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)). The addition of Herceptin prolonged overall survival (Norton et al., *Proc. Am. Soc. Clin. Oncol.* 18:127a (1999)).

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. However, 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.

Since cancer is still one of the most deadly threats, additional cancer treatments for patients are needed. The invention addresses these and other needs, as will be apparent upon review of the following disclosure.

SUMMARY

Uses of an anti-VEGF antibody for effectively treating breast cancer patients with previously treated metastic breast cancer are provided. In particular, the disclosure~~invention~~ provides data from a randomized phase III clinical trial of bevacizumab (AVASTIN®) in combination with chemotherapy regimes in subjects with previously treated metastic breast cancer in human subjects. Such chemotherapy regimes include taxane therapy (e.g., paclitaxel q3wk or weekly, paclitaxel protein-bound particles (e.g., Abraxane®) or docetaxel), gemcitabine, vinorelbine or capecitabine therapy. The success of the trial shows that adding anti-VEGF antibody to chemotherapy provides statistically significant and clinically meaningful benefits as a second line therapy for previously treated breast cancer patients.

In one aspect, there is provided the use of an anti-VEGF antibody in the manufacture of a medicament for treating a patient diagnosed with previously treated metastatic triple negative breast cancer, wherein said medicament is to be administered in a treatment regimen as a second line therapy in combination with a chemotherapy, wherein the chemotherapy of the treatment regimen comprises administration of at least one chemotherapeutic agent, and wherein the treatment regimen effectively extends the progression free survival of the patient.

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 chemotherapy (taxane therapy, capecitabine, gemcitabine or vinorelbine) had an increase in progression free survival compared to subjects treated with chemotherapy alone. The difference was significantly significant.

Accordingly, the disclosure~~invention~~ provides a method of treating a patient diagnosed with previously treated metastatic breast cancer, comprising subjecting the patient to a treatment regimen combining a chemotherapy with the administration of an effective amount of an anti-VEGF antibody. The treatment regimen combining the chemotherapy with the administration of the anti-VEGF effectively extends the progression free survival (PFS) of the patient.

In certain embodiments, the PFS is extended about 0.5 months, 1 month, 1.2 months, 2 months, 2.1 months, 2.2 months, 2.8 months, 3 months, etc. In one embodiment, the PFS is extended about 2.1 months. In one embodiment, the PFS is extended about 2.2 months. In one embodiment, the PFS is extended about 2.8 months.

Any chemotherapeutic agent exhibiting anticancer activity can be used according to the disclosure~~invention~~. 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, epipodopyyllotoxins, antibiotics, L-Asparaginase, topoisomerase inhibitor, interferons, platinum cooridnation 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, paclitaxel, docetaxel, paclitaxel protein-bound particles (e.g., Abraxane®), gemcitabine, vinorelbine or combinations thereof. In certain embodiments, the chemotherapeutic agent is for example, capecitabine, taxane, paclitaxel, docetaxel, paclitaxel protein-bound particles (e.g., Abraxane®), gemcitabine, or combinations thereof. In certain embodiments, the chemotherapeutic agent is for example, capecitabine, taxane, paclitaxel, docetaxel, paclitaxel protein-bound particles (e.g., Abraxane®), or combinations thereof. Two or more chemotherapeutic agents can be used in a cocktail to be administered in combination with administration of the anti-VEGF antibody.

Clinical benefits of the treatments according to the disclosure~~invention~~ can be measured by, for example, duration of progression free survival (PFS), time to treatment failure, objective response rate and duration of response.

Accordingly, the disclosure~~invention~~ features a method of instructing a human subject with previously treated, 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.

The disclosure~~invention~~ also provides a promotional method, comprising promoting the administration of an anti-VEGF antibody for treatment of previously treated, 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.

The disclosure~~invention~~ provides a business method, comprising marketing an anti-VEGF antibody for treatment of previously treated, 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.

Also provided is a business method, comprising marketing a chemotherapeutic agent in combination with an anti-VEGF antibody for treatment of previously treated, 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.

In each of the methods of the disclosure~~invention~~ 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 below. In certain embodiments of the methods of the disclosure~~invention~~ 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 disclosure~~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 NYGMNWVRQA PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTLVT VSS (SEQ ID No. 1)

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

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

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.

In one embodiment, the treatment is a combination of a VEGF-specific antagonist, e.g., anti-VEGF antibody, and at least one chemotherapeutic agent.

Each of the methods of the disclosure~~invention~~ 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, the subject has HER2-negative metastic previously treated breast cancer.

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.

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 1μg/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 of the disclosure~~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. However, other dosage regimens may be useful. The progress of the therapy of the disclosure~~invention~~ is easily monitored by conventional techniques and assays.

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 extended treatment phase or maintenance therapy, as assessed by the clinician or described herein.

In other embodiments, treatment with the VEGF-specific antagonist for previously treated metastatic breast cancer 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 an anti-proliferative compound. Treatment 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.

In the embodiments 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.

Other features and advantages of the disclosure~~invention~~ will be apparent from the following Detailed Description, the drawings, and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the study design for the previously treated metastatic breast cancer trial using bevacizumab (Arm A) or placebo (Arm B) with various chemotherapies.

Figure 2 depicts the primary endpoint analysis of PFS of the study in Figure 1.

Figure 3 depicts the cohort-specific analyses of PFS of the study in Figure 1.

Figure 4 depicts the objective response rate of the study in Figure 1.

DETAILED DESCRIPTION

I. DEFINITIONS

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 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. 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. 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