2 months to about 2.4 months (e.g., with taxane/anthracycline). In one aspect of the invention, PFS can be assessed by Response Evaluation Criteria in Solid Tumors (RECIST).

[0074] "Overall survival" refers to the subject remaining alive for a defined period of time, such as about 1 year, about 2 years, about 3 years, about 4 years, about 5 years, about 10 years, etc., from initiation of treatment or from initial diagnosis. In the studies underlying the invention the event used for survival analysis was death from any cause.

[0075] By "extending survival" or "increasing the likelihood of survival" is meant increasing PFS and/or OS in a treated subject relative to an untreated subject (i.e. relative to a subject not treated with a VEGF-specific antagonist, e.g., a VEGF antibody), or relative to a control treatment protocol, such as treatment only with the chemotherapeutic agent, such as those used in the standard of care for breast cancer, e.g., capecitabine, taxane, anthracycline, paclitaxel, docetaxel, paclitaxel protein-bound particles (e.g., Abraxane®), doxorubicin, epirubicin, 5-fluorouracil, cyclophosphamide or combinations thereof. Survival is monitored for at least about one month, two months, four months, six months, nine months, or at least about 1 year, or at least about 2 years, or at least about 3 years, or at least about 4 years, or at least about 5 years, or at least about 10 years, etc., following the initiation of treatment or following the initial diagnosis.

[0076] Hazard ratio (HR) is a statistical definition for rates of events. For the purpose of the invention, hazard ratio is

defined as representing the probability of an event in the experimental arm divided by the probability of an event in the control arm at any specific point in time. "Hazard ratio" in progression free survival analysis is a summary of the difference between two progression free survival curves, representing the reduction in the risk of death on treatment compared to control, over a period of follow-up.

[0077] The term "concurrently" is used herein to refer to administration of two or more therapeutic agents, where at least part of the administration overlaps in time. Accordingly, concurrent administration includes a dosing regimen when the administration of one or more agent(s) continues after discontinuing the administration of one or more other agent(s).

[0078] By "monotherapy" is meant a therapeutic regimen that includes only a single therapeutic agent for the treatment of the cancer or tumor during the course of the treatment period. Monotherapy using a VEGF-specific antagonist means that the VEGF-specific antagonist is administered in the absence of an additional anti-cancer therapy during treatment period.

[0079] By "maintenance therapy" is meant a therapeutic regimen that is given to reduce the likelihood of disease recurrence or progression. Maintenance therapy can be provided for any length of time, including extended time periods up to the life-span of the subject. Maintenance therapy can be provided after initial therapy or in conjunction with initial or additional therapies. Dosages used for maintenance therapy can vary and can include diminished dosages as compared to dosages used for other types of therapy. See also "maintenance" herein.

[0080] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Included in this definition are benign and malignant cancers as well as dormant tumors or micrometastases. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include breast cancer, squamous cell cancer, lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

[0081] By "metastasis" is meant the spread of cancer from its primary site to other places in the body. Cancer cells can break away from a primary tumor, penetrate into lymphatic and blood vessels, circulate through the bloodstream, and grow in a distant focus (metastasize) in normal tissues elsewhere in the body. Metastasis can be local or distant. Metastasis is a sequential process, contingent on tumor cells breaking off from the primary tumor, traveling through the bloodstream, and stopping at a distant site. At the new site, the cells establish a blood supply and can grow to form a life-threatening mass. Both stimulatory and inhibitory molecular pathways within the tumor cell regulate this behavior, and interactions between the tumor cell and host cells in the distant site are also significant.

[0082] By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline. Preferably, the subject is a human. Patients are also subjects herein.

[0083] For the methods of the present invention, the term "instructing" a subject means providing directions for applicable therapy, medication, treatment, treatment regimens, and the like, by any means, but preferably in writing, such as in the form of package inserts or other written promotional material.

[0084] For the methods of the present invention, the term "promoting" means offering, advertising, selling, or describing a particular drug, combination of drugs, or treatment modality, by any means, including writing, such as in the form of package inserts. Promoting herein refers to promotion of a therapeutic agent, such as a VEGF antagonist, e.g., anti-VEGF antibody or chemotherapeutic agent, for an indication, such as breast cancer treatment, where such promoting is authorized by the Food and Drug Administration (FDA) as having been demonstrated to be associated with statistically significant therapeutic efficacy and acceptable safety in a population of subjects.

[0085] The term "marketing" is used herein to describe the promotion, selling or distribution of a product (e.g., drug). Marketing specifically includes packaging, advertising, and any business activity with the purpose of commercializing a product.

[0086] A "population" of subjects refers to a group of subjects with cancer, such as in a clinical trial, or as seen by oncologists following FDA approval for a particular indication, such as breast cancer therapy. In one embodiment, the population comprises at least about 1200 subjects.

[0087] The term "anti-cancer therapy" refers to a therapy useful in treating cancer. Examples of anti-cancer therapeutic agents include, but are limited to, e.g., surgery, chemotherapeutic agents, growth inhibitory agents, cytotoxic agents,

agents used in radiation therapy, anti-angiogenesis agents, apoptotic agents, anti-tubulin agents, and other agents to treat cancer, such as anti-HER-2 antibodies (e.g., Herceptin®), anti-CD20 antibodies, an epidermal growth factor receptor (EGFR) antagonist (e.g., a tyrosine kinase inhibitor), HER1/EGFR inhibitor (e.g., erlotinib (Tarceva®)), platelet derived growth factor inhibitors (e.g., Gleevec™ (Imatinib Mesylate)), a COX-2 inhibitor (e.g., celecoxib), interferons, cytokines, antagonists (e.g., neutralizing antibodies) that bind to one or more of the following targets ErbB2, ErbB3, ErbB4, PDGFR-beta, BlyS, APRIL, BCMA or VEGF receptor(s), TRAIL/Apo2, and other bioactive and organic chemical agents, etc. Combinations thereof are also included in the invention.

[0088] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³² and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof.

[0089] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gamma11 and calicheamicin omega11 (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994))); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabacin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verrucurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE® Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumburg, Illinois), and TAXOTERE® doxetaxel (Rhone-Poulenc Rorer, Antony, France); chlorambucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; combretastatin; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); lapatinib (Tykerb®); inhibitors of PKC-alpha, Raf, H-Ras, EGFR (e.g., erlotinib (Tarceva®)) and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0090] Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen pro-

duction in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestane, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC- α , Ralf and H-Ras; ribozymes such as a VEGF expression inhibitor (e.g., ANGIOZYME® ribozyme) and a HER2 expression inhibitor; vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0091] The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); epidermal growth factor; hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- α and - β ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- α ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- α and TGF- β ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- α , - β and - γ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; a tumor necrosis factor such as TNF- α or TNF- β ; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

[0092] A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell in vitro and/or in vivo. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), TAXOL®, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13.

[0093] The term "prodrug" as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, e.g., Wilman, "Prodrugs in Cancer Chemotherapy" *Biochemical Society Transactions*, 14, pp. 375-382, 615th Meeting Belfast (1986) and Stella et al., "Prodrugs: A Chemical Approach to Targeted Drug Delivery," *Directed Drug Delivery*, Borchardt et al., (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β -lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

[0094] By "radiation therapy" is meant the use of directed gamma rays or beta rays to induce sufficient damage to a cell so as to limit its ability to function normally or to destroy the cell altogether. It will be appreciated that there will be many ways known in the art to determine the dosage and duration of treatment. Typical treatments are given as a one time administration and typical dosages range from 10 to 200 units (Grays) per day.

[0095] By "reduce or inhibit" is meant the ability to cause an overall decrease preferably of 20% or greater, more preferably of 50% or greater, and most preferably of 75%, 85%, 90%, 95%, or greater. Reduce or inhibit can refer to the symptoms of the disorder being treated, the presence or size of metastases or micrometastases, the size of the primary tumor, the presence or the size of the dormant tumor, or the size or number of the blood vessels in angiogenic disorders.

[0096] The term "intravenous infusion" refers to introduction of a drug into the vein of an animal or human subject over a period of time greater than approximately 5 minutes, preferably between approximately 30 to 90 minutes, although, according to the invention, intravenous infusion is alternatively administered for 10 hours or less.

[0097] The term "intravenous bolus" or "intravenous push" refers to drug administration into a vein of an animal or human such that the body receives the drug in approximately 15 minutes or less, preferably 5 minutes or less.

[0098] The term "subcutaneous administration" refers to introduction of a drug under the skin of an animal or human

subject, preferable within a pocket between the skin and underlying tissue, by relatively slow, sustained delivery from a drug receptacle. The pocket may be created by pinching or drawing the skin up and away from underlying tissue.

[0099] The term "subcutaneous infusion" refers to introduction of a drug under the skin of an animal or human subject, preferably within a pocket between the skin and underlying tissue, by relatively slow, sustained delivery from a drug receptacle for a period of time including, but not limited to, 30 minutes or less, or 90 minutes or less. Optionally, the infusion may be made by subcutaneous implantation of a drug delivery pump implanted under the skin of the animal or human subject, wherein the pump delivers a predetermined amount of drug for a predetermined period of time, such as 30 minutes, 90 minutes, or a time period spanning the length of the treatment regimen.

[0100] The term "subcutaneous bolus" refers to drug administration beneath the skin of an animal or human subject, where bolus drug delivery is preferably less than approximately 15 minutes, more preferably less than 5 minutes, and most preferably less than 60 seconds. Administration is preferably within a pocket between the skin and underlying tissue, where the pocket is created, for example, by pinching or drawing the skin up and away from underlying tissue.

[0101] A "disorder" is any condition that would benefit from treatment with the antibody. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include cancer; benign and malignant tumors; leukemias and lymphoid malignancies; neuronal, glial, astrocytic, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

[0102] The term "therapeutically effective amount" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the disorder. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy *in vivo* can, for example, be measured by assessing the duration of survival, duration of progression free survival (PFS), the response rates (RR), duration of response, and/or quality of life.

[0103] "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

[0104] The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the polypeptide. The label may be itself be detectable (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

II. ANTI-VEGF ANTIBODIES AND ANTAGONISTS

(i) VEGF Antigen

[0105] The VEGF antigen to be used for production of antibodies may be, e.g., the VEGF₁₆₅ molecule as well as other isoforms of VEGF or a fragment thereof containing the desired epitope. Other forms of VEGF useful for generating anti-VEGF antibodies of the invention will be apparent to those skilled in the art.

[0106] Human VEGF was obtained by first screening a cDNA library prepared from human cells, using bovine VEGF cDNA as a hybridization probe. Leung et al. (1989) Science, 246:1306. One cDNA identified thereby encodes a 165-amino acid protein having greater than 95% homology to bovine VEGF; this 165-amino acid protein is typically referred to as human VEGF (hVEGF) or VEGF₁₆₅. The mitogenic activity of human VEGF was confirmed by expressing the human VEGF cDNA in mammalian host cells. Media conditioned by cells transfected with the human VEGF cDNA promoted the proliferation of capillary endothelial cells, whereas control cells did not. Leung et al. (1989) Science, *supra*. Further efforts were undertaken to clone and express VEGF via recombinant DNA techniques. (See, e.g., Ferrara, Laboratory Investigation 72:615-618 (1995), and the references cited therein).

[0107] VEGF is expressed in a variety of tissues as multiple homodimeric forms (121, 145, 165, 189, and 206 amino acids per monomer) resulting from alternative RNA splicing. VEGF₁₂₁ is a soluble mitogen that does not bind heparin; the longer forms of VEGF bind heparin with progressively higher affinity. The heparin-binding forms of VEGF can be cleaved in the carboxy terminus by plasmin to release a diffusible form(s) of VEGF. Amino acid sequencing of the carboxy terminal peptide identified after plasmin cleavage is Arg₁₁₀-Ala₁₁₁. Amino terminal "core" protein, VEGF (1-110) isolated as a homodimer, binds neutralizing monoclonal antibodies (such as the antibodies referred to as 4.6.1 and 3.2E3.1.1) and soluble forms of VEGF receptors with similar affinity compared to the intact VEGF₁₆₅ homodimer.

[0108] Several molecules structurally related to VEGF have also been identified recently, including placenta growth factor (PlGF), VEGF-B, VEGF-C, VEGF-D and VEGF-E. Ferrara and Davis-Smyth (1987) Endocr. Rev., *supra*; Ogawa et al. J. Biological Chem. 273:31273-31281(1998); Meyer et al. EMBO J., 18:363-374(1999). A receptor tyrosine kinase, Flt-4 (VEGFR-3), has been identified as the receptor for VEGF-C and VEGF-D. Joukov et al. EMBO. J. 15:1751(1996);

Lee et al. Proc. Natl. Acad. Sci. USA 93:1988-1992(1996); Achen et al. (1998) Proc. Natl. Acad. Sci. USA 95:548-553. VEGF-C has been shown to be involved in the regulation of lymphatic angiogenesis. Jeltsch et al. Science 276:1423-1425(1997).

[0109] Two VEGF receptors have been identified, Flt-1 (also called VEGFR-1) and KDR (also called VEGFR-2). Shibuya et al. (1990) Oncogene 8:519-527; de Vries et al. (1992) Science 255:989-991; Terman et al. (1992) Biochem. Biophys. Res. Commun. 187:1579-1586. Neuropilin-1 has been shown to be a selective VEGF receptor, able to bind the heparin-binding VEGF isoforms (Soker et al. (1998) Cell 92:735-45).

(ii) Anti-VEGF Antibodies

[0110] Anti-VEGF antibodies that are useful in the methods of the invention include any antibody, or antigen binding fragment thereof, that bind with sufficient affinity and specificity to VEGF and can reduce or inhibit the biological activity of VEGF. An anti-VEGF antibody will usually not bind to other VEGF homologues such as VEGF-B or VEGF-C, nor other growth factors such as PIGF, PDGF, or bFGF.

[0111] In certain embodiments of the invention, the anti-VEGF antibodies include, but are not limited to, a monoclonal antibody that binds to the same epitope as the monoclonal anti-VEGF antibody A4.6.1 produced by hybridoma ATCC HB 10709; a recombinant humanized anti-VEGF monoclonal antibody generated according to Presta et al. (1997) Cancer Res. 57:4593-4599. In one embodiment, the anti-VEGF antibody is "Bevacizumab (BV)", also known as "rhuMAb VEGF" or "AVASTIN®". It comprises mutated human IgG1 framework regions and antigen-binding complementarity-determining regions from the murine anti-hVEGF monoclonal antibody A.4.6.1 that blocks binding of human VEGF to its receptors. Approximately 93% of the amino acid sequence of bevacizumab, including most of the framework regions, is derived from human IgG1, and about 7% of the sequence is derived from the murine antibody A4.6.1.

[0112] Bevacizumab (AVASTIN®) was the first anti-angiogenesis therapy approved by the FDA and is approved for the treatment metastatic colorectal cancer (first- and second-line treatment in combination with intravenous 5-FU-based chemotherapy), advanced non-squamous, non-small cell lung cancer (NSCLC) (first-line treatment of unresectable, locally advanced, recurrent or metastatic NSCLC in combination with carboplatin and paclitaxel) and metastatic HER2-negative breast cancer (previously untreated, metastatic HER2-negative breast cancer in combination with paclitaxel).

[0113] Bevacizumab and other humanized anti-VEGF antibodies are further described in U.S. Pat. No. 6,884,879 issued Feb. 26, 2005. Additional antibodies include the G6 or B20 series antibodies (e.g., G6-31, B20-4.1), as described in PCT Publication No. WO2005/012359, PCT Publication No. WO2005/044853, and US Patent Application 60/991,302. For additional antibodies see U.S. Pat. Nos. 7,060,269, 6,582,959, 6,703,020; 6,054,297; WO98/45332; WO 96/30046; WO94/10202; EP 0666868B1; U.S. Patent Application Publication Nos. 2006009360, 20050186208, 20030206899, 20030190317, 20030203409, and 20050112126; and Popkov et al., Journal of Immunological Methods 288:149-164 (2004). Other antibodies include those that bind to a functional epitope on human VEGF comprising of residues F17, M18, D19, Y21, Y25, Q89, I91, K101, E103, and C104 or, alternatively, comprising residues F17, Y21, Q22, Y25, D63, I83 and Q89.

[0114] In one embodiment of the invention, the anti-VEGF antibody has a heavy chain variable region comprising the following amino acid sequence:

EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGVW

INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP

HYYGSSHWYF DVWGQGTSLVTVSS (SEQ ID No. 1)

and a light chain variable region comprising the following amino acid sequence:

DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIIYF

TSSLHSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ

GTKVEIKR (SEQ ID No. 2).

[0115] A "G6 series antibody" according to this invention, is an anti-VEGF antibody that is derived from a sequence

of a G6 antibody or G6-derived antibody according to any one of Figures 7, 24-26, and 34-35 of PCT Publication No. WO2005/012359. See also PCT Publication No. WO2005/044853. In one embodiment, the G6 series antibody binds to a functional epitope on human VEGF comprising residues F17, Y21, Q22, Y25, D63, I83 and Q89.

[0116] A "B20 series antibody" according to this invention is an anti-VEGF antibody that is derived from a sequence of the B20 antibody or a B20-derived antibody according to any one of Figures 27-29 of PCT Publication No. WO2005/012359. See also PCT Publication No. WO2005/044853, and US Patent Application 60/991,302. In one embodiment, the B20 series antibody binds to a functional epitope on human VEGF comprising residues F17, M18, D19, Y21, Y25, Q89, I91, K101, E103, and C104.

[0117] A "functional epitope" according to this invention refers to amino acid residues of an antigen that contribute energetically to the binding of an antibody. Mutation of any one of the energetically contributing residues of the antigen (for example, mutation of wild-type VEGF by alanine or homolog mutation) will disrupt the binding of the antibody such that the relative affinity ratio (IC₅₀mutant VEGF/IC₅₀wild-type VEGF) of the antibody will be greater than 5 (see Example 2 of WO2005/012359). In one embodiment, the relative affinity ratio is determined by a solution binding phage displaying ELISA. Briefly, 96-well Maxisorp immunoplates (NUNC) are coated overnight at 4°C with an Fab form of the antibody to be tested at a concentration of 2 µg/ml in PBS, and blocked with PBS, 0.5% BSA, and 0.05% Tween20 (PBT) for 2h at room temperature. Serial dilutions of phage displaying hVEGF alanine point mutants (residues 8-109 form) or wild type hVEGF (8-109) in PBT are first incubated on the Fab-coated plates for 15 min at room temperature, and the plates are washed with PBS, 0.05% Tween20 (PBST). The bound phage is detected with an anti-M13 monoclonal antibody horseradish peroxidase (Amersham Pharmacia) conjugate diluted 1:5000 in PBT, developed with 3,3', 5,5'-tetramethylbenzidine (TMB, Kirkegaard & Perry Labs, Gaithersburg, MD) substrate for approximately 5 min, quenched with 1.0 M H₃PO₄, and read spectrophotometrically at 450 nm. The ratio of IC₅₀ values (IC₅₀,ala/IC₅₀,wt) represents the fold of reduction in binding affinity (the relative binding affinity).

(iii) VEGF receptor molecules

[0118] The two best characterized VEGF receptors are VEGFR1 (also known as Flt-1) and VEGFR2 (also known as KDR and FLK-1 for the murine homolog). The specificity of each receptor for each VEGF family member varies but VEGF-A binds to both Flt-1 and KDR. Both Flt-1 and KDR belong to the family of receptor tyrosine kinases (RTKs). The RTKs comprise a large family of transmembrane receptors with diverse biological activities. At least nineteen (19) distinct RTK subfamilies have been identified. The receptor tyrosine kinase (RTK) family includes receptors that are crucial for the growth and differentiation of a variety of cell types (Yarden and Ullrich (1988) Ann. Rev. Biochem. 57:433-478; Ullrich and Schlessinger (1990) Cell 61:243-254). The intrinsic function of RTKs is activated upon ligand binding, which results in phosphorylation of the receptor and multiple cellular substrates, and subsequently in a variety of cellular responses (Ullrich & Schlessinger (1990) Cell 61:203-212). Thus, receptor tyrosine kinase mediated signal transduction is initiated by extracellular interaction with a specific growth factor (ligand), typically followed by receptor dimerization, stimulation of the intrinsic protein tyrosine kinase activity and receptor trans-phosphorylation. Binding sites are thereby created for intracellular signal transduction molecules and lead to the formation of complexes with a spectrum of cytoplasmic signaling molecules that facilitate the appropriate cellular response. (e.g., cell division, differentiation, metabolic effects, changes in the extracellular microenvironment) see, Schlessinger and Ullrich (1992) Neuron 9:1-20. Structurally, both Flt-1 and KDR have seven immunoglobulin-like domains in the extracellular domain, a single transmembrane region, and a consensus tyrosine kinase sequence which is interrupted by a kinase-insert domain. Matthews et al. (1991) Proc. Natl. Acad. Sci. USA 88:9026-9030; Terman et al. (1991) Oncogene 6:1677-1683. The extracellular domain is involved in the binding of VEGF and the intracellular domain is involved in signal transduction.

[0119] VEGF receptor molecules, or fragments thereof, that specifically bind to VEGF can be used in the methods of the invention to bind to and sequester the VEGF protein, thereby preventing it from signaling. In certain embodiments, the VEGF receptor molecule, or VEGF binding fragment thereof, is a soluble form, such as sFlt-1. A soluble form of the receptor exerts an inhibitory effect on the biological activity of the VEGF protein by binding to VEGF, thereby preventing it from binding to its natural receptors present on the surface of target cells. Also included are VEGF receptor fusion proteins, examples of which are described below.

[0120] A chimeric VEGF receptor protein is a receptor molecule having amino acid sequences derived from at least two different proteins, at least one of which is a VEGF receptor protein (e.g., the flt-1 or KDR receptor), that is capable of binding to and inhibiting the biological activity of VEGF. In certain embodiments, the chimeric VEGF receptor proteins of the disclosure consist of amino acid sequences derived from only two different VEGF receptor molecules; however, amino acid sequences comprising one, two, three, four, five, six, or all seven Ig-like domains from the extracellular ligand-binding region of the flt-1 and/or KDR receptor can be linked to amino acid sequences from other unrelated proteins, for example, immunoglobulin sequences. Other amino acid sequences to which Ig-like domains are combined will be readily apparent to those of ordinary skill in the art. Examples of chimeric VEGF receptor proteins include, e.g., soluble Flt-1/Fc, KDR/Fc, or FLt-1/KDR/Fc (also known as VEGF Trap). (See for example PCT Application Publication

No. WO97/44453)

[0121] A soluble VEGF receptor protein or chimeric VEGF receptor proteins of the disclosure includes VEGF receptor proteins which are not fixed to the surface of cells via a transmembrane domain. As such, soluble forms of the VEGF receptor, including chimeric receptor proteins, while capable of binding to and inactivating VEGF, do not comprise a transmembrane domain and thus generally do not become associated with the cell membrane of cells in which the molecule is expressed.

III. THERAPEUTIC USES AND COMPOSITIONS OF ANTI-VEGF ANTIBODIES

[0122] The disclosure encompasses antiangiogenic therapy, a novel cancer treatment strategy aimed at inhibiting the development of tumor blood vessels required for providing nutrients to support tumor growth. Because angiogenesis is involved in both primary tumor growth and metastasis, the antiangiogenic treatment provided by the invention is capable of inhibiting the neoplastic growth of tumor at the primary site as well as preventing metastasis of tumors at the secondary sites, therefore allowing attack of the tumors by other therapeutics.

[0123] Specifically, described herein are methods of treating a subject diagnosed with previously untreated metastatic breast cancer, comprising administering to the subject a treatment regimen combining an effective amount of at least one chemotherapeutic agent and an anti-VEGF antibody, wherein said subject has not received any chemotherapy for locally recurrent or metastatic breast cancer. Optionally, the subject has not received prior adjuvant chemotherapy in recurrence less than or equal to 12 months since last dose. The treatment regimen combining the chemotherapy and the administration of the anti-VEGF effectively extends the progression free survival (PFS) of the subject. Further described herein are uses of an anti-VEGF antibody with at least one chemotherapeutic agent in the manufacture of a medicament for treating previously untreated metastatic breast cancer in a subject, wherein said subject has not received any chemotherapy for locally recurrent or metastatic breast cancer. Optionally, the subject has not received prior adjuvant chemotherapy in recurrence less than or equal to 12 months since last dose. The use of the anti-VEGF and the chemotherapeutic agent effectively extends the progression free survival (PFS) of the subject. Provided also herein are anti-VEGF antibodies for use in a method of treating locally recurrent or metastatic breast cancer in a subject, the method comprising administering to the subject a treatment regimen combining an effective amount of at least one chemotherapeutic agent and an anti-VEGF antibody, wherein said subject has not received any chemotherapy for locally recurrent or metastatic breast cancer. Optionally, the subject has not received prior adjuvant chemotherapy in recurrence less than or equal to 12 months since last dose. The use of the anti-VEGF and the chemotherapeutic agent effectively extends the progression free survival (PFS) of the subject. In certain embodiments, in any of the methods, uses, and compositions of the invention, the administration of the chemotherapy and the anti-VEGF antibody has a safety profile that is consistent with results of prior bevacizumab trials (see, e.g., the bevacizumab product insert).

[0124] In some embodiments of the invention, the subject is HER2 negative. In some embodiments of the invention, the subject is HER2 positive. HER2 is recognized as an important predictive and prognostic factor in some breast cancers. See, e.g., Slamon DJ, et al. *Science*. 1989;244:707-712; and Sjögren S, et al. *J Clin Oncol*. 1998;16:462-469. HER2 gene amplification is a permanent genetic change that results in the continuous overexpression of the HER2 receptor (HER2 protein). See, e.g., Simon R, et al. *J Natl Cancer Inst*. 2001;93:1141-1146; and, Sliwkowski MX, et al. *Semin Oncol*. 1999;26(suppl 12):60-70. Several studies have shown that HER2 overexpression (either extra copies of the gene itself, or an excess amount of the gene's protein product) is associated with decreased overall survival. See, e.g., Slamon DJ, et al. *Science*. 1987;235:177-182; and, Paik S, et al. *J Clin Oncol*. 1990;8:103-112. Several commercial assays are available to determine HER2 status, e.g., HercepTest® and Pathway™ for protein and PathVysion® and HER2 FISH pharmDx™ for gene alteration.

Combination Therapies

[0125] The invention features the use or compositions of a combination of at least one VEGF-specific antagonist with one or more additional anti-cancer therapies. Examples of anti-cancer therapies include, without limitation, surgery, radiation therapy (radiotherapy), biotherapy, immunotherapy, chemotherapy, or a combination of these therapies. In addition, cytotoxic agents, anti-angiogenic and anti-proliferative agents can be used in combination with the VEGF-specific antagonist.

[0126] In certain aspects of any of the methods and uses, the disclosure includes treating breast cancer, by administering effective amounts of an anti-VEGF antibody and one or more chemotherapeutic agents to a subject susceptible to, or diagnosed with, locally recurrent or previously untreated metastatic cancer. A variety of chemotherapeutic agents may be used in the combined treatment methods and uses of the disclosure. An exemplary and non-limiting list of chemotherapeutic agents contemplated is provided herein under "Definition", or described herein.

[0127] In one example, the disclosure includes the methods and uses of a VEGF-specific antagonist with one or more chemotherapeutic agents (e.g., a cocktail) or any combination thereof. In certain embodiments, the chemotherapeutic

agent is for example, capecitabine, taxane, anthracycline, paclitaxel, docetaxel, paclitaxel protein-bound particles (e.g., Abraxane®), doxorubicin, epirubicin, 5-fluorouracil, cyclophosphamide or combinations thereof therapy. In certain embodiments, VEGF antagonist (e.g., anti-VEGF antibody) is combined with lapatinib (Tykerb®). The combined administration includes simultaneous administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for chemotherapy are also described in Chemotherapy Service Ed., M. C. Perry, Williams & Wilkins, Baltimore, Md. (1992). The chemotherapeutic agent may precede, or follow administration of the VEGF-specific antagonist or may be given simultaneously therewith.

[0128] In some other aspects of any of the methods and uses, other therapeutic agents useful for combination tumor therapy with the antibody of the invention include antagonist of other factors that are involved in tumor growth, such as EGFR, ErbB2 (also known as Her2), ErbB3, ErbB4, or TNF. Sometimes, it may be beneficial to also administer one or more cytokines to the subject. In one embodiment, the VEGF antibody is co-administered with a growth inhibitory agent. For example, the growth inhibitory agent may be administered first, followed by the VEGF antibody. However, simultaneous administration or administration of the VEGF antibody first is also contemplated. Suitable dosages for the growth inhibitory agent are those presently used and may be lowered due to the combined action (synergy) of the growth inhibitory agent and anti-VEGF antibody.

[0129] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide antibodies which bind to EGFR, VEGF (e.g. an antibody which binds a different epitope or same epitope on VEGF), VEGFR, or ErbB2 (e.g., Herceptin®) in the one formulation. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine, growth inhibitory agent and/or VEGFR antagonist. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0130] In certain aspects of any of the methods and uses, other therapeutic agents useful for combination cancer therapy with the antibody of the invention include other anti-angiogenic agents. Many anti-angiogenic agents have been identified and are known in the arts, including those listed by Carmeliet and Jain (2000). In one embodiment, the anti-VEGF antibody of the invention is used in combination with another VEGF antagonist or a VEGF receptor antagonist such as VEGF variants, soluble VEGF receptor fragments, aptamers capable of blocking VEGF or VEGFR, neutralizing anti-VEGFR antibodies, low molecule weight inhibitors of VEGFR tyrosine kinases and any combinations thereof. Alternatively, or in addition, two or more anti-VEGF antibodies may be co-administered to the subject.

[0131] For the prevention or treatment of disease, the appropriate dosage of VEGF-specific antagonist will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the VEGF-specific antagonist is administered for preventive or therapeutic purposes, previous therapy, the subject's clinical history and response to the VEGF-specific antagonist, and the discretion of the attending physician. The VEGF-specific antagonist is suitably administered to the subject at one time or over a series of treatments. In a combination therapy regimen, the VEGF-specific antagonist and the one or more anti-cancer therapeutic agent of the invention are administered in a therapeutically effective or synergistic amount. As used herein, a therapeutically effective amount is such that co-administration of a VEGF-specific antagonist and one or more other therapeutic agents, or administration of a composition of the invention, results in reduction or inhibition of the cancer as described above. A therapeutically synergistic amount is that amount of a VEGF-specific antagonist and one or more other therapeutic agents necessary to synergistically or significantly reduce or eliminate conditions or symptoms associated with a particular disease.

[0132] The VEGF-specific antagonist and the one or more other therapeutic agents can be administered simultaneously or sequentially in an amount and for a time sufficient to reduce or eliminate the occurrence or recurrence of a tumor, a dormant tumor, or a micrometastases. The VEGF-specific antagonist and the one or more other therapeutic agents can be administered as maintenance therapy to prevent or reduce the likelihood of recurrence of the tumor.

[0133] As will be understood by those of ordinary skill in the art, the appropriate doses of chemotherapeutic agents or other anti-cancer agents will be generally around those already employed in clinical therapies, e.g., where the chemotherapeutics are administered alone or in combination with other chemotherapeutics. Variation in dosage will likely occur depending on the condition being treated. The physician administering treatment will be able to determine the appropriate dose for the individual subject.

[0134] In addition to the above therapeutic regimes, the subject may be subjected to radiation therapy.

[0135] In certain embodiments of any of the methods, uses and compositions, the administered VEGF antibody is an intact, naked antibody. However, the VEGF antibody may be conjugated with a cytotoxic agent. In certain embodiments of any of the methods and uses, the conjugated antibody and/or antigen to which it is bound is/are internalized by the cell, resulting in increased therapeutic efficacy of the conjugate in killing the cancer cell to which it binds. In one embodiment, the cytotoxic agent targets or interferes with nucleic acid in the cancer cell. Examples of such cytotoxic agents include maytansinoids, calicheamicins, ribonucleases and DNA endonucleases.

[0136] The disclosure also includes a method of instructing a human subject with breast cancer or a health care provider by providing instructions to receive treatment with an anti-VEGF antibody so as to increase the time for progression free survival, to decrease the subject's risk of cancer recurrence or to increase the subject's likelihood of survival. In some embodiments the method further comprises providing instructions to receive treatment with at least one chemotherapeutic agent. The treatment with the anti-VEGF antibody may be concurrent with or sequential to the treatment with the chemotherapeutic agent. In certain embodiments the subject is treated as instructed by the method of instructing. Treatment of breast cancer by administration of an anti-VEGF antibody with or without chemotherapy may be continued until cancer recurrence or death.

[0137] The disclosure further includes a promotional method, comprising promoting the administration of an anti-VEGF antibody for treatment of breast cancer in a human subject. In some embodiments the method further comprises promoting the administration of at least one chemotherapeutic agent. Administration of the anti-VEGF antibody may be concurrent with or sequential to administration of the chemotherapeutic agent. Promotion may be conducted by any means available. In some embodiments the promotion is by a package insert accompanying a commercial formulation of the anti-VEGF antibody. The promotion may also be by a package insert accompanying a commercial formulation of the chemotherapeutic agent. Promotion may be by written or oral communication to a physician or health care provider. In some embodiments the promotion is by a package insert where the package insert provides instructions to receive breast cancer therapy with anti-VEGF antibody. In a further embodiment, the package insert include some or all of the results under Example 1. In some embodiments the promotion is followed by the treatment of the subject with the anti-VEGF antibody with or without the chemotherapeutic agent.

[0138] The disclosure includes a business method, comprising marketing an anti-VEGF antibody for treatment of breast cancer in a human subject so as to increase the subject's time for progression free survival, to decrease the subject's likelihood of cancer recurrence or increase the subject's likelihood of survival. In some embodiments the method further comprises marketing a chemotherapeutic agent for use in combination with the anti-VEGF antibody. In some embodiments the marketing is followed by treatment of the subject with the anti-VEGF antibody with or without the chemotherapeutic agent.

[0139] Also described is a business method, comprising marketing a chemotherapeutic agent in combination with an anti-VEGF antibody for treatment of breast cancer in a human subject so as to increase the subject's time for progression free survival, to decrease the subject's likelihood of cancer recurrence or increase the subject's likelihood of survival. In some embodiments, the marketing is followed by treatment of the subject with the combination of the chemotherapeutic agent and the anti-VEGF antibody.

IV. DOSAGES AND DURATION

[0140] The VEGF-specific antagonist composition will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular subject being treated, the clinical condition of the individual subject, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of the VEGF-specific antagonist to be administered will be governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat, or stabilize, the cancer; to increase the time until progression (duration of progression free survival) or to treat or prevent the occurrence or recurrence of a tumor, a dormant tumor, or a micrometastases. The VEGF-specific antagonist need not be, but is optionally, formulated with one or more agents currently used to prevent or treat cancer or a risk of developing a cancer. The effective amount of such other agents depends on the amount of VEGF-specific antagonist present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

[0141] Depending on the type and severity of the disease, about 1 μ g/kg to 100 mg/kg (e.g., 0.1-20 mg/kg) of VEGF-specific antagonist is an initial candidate dosage for administration to the subject, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 μ g/kg to about 100 mg/kg or more, depending on the factors mentioned above. Particularly desirable dosages include, for example, 5 mg/kg, 7.5 mg/kg, 10 mg/kg, and 15 mg/kg. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until the cancer is treated, as measured by the methods described above or known in the art. However, other dosage regimens may be useful. In one example, if the VEGF-specific antagonist is an antibody, the antibody of the invention is administered once every week, every two weeks, or every three weeks, at a dose range from about 5 mg/kg to about 15 mg/kg, including but not limited to 5 mg/kg, 7.5 mg/kg, 10 mg/kg or 15 mg/kg. The progress of the therapy of the invention is easily monitored by conventional techniques and assays. In other embodiments, such dosing regimen is used in combination with a chemotherapy regimen as the first line therapy for treating locally recurrent or metastatic breast cancer. Further information about suitable dosages is provided in the Example below.

[0142] The duration of therapy will continue for as long as medically indicated or until a desired therapeutic effect (e.g.,

those described herein) is achieved. In certain embodiments, the VEGF-specific antagonist therapy is continued for 1 month, 2 months, 4 months, 6 months, 8 months, 10 months, 1 year, 2 years, 3 years, 4 years, 5 years, or for a period of years up to the lifetime of the subject.

[0143] The VEGF-specific antagonists of the invention are administered to a subject, e.g., a human subject, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Local administration is particularly desired if extensive side effects or toxicity is associated with the VEGF antagonist. An *ex vivo* strategy can also be used for therapeutic applications. *Ex vivo* strategies involve transfecting or transducing cells obtained from the subject with a polynucleotide encoding a VEGF antagonist. The transfected or transduced cells are then returned to the subject. The cells can be any of a wide range of types including, without limitation, hematopoietic cells (e.g., bone marrow cells, macrophages, monocytes, dendritic cells, T cells, or B cells), fibroblasts, epithelial cells, endothelial cells, keratinocytes, or muscle cells.

[0144] For example, if the VEGF-specific antagonist is an antibody, the antibody is administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local immunosuppressive treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the antibody is suitably administered by pulse infusion, particularly with declining doses of the antibody. Preferably the dosing is given by injections, most preferably intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

[0145] In another example, the VEGF-specific antagonist compound is administered locally, e.g., by direct injections, when the disorder or location of the tumor permits, and the injections can be repeated periodically. The VEGF-specific antagonist can also be delivered systemically to the subject or directly to the tumor cells, e.g., to a tumor or a tumor bed following surgical excision of the tumor, in order to prevent or reduce local recurrence or metastasis, for example of a dormant tumor or micrometastases.

[0146] Alternatively, an inhibitory nucleic acid molecule or polynucleotide containing a nucleic acid sequence encoding a VEGF-specific antagonist can be delivered to the appropriate cells in the subject. In certain embodiments, the nucleic acid can be directed to the tumor itself.

[0147] The nucleic acid can be introduced into the cells by any means appropriate for the vector employed. Many such methods are well known in the art (Sambrook et al., *supra*, and Watson et al., *Recombinant DNA*, Chapter 12, 2d edition, Scientific American Books, 1992). Examples of methods of gene delivery include liposome mediated transfection, electroporation, calcium phosphate/DEAE dextran methods, gene gun, and microinjection.

V. PHARMACEUTICAL FORMULATIONS

[0148] Therapeutic formulations of the agents (e.g., antibodies) used in accordance with the invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Lyophilized anti-VEGF antibody formulations are described in WO 97/04801.

[0149] Optionally, but preferably, the formulation contains a pharmaceutically acceptable salt, typically, e.g., sodium chloride, and preferably at about physiological concentrations. Optionally, the formulations of the invention can contain a pharmaceutically acceptable preservative. In some embodiments the preservative concentration ranges from 0.1 to 2.0%, typically v/v. Suitable preservatives include those known in the pharmaceutical arts. Benzyl alcohol, phenol, m-cresol, methylparaben, and propylparaben are examples of preservatives. Optionally, the formulations of the invention can include a pharmaceutically acceptable surfactant at a concentration of 0.005 to 0.02%.

[0150] Typically, bevacizumab is supplied for therapeutic uses in 100 mg and 400 mg preservative-free, single-use vials to deliver 4 ml or 16 ml of bevacizumab (25 mg/ml). The 100 mg product is formulated in 240 mg α , α -trehalose dehydrate, 23.2 mg sodium phosphate (monobasic, monohydrate), 4.8 mg sodium phosphate (dibasic, anhydrous), 1.6 mg polysorbate 20, and Water for Injection, USP. The 400 mg product is formulated in 960 mg α , α -trehalose dehydrate, 92.8 mg sodium phosphate (monobasic, monohydrate), 19.2 mg sodium phosphate (dibasic, anhydrous), 6.4 mg polys-

orbate 20, and Water for Injection, USP. See also the label for bevacizumab.

[0151] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide antibodies which bind to EGFR, VEGF (e.g. an antibody which binds a different epitope on VEGF), VEGFR, or ErbB2 (e.g., Herceptin®) in the one formulation. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine, growth inhibitory agent and/or VEGFR antagonist. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0152] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0153] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

[0154] The formulations to be used *for in vivo* administration may be sterile. This is readily accomplished by filtration through sterile filtration membranes.

VI. EFFICACY OF THE TREATMENT

[0155] The main advantage of the of any of the methods, uses and compositions provided herein is the ability of producing marked anti-cancer effects in a human subject without causing significant toxicities or adverse effects, so that the subject benefited from the treatment overall. In one embodiment of any of the methods, uses or compositions, the safety profile is comparable to previous bevacizumab phase III studies. The efficacy of the treatment of the invention can be measured by various endpoints commonly used in evaluating cancer treatments, including but not limited to, tumor regression, tumor weight or size shrinkage, time to progression, duration of survival, progression free survival, overall response rate, duration of response, and quality of life. Because the anti-angiogenic agents of the invention target the tumor vasculature and not necessarily the neoplastic cells themselves, they represent a unique class of anticancer drugs, and therefore may require unique measures and definitions of clinical responses to drugs. For example, tumor shrinkage of greater than 50% in a 2-dimensional analysis is the standard cut-off for declaring a response. However, the anti-VEGF antibody of the invention may cause inhibition of metastatic spread without shrinkage of the primary tumor, or may simply exert a tumouristatic effect. Accordingly, novel approaches to determining efficacy of an anti-angiogenic therapy should be employed, including for example, measurement of plasma or urinary markers of angiogenesis and measurement of response through radiological imaging.

[0156] In another embodiment, the disclosure includes methods for increasing progression free survival of a human subject susceptible to or diagnosed with a cancer. Time to disease progression is defined as the time from administration of the drug until disease progression or death. In a preferred embodiment, the combination treatment of the invention using anti-VEGF antibody and one or more chemotherapeutic agents significantly increases progression free survival by at least about 1 month, 1.2 months, 2 months, 2.4 months, 2.9 months, 3.5 months, preferably by about 1 to about 5 months, when compared to a treatment with chemotherapy alone. In one embodiment, the PFS median in months (95% CI) is 9.2 months (8.6, 10.1) in the subjects treated with bevacizumab and taxane therapy (e.g., docetaxel or paclitaxel protein-bound particles (e.g., Abraxane®)/anthracycline therapy (e.g., doxorubicin, epirubicin or combinations thereof) compared to 8.0 months (6.7, 8.4) the taxane/anthracycline therapy without bevacizumab, with a HR (95% CI) 0.644 (0.522, 0.795), p-value (log-rank) less than 0.0001. In one embodiment, the PFS in the subjects treated with bevacizumab and taxane/anthracycline is 10.7 months compared to 8.3 in subjects treated with placebo and taxane/anthracycline. In one embodiment, the PFS median in months (95% CI) is 8.6 months (8.1, 9.5) in the subjects treated with bevacizumab and capecitabine compared to 5.7 months (4.3, 6.2) with capecitabine therapy without bevacizumab,

with a HR (95% CI) 0.688 (0.564, 0.840), p-value (log-rank) 0.0002. In one embodiment, the PFS in the subjects treated with bevacizumab and capecitabine is 9.7 months compared to 6.2 in subjects treated with placebo and capecitabine.

[0157] In yet another embodiment, the treatment of the invention significantly increases response rate in a group of human subjects susceptible to or diagnosed with a cancer who are treated with various therapeutics. Response rate is defined as the percentage of treated subjects who responded to the treatment. In one aspect, the combination treatment of the invention using anti-VEGF antibody and one or more chemotherapeutic agents significantly increases response rate in the treated subject group compared to the group treated with chemotherapy alone.

[0158] In one aspect, the disclosure includes methods for increasing duration of response in a human subject or a group of human subjects susceptible to or diagnosed with a cancer. Duration of response is defined as the time from the initial response to disease progression.

[0159] In one embodiment, the invention can be used for increasing the duration of survival of a human subject susceptible to or diagnosed with a cancer.

VII. Antibody Production

(i) Polyclonal antibodies

[0160] Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}^1\text{N}=\text{C}=\text{NR}$, where R and R^1 are different alkyl groups.

[0161] Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) Monoclonal antibodies

[0162] Various methods for making monoclonal antibodies herein are available in the art. For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), or by recombinant DNA methods (U.S. Patent No. 4,816,567).

[0163] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster or macaque monkey, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)).

[0164] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[0165] Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0166] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked

immunoabsorbent assay (ELISA).

[0167] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

[0168] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[0169] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Recombinant production of antibodies will be described in more detail below.

[0170] In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., *Nature*, 348:552-554 (1990). Clackson et al., *Nature*, 352:624-628 (1991) and Marks et al., *J. Mol. Biol.*, 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks et al., *BioTechnology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse et al., *Nuc. Acids. Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[0171] The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, et al., *Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

[0172] Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

(iii) *Humanized and human antibodies*

[0173] A humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-327 (1988); Verhoeyen et al., *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0174] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims et al., *J. Immunol.*, 151:2296 (1993); Chothia et al., *J. Mol. Biol.*, 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta et al., *J. Immunol.*, 151:2623 (1993)).

[0175] It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays

permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

[0176] Humanized anti-VEGF antibodies and affinity matured variants thereof are described in, for example, U.S. Pat. No. 6,884,879 issued February 26, 2005.

[0177] It is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551 (1993); Jakobovits et al., Nature, 362:255-258 (1993); Bruggermann et al., Year in Immuno., 7:33 (1993); and Duchosal et al. Nature 355:258 (1992).

[0178] Alternatively, phage display technology (McCafferty et al., Nature 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M 13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, *e.g.*, Johnson, Kevin S. and Chiswell, David J., Current Opinion in Structural Biology 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., Nature, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., J. Mol. Biol. 222:581-597 (1991), or Griffith et al., EMBO J. 12:725-734 (1993). See, also, U.S. Patent Nos. 5,565,332 and 5,573,905.

[0179] As discussed above, human antibodies may also be generated by *in vitro* activated B cells (see U.S. Patents 5,567,610 and 5,229,275).

[0180] Human monoclonal anti-VEGF antibodies are described in U.S. Patent No. 5,730,977, issued March 24, 1998.

(iv) Antibody fragments

[0181] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992) and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter et al., Bio/Technology 10:163-167 (1992)). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185.

(v) Other amino acid sequence modifications

[0182] Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

[0183] A useful method for identification of certain residues or regions of the antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells Science, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the

interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed antibody variants are screened for the desired activity.

[0184] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

[0185] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated.

[0186] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Amino acids may be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)):

(1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)

(3) acidic: Asp (D), Glu (E)

(4) basic: Lys (K), Arg (R), His(H)

[0187] Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0188] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0189] Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

[0190] A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g. 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g. binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the

antigen-antibody complex to identify contact points between the antibody and human VEGF. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0191] Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

[0192] Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0193] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0194] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 A1, Presta, L. See also US 2004/0093621 A1 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO03/011878, Jean-Mairet et al. and US Patent No. 6,602,684, Umana et al. Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO97/30087, Patel et al. See, also, WO98/58964 (Raju, S.) and WO99/22764 (Raju, S.) concerning antibodies with altered carbohydrate attached to the Fc region thereof.

[0195] It may be desirable to modify the antibody of the invention with respect to effector function, e.g. so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., J. Exp Med. 176:1191-1195 (1992) and Shopes, B. J. Immunol. 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff et al. Cancer Research 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al. Anti-Cancer Drug Design 3:219-230 (1989).

[0196] WO00/42072 (Presta, L.) describes antibodies with improved ADCC function in the presence of human effector cells, where the antibodies comprise amino acid substitutions in the Fc region thereof. Preferably, the antibody with improved ADCC comprises substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Preferably the altered Fc region is a human IgG1 Fc region comprising or consisting of substitutions at one, two or three of these positions. Such substitutions are optionally combined with substitution(s) which increase C1q binding and/or CDC.

[0197] Antibodies with altered C1q binding and/or complement dependent cytotoxicity (CDC) are described in WO99/51642, US Patent No. 6,194,551B1, US Patent No. 6,242,195B1, US Patent No. 6,528,624B1 and US Patent No. 6,538,124 (Idusogie et al.). The antibodies comprise an amino acid substitution at one or more of amino acid positions 270, 322, 326, 327, 329, 313, 333 and/or 334 of the Fc region thereof (Eu numbering of residues).

[0198] To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in US Patent 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG₁, IgG₂, IgG₃, or IgG₄) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

[0199] Antibodies with improved binding to the neonatal Fc receptor (FcRn), and increased half-lives, are described in WO00/42072 (Presta, L.) and US2005/0014934A1 (Hinton et al.). These antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. For example, the Fc region may have substitutions at one or more of positions 238, 250, 256, 265, 272, 286, 303, 305, 307, 311, 312, 314, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424, 428 or 434 (Eu numbering of residues). The preferred Fc region-comprising antibody variant with improved FcRn binding comprises amino acid substitutions at one, two or three of positions 307, 380 and 434 of the Fc region thereof (Eu numbering of residues). In one embodiment, the antibody has 307/434 mutations.

[0200] Engineered antibodies with three or more (preferably four) functional antigen binding sites are also contemplated

(US Appln No. US2002/0004587 A1, Miller et al.).

[0201] Nucleic acid molecules encoding amino acid sequence variants of the antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody.

(vi) *Immunoconjugates*

[0202] The disclosure also includes immunoconjugates comprising the antibody described herein conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g. an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

[0203] Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. A variety of radionuclides are available for the production of radioconjugate antibodies. Examples include ²¹²Bi, ¹³¹I, ¹³¹In, ⁹⁰Y and ¹⁸⁶Re.

[0204] Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al. Science 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

[0205] In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the subject, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide).

(vii) *Immunoliposomes*

[0206] The antibody disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82:3688 (1985); Hwang et al., Proc. Natl. Acad. Sci. USA, 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

[0207] Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the invention can be conjugated to the liposomes as described in Martin et al. J. Biol. Chem. 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon et al. J. National Cancer Inst. 81(19):1484 (1989).

VIII. ARTICLES OF MANUFACTURE AND KITS

[0208] Also disclosed is, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container, a label and a package insert. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-VEGF antibody. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes. In addition, the article of manufacture comprises a package inserts with instructions for use, including for example instructing the user of the

composition to administer the anti-VEGF antibody composition and a chemotherapeutic agent to the subject, e.g., capecitabine, taxane, anthracycline, paclitaxel, docetaxel, paclitaxel protein-bound particles (e.g., Abraxane®), doxorubicin, epirubicin, 5-fluorouracil, cyclophosphamide or combinations thereof. The package insert may optionally contain some or all of the results found in Example 1.

[0209] The VEGF-specific antagonist can be packaged alone or in combination with other anti-cancer therapeutic compounds as a kit. The kit can include optional components that aid in the administration of the unit dose to subjects, such as vials for reconstituting powder forms, syringes for injection, customized IV delivery systems, inhalers, etc. Additionally, the unit dose kit can contain instructions for preparation and administration of the compositions. In certain embodiments, the instructions comprises instructions for use, including for example instructing the user of the composition to administer the anti-VEGF antibody composition and a chemotherapeutic agent to the subject, e.g., capecitabine, taxane, anthracycline, paclitaxel, docetaxel, paclitaxel protein-bound particles (e.g., Abraxane®), doxorubicin, epirubicin, 5-fluorouracil, cyclophosphamide or combinations thereof. The instructions may optionally contain some or all of the results found in Example 1. The kit may be manufactured as a single use unit dose for one subject, multiple uses for a particular subject (at a constant dose or in which the individual compounds may vary in potency as therapy progresses); or the kit may contain multiple doses suitable for administration to multiple subjects ("bulk packaging"). The kit components may be assembled in cartons, blister packs, bottles, tubes, and the like.

Deposit of Materials

[0210] The following hybridoma cell line has been deposited under the provisions of the Budapest Treaty with the American Type Culture Collection (ATCC), Manassas, VA, USA:

Antibody Designation	ATCC No.	Deposit Date
A4.6.1	ATCC HB-10709	March 29, 1991

[0211] The following example is intended merely to illustrate the practice of the invention and is not provided by way of limitation.

EXAMPLE

Example 1. Bevacizumab in Combination with Chemotherapy Regimens in Subjects with Previously Untreated Metastatic Breast Cancer

[0212] Metastatic breast cancer (MBC) is an incurable disease, with the majority of patients succumbing to their disease within 2 year of diagnosis (Greenberg, et al., 1996, J. Clin. Oncol. 14:2197-205; Dawood, et al., 2008, J. Clin. Oncol. 26:4891-8; and Chia et al., Cancer, 2007, 110:973-9). Of the patients presenting with MBC, approximately 60% will have previously presented with localized disease that has recurred; approximately 40% of patients will present with metastatic disease de novo.

[0213] Two prior randomized Phase III trials in MBC have demonstrated benefit from addition of bevacizumab to initial chemotherapy with taxanes. In the pivotal Phase III E2100 trial, progression-free survival (PFS) was significantly longer in patients treated with weekly paclitaxel+ bevacizumab than in those treated with paclitaxel alone (Miller et al., N. Engl J. Med., 2007, 357:2666-76). Similarly, the AVADO trial, which investigated the combination of bevacizumab (at 7.5 and 15 mg/kg q3w) with docetaxel, found that patients treated with docetaxel+ bevacizumab had progression-free survival (PFS) that was longer than in those treated with docetaxel alone (Miles et al., 2008 ASCO Annual Meeting Chicago, IL). Previously, a randomized Phase III trial (AVF2119g) for previously treated MBC that evaluated the combination of bevacizumab with capecitabine demonstrated that overall response rate (ORR) was higher in those treated with capecitabine + bevacizumab than capecitabine alone, but it failed to meet its primary objective of improving PFS (Miller et al., J. Clin. Oncol. 2005, 23:792-9).

[0214] This example concerns analysis of results obtained with previously untreated metastatic breast cancer subjects treated in the RIBBON 1 clinical trial using taxanes and non-taxane chemotherapies. The primary objective of the study was to determine the clinical benefit of the addition of bevacizumab to standard chemotherapy regimes for previously untreated metastatic breast cancer, as measured by PFS based on investigator tumor assessment. See, e.g., O'Shaughnessy and Brufsky, (2008), Clinical Breast Cancer, 8(4): 370-373. The trial comprised two study groups that evaluated AVASTIN® with different types of chemotherapies in women who had not previously received chemotherapy for their advanced HER2-negative breast cancer. In the first study group, women received either AVASTIN or placebo in combination with taxane or anthracycline-based chemotherapies. In the second study group, women received either AVASTIN or placebo in combination with capecitabine chemotherapy. The analysis of this example was based on information from

1237 patients. These trials evaluated the efficacy of bevacizumab (AVASTIN®) as therapy for patients previously untreated metastatic breast cancer.

Study Design

[0215] The design of the RIBBON1 study is depicted in **Figure 1**.

[0216] In the RIBBON1 trial, the following treatment protocol was used:

Arm A: bevacizumab 15mg/kg IV on day 1 of each 21-day cycle and either cohort 1, cohort 2 or cohort 3;

Arm B: placebo IV on day 1 of each 21-day cycle and either cohort 1, cohort 2 or cohort 3.

Cohort 1: Either of the following taxanes administered every 3 weeks

Docetaxel 75-100 mg/m² IV

Paclitaxel protein-bound particles (Abraxane®) 260 mg/m² IV

Cohort 2: Any of the following anthracycline-based combination chemotherapies, for subjects previously untreated with anthracyclines, every 3 weeks:

FEC: 5-fluorouracil 500 mg/m² IV, epirubicin 90-100 mg/m² IV and cyclophosphamide 500 mg/m² IV on Day 1

FAC: 5-fluorouracil 500 mg/m² IV, doxorubicin 50 mg/m² IV and cyclophosphamide 500 mg/m² IV on Day 1

AC: Doxorubicin 50-60 mg/m² IV and cyclophosphamide 500-600 mg/m² IV on Day 1

EC: Epirubicin 90-100 mg/m² IV and cyclophosphamide 500-600 mg/m² IV on Day 1

Cohort 3: Capecitabine 1000 mg/m² oral twice daily on Days 1-14 of each 3-week cycle.

[0217] In addition, after the blinded treatment phase, some subjects were given bevacizumab either 15 mg/kg IV every three weeks or 10 mg/ml IV every 2 weeks; given concurrently with chemotherapy.

[0218] Bevacizumab (AVASTIN®) was supplied as a clear to slightly opalescent, colorless to pale brown, sterile liquid concentrate for solution for IV infusion. Bevacizumab was supplied in either a 5-ml (100 mg) or 20-ml (400 mg) glass vials containing 4 mL or 16 mL bevacizumab, respectively (25 mg/ml for either vial). Vials contain bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection (SWFI), USP. Vials contained no preservative. AVASTIN® was diluted in 0.9% Sodium Chloride Injection, USP, to a total volume of 100 ml before continuous intravenous administration.

Methods

[0219] Eligible Subjects/Patients had the following key eligibility criteria: Age > 18 years, ECOG 0 or 1 (ECOG Performance Status Scale), no prior chemotherapy for locally recurrent or metastatic breast cancer, Her2 negative (unless Her2 positive and trastuzumab contraindicated or unavailable) and/or prior adjuvant chemotherapy allowed if recurrence > (or equal to) 12 months since last dose. All subjects had histologically or cytologically confirmed adenocarcinoma of the breast, subjects may have had either measureable (per the Response Evaluation Criteria in Solid Tumors (RECIST)) or non-measureable locally recurrent or metastatic disease. The locally recurrent disease was not amenable to resection with curative intent.

[0220] Subjects may have received prior hormonal therapy in either the adjuvant or metastatic setting if discontinued greater than or equal to 1 week prior to Day 0, or adjuvant chemotherapy if discontinued greater than or equal to 12 months prior to Day 0.

[0221] Exclusion criteria included known HER2-positive status (unless the patient had progressed on trastuzumab therapy or trastuzumab therapy was contraindicated or unavailable); prior adjuvant or neo adjuvant chemotherapy within 12 months; known central nervous system metastases; blood pressure >150/100 mmHg; unstable angina; New York Heart Association Grade II or greater congestive heart failure (CHF); history of myocardial infarction within 6 months; history of stroke or transient ischemic attack within 6 months; clinically significant peripheral vascular disease; evidence of bleeding diathesis or coagulopathy; history of abdominal fistula, gastrointestinal (GI) perforation, or intra abdominal abscess within 6 months; history of anaphylactic reaction to monoclonal antibody therapy not controlled with premedication; serious non healing wound; inadequate organ function; locally recurrent disease amenable to resection with curative intent; history of other malignancies within 5 years. If anthracycline chosen as chemotherapy, patients were also required to have left ejection fraction ≥50% and no prior history of anthracycline treatment.

[0222] The trial was conducted worldwide (at least 22 countries) and accrued 1237 subjects/patients (Taxane (T): 307; Anthracycline (Anthra): 315; and Capecitabine (Cap): 615).

[0223] The primary endpoint of the study was progression free survival (PFS), defined as the time from randomization to disease progression or to death, based on investigator assessment. Kaplan-Meier methodology can be used to estimate median PFS for each treatment arm. In certain embodiments, the hazard ratio for PFS will be estimated using a stratified Cox regression model with the same stratification factors used in the stratified log-rank test. Analyses of PFS

in each cohort is performed at the two-sided $\alpha=0.05$ level. Time-to-event data are compared between treatment arms using a stratified log-rank test. The Kaplan-Meier method is used to estimate duration of time-to-event data. The 95% confidence intervals for median time-to-event are computed using the Brookmeyer-Crowley method. The HR for time-to-event data are estimated using a stratified Cox regression model.

[0224] The secondary endpoints included objective response rate (ORR), one-year survival rate, overall survival (OS), and PFS based on IRC assessment and safety. OS is defined as the time from randomization until death from any cause. ORR is defined as the percentage of patients who achieved a complete or partial response confirmed ≥ 28 days after initial documentation of response. One-year survival rate is assessed between treatment arms using the normal approximation method. ORR in patients with measurable disease at baseline is compared using the stratified Mantel-Haenszel χ^2 test. Randomization stratification factors are included in all stratified analyses.

Results

[0225] RIBBON1 was an international, multicenter, randomized, double-blind, placebo-controlled clinical study that enrolled 1,237 subjects/patients with locally recurrent or metastatic HER2-negative breast cancer who had not received chemotherapy for their metastatic disease. See Table 1 for Subject/Patient Characteristics from the trial. The primary endpoint of these trials was progression free survival (PFS), defined as the time from randomization to disease progression or death, based on investigator assessment. The results from the trial indicate that AVASTIN® in combination with the following used chemotherapies for first-line metastatic HER2-negative breast cancer increased the time women lived without their disease advancing, as defined as the primary endpoint of progression-free survival (PFS), compared to chemotherapies alone.

Table 1: Subject/Patient Characteristics

	Cap		T/Anthra	
	PL (n=206)	BV (n=409)	PL (n=207)	BV (n=415)
Median age, yr	57	56	55	55
ECOG PS 0	53	52	53	52
HR positive	71	76	74	74
Triple negative	24	21	22	23
Disease-free ≤ 12 months	22	27	41	37
Adjuvant chemotherapy	76	70	47	45
Taxane	41	39	15	15
Anthracycline	69	60	30	30
≥ 3 metastatic sites	45	43	45	45
Measurable dx	78	80	86	83

[0226] The results of this phase III study provide direct support for use of antiangiogenic agents as first line therapy for patients with previously untreated breast cancer. The addition of bevacizumab, an anti-VEGF antibody, to the taxane therapy (e.g., docetaxel or paclitaxel protein-bound particles (e.g., Abraxane®))/anthracycline therapy (e.g., doxorubicin, epirubicin or combinations thereof) or capecitabine therapy chemotherapy conferred a clinically meaningful and statistically significant improvement in breast cancer patients as measured by, for example, progression-free survival. The PFS median in months (95% CI) is 9.2 months (8.6, 10.1) in the patients treated with bevacizumab and taxane therapy (e.g., docetaxel or paclitaxel protein-bound particles (e.g., Abraxane®))/anthracycline therapy (e.g., doxorubicin, epirubicin or combinations thereof) compared to 8.0 months (6.7, 8.4) in the taxane/anthracycline therapy without bevacizumab, with a HR (95% CI) 0.644 (0.522, 0.795), p-value (log-rank) less than 0.0001. See Table 2. See **Figure 3** to see investigator (INV) determined PFS values and independent review committee (IRC) determined PFS values. The PFS median in months (95% CI) is 8.6 months (8.1, 9.5) in the patients treated with bevacizumab and capecitabine compared to 5.7 months (4.3, 6.2) in capecitabine therapy without bevacizumab, with a HR (95% CI) 0.688 (0.564, 0.840), p-value (log-rank) 0.0002. See Table 2. See **Figure 2** to see investigator (INV) determined PFS values and independent review committee (IRC) determined PFS values. See Table 3 for secondary endpoints, where the PFS is divided by chemotherapy subgroups. See **Figures 4 and 6** for subgroup analyses of PFS by various cohorts, e.g., capecitabine and T/anthracycline in **Figure 4**, and T/anthracycline in **Figure 6**. See **Figure 5** for objective response rate (ORR) and Table

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2. Among responders, median duration of objective response was longer in the bevacizumab arms for both cohorts : Capecitabine cohort, 9.2 months (95% CI: 8.5-10.4) vs. 7.2 months (95% CI: 5.1-9.3); and for the taxane / anthracycline cohort, 8.3 months (95% CI: 7.2-10.7) vs. 7.1 months (95% CI: 6.2-8.8). See Table 4 for Overall survival details. There is no unexpected safety signal. Safety was consistent with results of prior bevacizumab trials. See Table 5 for safety summary. This improvement is clinically meaningful.

Table 2 PFS and OS

	T/Anthr n=622		Cap n=615	
	pl n=207	B n=415	pl n=206	B N=409
PFS (HR, 95% CI)	0.644		0.688	
	(0.522, 0.795)		(0.564, 0.840)	
p-value (Log-rank)	<0.0001		0.0002	
Median (months)	8.0	9.2	5.7	8.6
ORR (%)	67 (37.9%)	177 (51.3%)	38 (23.6%)	115 (35.4%)
p-value	0.0054		0.0097	
OS (HR, 95% CI)	1.032 (0.774, 1.376)		0.847 (0.631, 1.138)	
p-value (Log-rank)	0.8298		0.2706	
Median (months)	23.8	25.2	21.2	29.0
HR=hazard ratio				

Table 3 Secondary Endpoint: PFS by Chemotherapy Subgroups (mPFS=median PFS)

	Taxane		Anthra	
	PL(n=104)	BV(n=203)	PL(n=103)	BV(n=212)
mPFS, mo	8.2	9.2	7.9	9.2
HR (95% CI)	0.75 (0.56-1.01)		0.55 (0.40-0.74)	
p-value	0.0547		<0.0001	

Table 4: Overall Survival

	Cap		T/Anthra	
	PL (n=206)	BV (n=409)	PL (n=207)	BV (n=415)
% of deaths	35	30	35	34
Median OS, mo	21.2	29.0	23.8	25.2
HR (95% CI)	0.85 (0.63-1.14)		1.03 (0.77-1.38)	
p-value	0.27		0.83	
1-yr survival rate (%)	74	81	83	81
p-value	0.076		0.44	

Table 5: Safety Summary

	Cape		Taxane		Anthra	
Event (%)	PL (n=201)	BV (n=404)	PL (n=102)	BV (n=203)	PL (n=100)	BV (n=210)
Selected AEs*	9.0	22.0	22.5	43.8	16.0	28.1

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(continued)

	Cape		Taxane		Anthra	
Event (%)	PL (n=201)	BV (n=404)	PL (n=102)	BV (n=203)	PL (n=100)	BV (n=210)
SAEs	18.9	24.3	26.5	41.4	16.0	22.4
AEs leading to study drug (PL or BV) discontinuation	11.9	11.9	7.8	24.1	4.0	14.3
AEs leading to death**	2.5	2.0	2.9	3.4	3.0	1.4
* Adverse Events (AEs) previously shown to be associated with bevacizumab **Excludes AEs related to metastatic breast cancer progression SAE-severe adverse events						

[0227] The addition of bevacizumab to capecitabine, taxane or anthracycline-based chemotherapy regimens used in 1st-line treatment of metastatic breast cancer, resulted in statistically-significant improvement in PFS with a safety profile comparable to prior Phase III studies.

Sequence Listing

[0228]

<110> GENENTECH, INC. et al.

<120> ANTI-ANGIOGENESIS THERAPY FOR THE TREATMENT OF BREAST CANCER

<130> P4331R1 WO

<141> 2009-11-20

<150> US 61/117,102

<151> 2008-11-22

<150> US 61/178,009

<151> 2009-05-13

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					95					100					105

Ile Lys Arg

55

Claims

1. An anti-VEGF antibody for use in a method of treating locally recurrent or metastatic breast cancer in a subject, the method comprising administering to the subject a treatment regimen comprising an effective amount of at least one chemotherapy and an anti-VEGF antibody, wherein said subject has not received any chemotherapy for locally recurrent or metastatic breast cancer, and/or has not received prior adjuvant chemotherapy in recurrence less than or equal to 12 months since last dose; and wherein the chemotherapeutic agent is capecitabine; and wherein the treatment regimen effectively extends the progression free survival of the subject.
2. The anti-VEGF antibody for use of claim 1, wherein said anti-VEGF antibody binds the same epitope as the monoclonal anti-VEGF antibody A4.6.1 produced by hybridoma ATCC HB 10709.
3. The anti-VEGF antibody for use of claim 1, wherein the anti-VEGF antibody is a humanized antibody.
4. The anti-VEGF antibody for use of claim 1, wherein the anti-VEGF antibody is bevacizumab.
5. The anti-VEGF antibody for use of claim 1, wherein the subject is HER2 negative.
6. The anti-VEGF antibody for use of claim 4, wherein the administration of capecitabine is 1000 mg/m² oral twice daily on Days 1-14 of each 3-week cycle and the administration of bevacizumab is 15mg/kg IV on day 1 of each 21-day cycle.
7. The anti-VEGF antibody for use of claim 1, wherein the treatment regimen extends the progression free survival of the subject by at least about 2.9 months when compared to another subject treated with the chemotherapy alone.
8. The anti-VEGF antibody for use of claim 1, wherein the anti-VEGF antibody has a heavy chain variable region comprising the following amino acid sequence:

EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW
 INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP
 HYYGSSHWYF DVWGQGTLLV VSS (SEQ ID NO.1)

and a light chain variable region comprising the following amino acid sequence:

DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIIYF
 TSSLHSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ
 GTKVEIKR (SEQ ID NO.2).

Patentansprüche

1. Anti-VEGF-Antikörper zur Verwendung in einem Verfahren zur Behandlung von lokal wiederkehrendem oder metastatischem Brustkrebs bei einem Individuum, wobei das Verfahren das Verabreichen eines Behandlungsregimes an das Individuum umfasst, das eine wirksame Menge zumindest einer Chemotherapie und eines Anti-VEGF-Antikörpers umfasst, worin das Individuum noch keine Chemotherapie gegen lokal wiederkehrenden oder metastatischen Brustkrebs erhalten hat und/oder 12 Monate oder weniger seit der letzten Dosis keine vorhergehende adjuvante Chemotherapie in Rekurrenz mehr erhalten hat; und worin das Chemotherapeutikum Capecitabin ist; und worin das Behandlungsregime das progressionsfreie Überleben des Individuums wirksam verlängert.
2. Anti-VEGF-Antikörper zur Verwendung nach Anspruch 1, worin der Anti-VEGF-Antikörper das gleiche Epitop bindet

wie der von dem Hybridom ATCC HB 10709 produzierte monoklonale Anti-VEGF-Antikörper A4.6.1.

3. Anti-VEGF-Antikörper zur Verwendung nach Anspruch 1, worin der Anti-VEGF-Antikörper ein humanisierter Anti-körper ist.
4. Anti-VEGF-Antikörper zur Verwendung nach Anspruch 1, worin der Anti-VEGF-Antikörper Bevacizumab ist.
5. Anti-VEGF-Antikörper zur Verwendung nach Anspruch 1, worin das Individuum HER2-negativ ist.
6. Anti-VEGF-Antikörper zur Verwendung nach Anspruch 4, worin die Verabreichung von Capecitabin 1000 mg/m² oral zweimal täglich an den Tagen 1 bis 14 jedes 3-wöchigen Zyklus umfasst und die Verabreichung von Bevacizumab 15 mg/kg IV an Tag 1 jedes 21-tägigen Zyklus umfasst.
7. Anti-VEGF-Antikörper zur Verwendung nach Anspruch 1, worin das Behandlungsregime das progressionsfreie Überleben des Individuums um zumindest etwa 2,9 Monate im Vergleich zu einem anderen Individuum, das nur mit der Chemotherapie behandelt wird, verlängert.
8. Anti-VEGF-Antikörper zur Verwendung nach Anspruch 1, worin der Anti-VEGF-Antikörper eine variable Schwerkettenregion, die die folgende Aminosäuresequenz umfasst:

EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW
INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP
HYYGSSHWYF DVWGQGTTLVT VSS (Seq.-ID Nr. 1),

und eine variable Leichtkettenregion aufweist, die die folgende Aminosäuresequenz umfasst:

DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIIYF
TSSLHSGVPS RFGSGSGSGTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ
GTKVEIKR (Seq.-ID Nr. 2).

Revendications

1. Anticorps anti-VEGF pour une utilisation dans un procédé de traitement d'un cancer du sein localement récidivant ou métastatique chez un sujet, le procédé comprenant l'administration au sujet d'un schéma thérapeutique comprenant une quantité efficace d'au moins une chimiothérapie et d'un anticorps anti-VEGF, dans lequel ledit sujet n'a reçu aucune chimiothérapie pour un cancer du sein localement récidivant ou métastatique, et/ou n'a reçu aucune chimiothérapie adjuvante antérieure lors d'une récurrence inférieure ou égale à 12 mois depuis la dernière dose ; et dans lequel l'agent chimiothérapeutique est la capécitabine ; et dans lequel le schéma thérapeutique prolonge de manière efficace la survie sans progression du sujet.
2. Anticorps anti-VEGF pour une utilisation selon la revendication 1, dans lequel ledit anticorps anti-VEGF se lie au même épitope que l'anticorps A4.6.1 anti-VEGF monoclonal produit par l'hybridome ATCC HB 10709.
3. Anticorps anti-VEGF pour une utilisation selon la revendication 1, dans lequel l'anticorps anti-VEGF est un anticorps humanisé.
4. Anticorps anti-VEGF pour une utilisation selon la revendication 1, dans lequel l'anticorps anti-VEGF est bévacicumab.
5. Anticorps anti-VEGF pour une utilisation selon la revendication 1, dans lequel le sujet est HER2-négatif.
6. Anticorps anti-VEGF pour une utilisation selon la revendication 4, dans lequel l'administration de capécitabine est de 1 000 mg/m² par voie orale deux fois par jour les jours 1 à 14 de chaque cycle de 3 semaines et l'administration de bévacicumab est de 15 mg/kg par voie intraveineuse le jour 1 de chaque cycle de 21 jours.

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7. Anticorps anti-VEGF pour une utilisation selon la revendication 1, dans lequel le schéma thérapeutique prolonge la survie sans progression du sujet d'au moins environ 2,9 mois par comparaison à un autre sujet traité à l'aide de la seule chimiothérapie.

5 8. Anticorps anti-VEGF pour une utilisation selon la revendication 1, dans lequel l'anticorps anti-VEGF a une région variable de la chaîne lourde comprenant la séquence d'acides aminés suivante :

10 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW
INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP
HYYGSSHWYF DVWGQGTLVT VSS (SEQ ID NO.1)

15 et une région variable de la chaîne légère comprenant la séquence d'acides aminés suivante :

20 DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIIYF
TSSLHSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ
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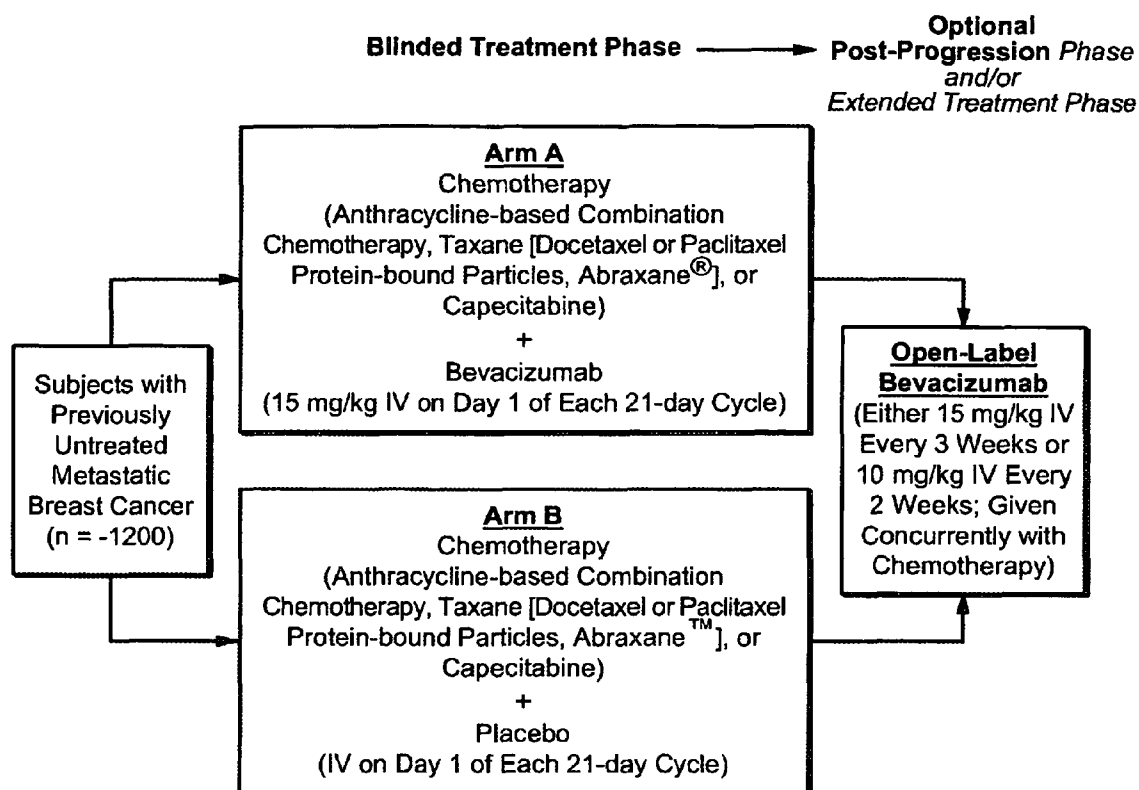
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50

55



Cohort 1: Either of the following taxanes administered every 3 weeks:

Docetaxel 75-100 mg/m² IV

Paclitaxel protein-bound particles (Abraxane®) 260 mg/m² IV

Cohort 2: Any of the following anthracycline-based combination chemotherapies, for subjects previously untreated with anthracyclines, every 3 weeks:

FEC: 5-fluorouracil 500 mg/m² IV, epirubicin 90-100 mg/m² IV, and cyclophosphamide 500 mg/m² IV on Day 1

FAC: 5-fluorouracil 500 mg/m² IV, doxorubicin 50 mg/m² IV, and cyclophosphamide 500 mg/m² IV on Day 1

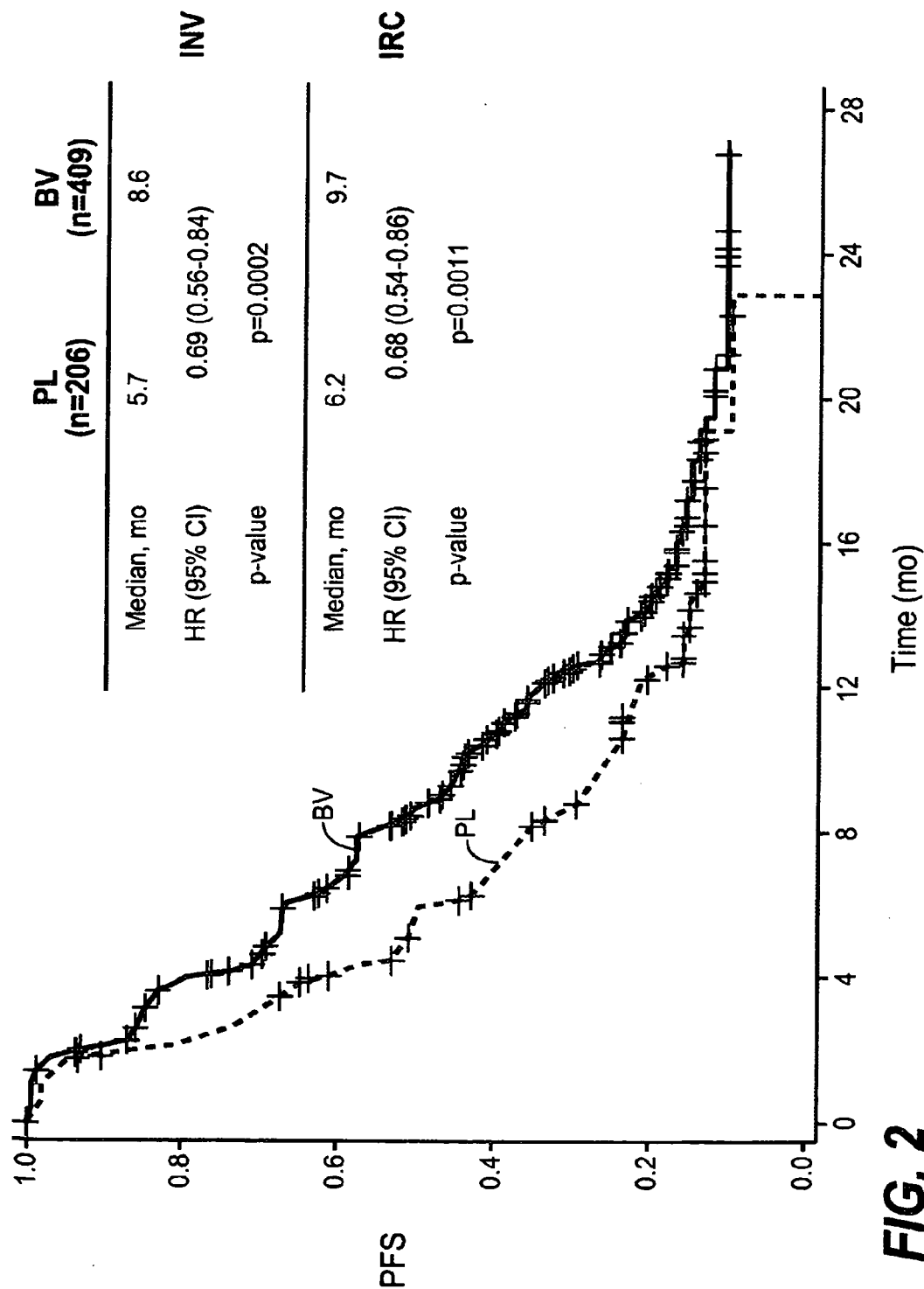
AC: Doxorubicin 50-60 mg/m² IV and cyclophosphamide 500-600 mg/m² IV on Day 1

EC: Epirubicin 90-100 mg/m² IV and cyclophosphamide 500-600 mg/m² IV on Day 1

Cohort 3: Capecitabine 1000 mg/m² oral twice daily on Days 1-14 of each 3-week cycle

FIG. 1

Capecitabine: PFS by Investigator



Taxane/Anthra: PFS by Investigator

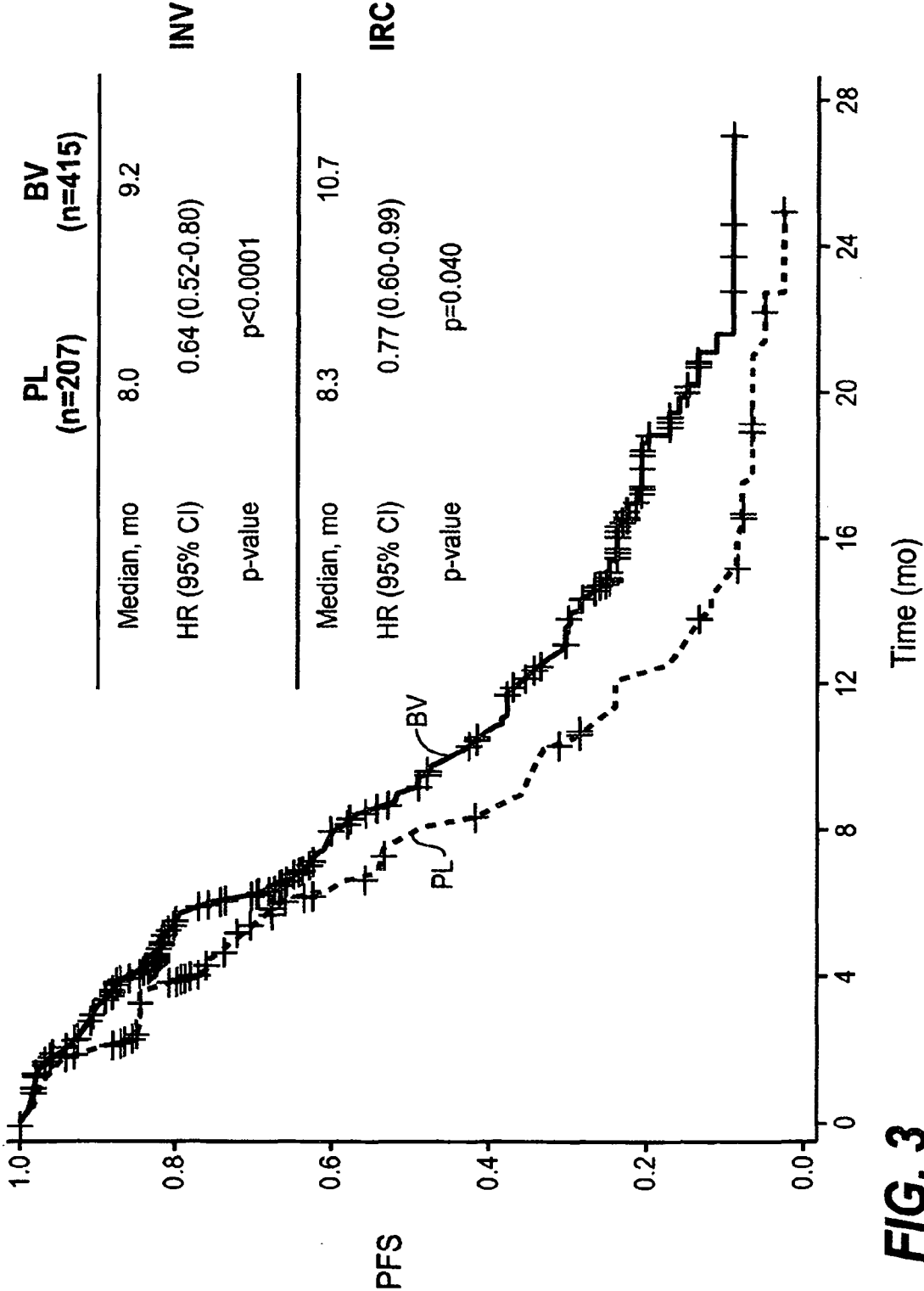


FIG. 3

Subgroup Analyses of PFS, Cape and T/Anthra Cohorts

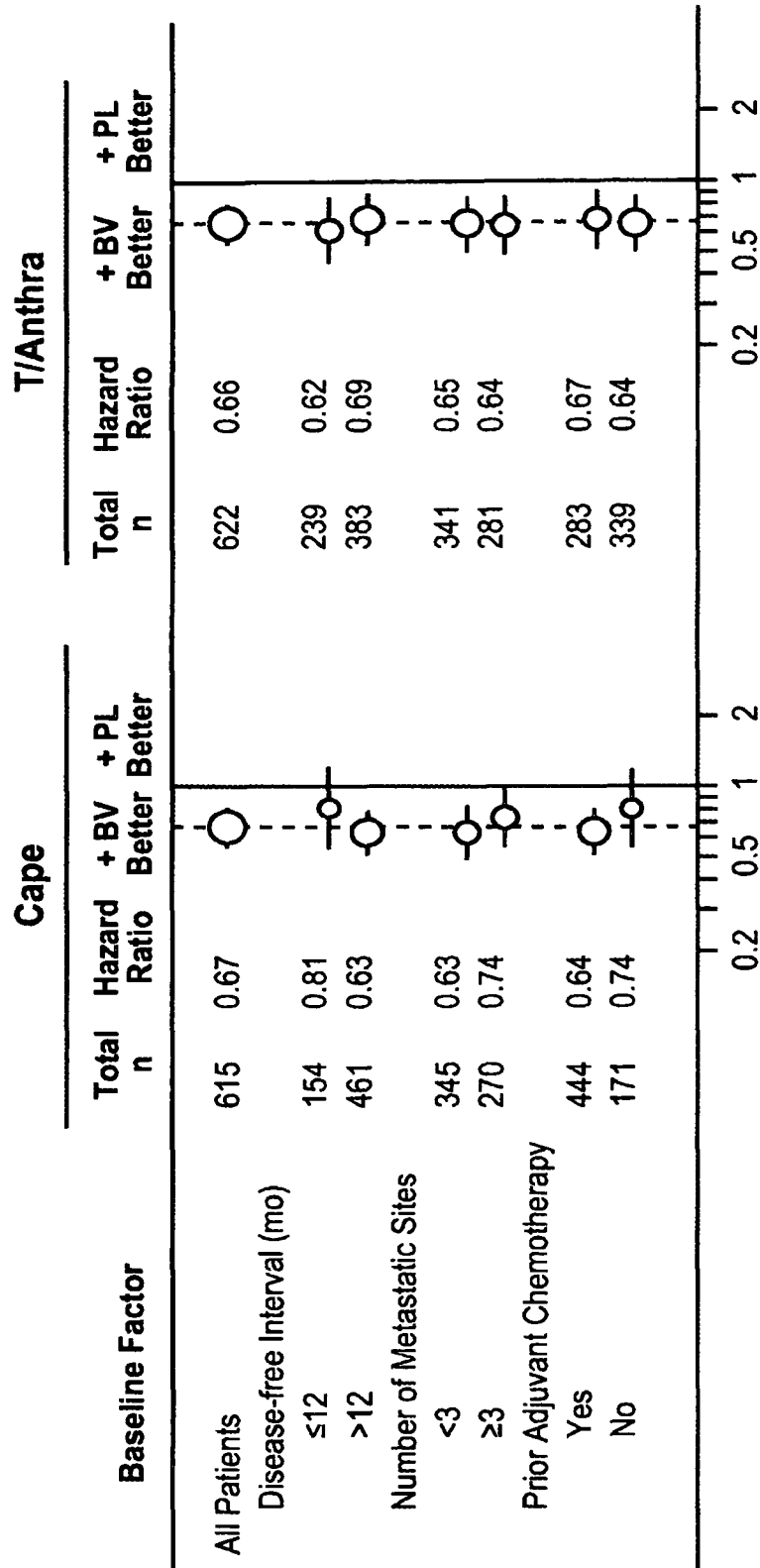
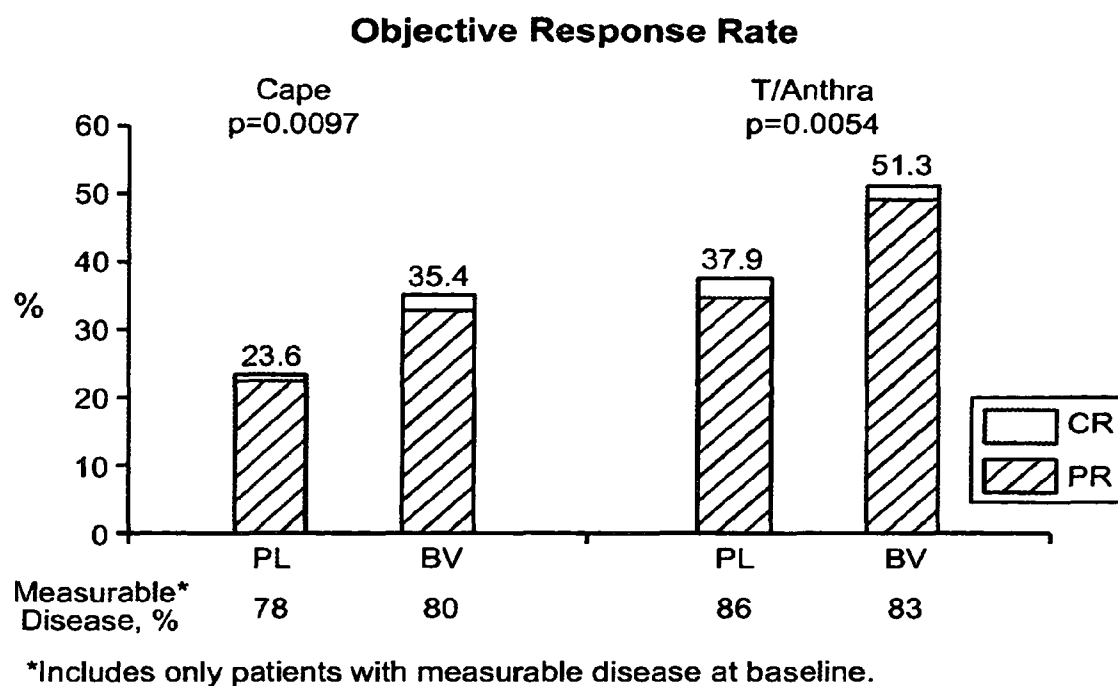
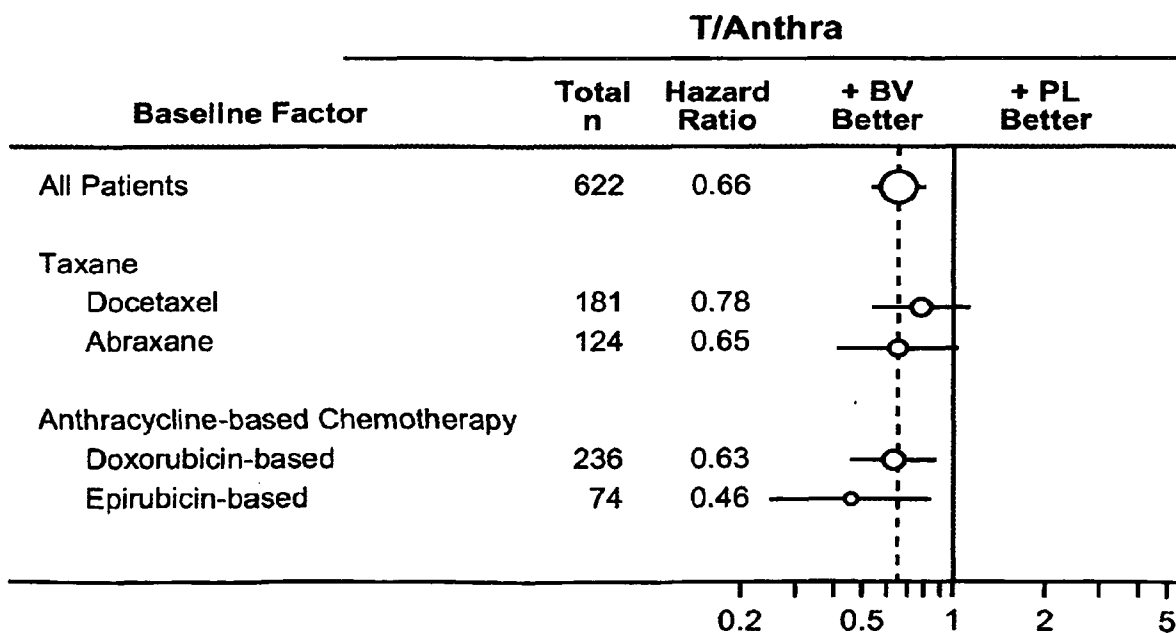


FIG. 4

**FIG. 5****Subgroup Analyses of PFS T/Anthra Cohorts****FIG. 6**

REFERENCES CITED IN THE DESCRIPTION

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Az EP 2 752 189 lajstromszámú európai szabadalom igénypontjainak magyar fordítása:

1. Anti-VEGF antitest lokálisan kiújuló vagy áttételes emlőrák kezelési eljárásban való alkalmazásra páciensben, az eljárás során a páciensnek olyan kezelési rendet adagolunk, amely tartalmazza legalább egy kemoterápia hatásos mennyiségét és tartalmaz egy anti-VEGF antitestet, ahol az említett páciens nem kapott semmilyen kemoterápiát a lokálisan kiújuló vagy áttételes emlőrákra és/vagy nem kapott előzőleg adjuváns kemoterápiát a kiújulás során az utolsó dózist követő 12 vagy annál kevesebb hónap alatt; és ahol a kemoterápiás szer kapecitabin; és ahol a kezelési rend hatékonyan meghosszabbítja a páciens terjedéstől (progression) mentes túlélését.
2. Alkalmazásra szolgáló anti-VEGF antitest az 1. igénypont szerint, ahol az említett anti-VEGF antitest ugyanahhoz az epitophoz kötődik, mint az A4.6.1. monoklonális anti-VEGF antitest, amelyet az ATCC HB 10709 hibridoma termel.
3. Alkalmazásra szolgáló anti-VEGF antitest az 1. igénypont szerint, ahol az anti-VEGF antitest humanizált antitest.
4. Alkalmazásra szolgáló anti-VEGF antitest az 1. igénypont szerinti, ahol az anti-VEGF antitest bevacizumab.
5. Alkalmazásra szolgáló anti-VEGF antitest az 1. igénypont szerint, ahol a páciens HER2 negatív.
6. Alkalmazásra szolgáló anti-VEGF antitest a 4. igénypont szerint, ahol a kapecitabin adagolása 1000 mg/m^2 orálisan napi két alkalommal mindegyik háromhetes ciklus 1-14. napján, és a bevacizumab adagolása 15 mg/kg IV mindegyik 21-napos ciklus 1. napján.
7. Alkalmazásra szolgáló anti-VEGF antitest az 1. igénypont szerint, ahol a kezelési rend meghosszabbítja a páciens terjedéstől mentes túlélését legalább körülbelül 2,9 hónappal egy másik, önmagában kemoterápiával kezelt pácienshez hasonlítva.
8. Alkalmazásra szolgáló anti-VEGF antitest az 1. igénypont szerint, ahol az anti-VEGF antitestnek van egy nehézlánc variábilis régiója, amely tartalmazza a következő aminosav-szekvenciát:




EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW
INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP
HYYGSSHWYF DVWGQGTLVT VSS (SEQ ID NO.1)

és egy könnyűlánc variábilis régiója, amely tartalmazza a következő aminosav-
szekvenciát:

DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIFY
TSSLHSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ
GTKVEIKR (SEQ ID NO.2).

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