(54) Title: ADJUVANT CANCER THERAPY

Arm A.

<table>
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<tr>
<th>Drug</th>
<th>Dose</th>
<th>Administration</th>
<th>Dosing Interval</th>
<th>Planned Duration</th>
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</thead>
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<tr>
<td>Oxaliplatin</td>
<td>80 mg/m²</td>
<td>IV bolus over 24 min + continuous infusion + bolus every 3 weeks (q3weeks)</td>
<td>Day 1, 8, 15, 22</td>
<td>q3weeks</td>
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<tr>
<td>5-FU</td>
<td>1000 mg/m²</td>
<td>IV continuous infusion over 4 h days</td>
<td>Days 1-4 q14 days</td>
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<tr>
<td>5-FU</td>
<td>1000 mg/m²</td>
<td>IV continuous infusion over 4 h days</td>
<td>Days 1-4 q14 days</td>
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Arm B.

<table>
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<tr>
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<th>Dose</th>
<th>Administration</th>
<th>Dosing Interval</th>
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<tr>
<td>Bevacizumab</td>
<td>5 mg/kg</td>
<td>IV bolus over 15 min into 0.9% NaCl solution every 2 weeks (q2weeks)</td>
<td>Day 1, q2weekly</td>
<td>q2weekly</td>
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<tr>
<td>Oxaliplatin</td>
<td>80 mg/m²</td>
<td>IV continuous infusion over 3 weeks (q3weeks)</td>
<td>Day 1, q3weekly</td>
<td>q3weekly</td>
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<tr>
<td>Lusevaxam</td>
<td>400 mg/m²</td>
<td>SQ bolus over 24 h days</td>
<td>Days 1-4 q14 days</td>
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<tr>
<td>5-FU</td>
<td>400 mg/m²</td>
<td>IV bolus over 2 min days</td>
<td>Days 1-4 q14 days</td>
<td></td>
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<tr>
<td>5-FU</td>
<td>200 mg/m²</td>
<td>IV bolus over 2 min days</td>
<td>Days 1-4 q14 days</td>
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(57) Abstract: Disclosed herein are methods and compositions comprising anti-VEGF antibodies for use in adjuvant cancer therapy.

(84) Published:
— with international search report (Art. 21(3))
— with sequence listing part of description (Rule 5.2(a))
ADJUVANT CANCER THERAPY

Related Applications

This application claims priority to and the benefit of United States provisional application No. 61/171,008 filed April 20, 2009; United States provisional application No. 61/171,318 filed April 21, 2009; and United States provisional application No. 61/181,195 filed May 26, 2009, the contents of each of which are incorporated herein by reference.

Field of the Invention

This invention relates in general to treatment of human diseases and pathological conditions. More specifically, the invention relates to use of anti-angiogenesis agents in adjuvant cancer therapy.

Background

Cancer is one of the most deadly threats to human health. In the U.S. alone, cancer affects nearly 1.3 million new patients each year, and is the second leading cause of death after cardiovascular disease, accounting for approximately 1 in 4 deaths. 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.

The majority of current methods of cancer treatment are relatively non-selective and generally target the tumor after the cancer has progressed to a more malignant state. Surgery removes the diseased tissue; radiotherapy shrinks solid tumors; and chemotherapy kills rapidly dividing cells. Chemotherapy, in particular, results in numerous side effects, in some cases so severe as to limit the dosage that can be given and thus preclude the use of potentially effective drugs. Moreover, cancers often develop resistance to chemotherapeutic drugs.

For most patients newly diagnosed with operable cancer, the standard treatment is definitive surgery followed by chemotherapy. Such treatment aims at
removing as much primary and metastatic disease as possible in order to prevent recurrence and improve survival. Indeed, most of these patients have no macroscopic evidence of residual tumor after surgery. However, many of them would later develop recurrence and may eventually die of their diseases. This can occur, for example, where a small number of viable tumor cells became metastasized prior to the surgery, escaped the surgery and went undetected after the surgery due to the limitation of current detection techniques.

Therefore, postoperative adjuvant treatments become important as auxiliary weapons to surgery in order to eliminate these residual micrometastatic cancer cells before they become repopulated and refractory. Over the past several decades, advances in adjuvant therapy have generally been incremental, centering on use of various chemotherapeutic agents. Many chemotherapy regimens have shown clinical benefits in adjuvantly treating patients with early stage major cancer indications such as lung, breast and colorectal cancers. Strauss et al. *J Clin Oncol* 22:7019 (2004); International Adjuvant Lung Cancer Trial (1995); Moertel et al. *Ann Intern Med* 122:321-6 (1995); IMPACT *Lancet* 345:939-44 (1995); Citron et al *J Clin Oncol* 21:1431-9 (2003).

Despite established benefits of chemo-based adjuvant therapy, one major limitation associated with chemotherapy of any kind is the significant toxicities. Generally, chemotherapeutic drugs are not targeted to the tumor site, and are unable to discriminate between normal and tumor cells. The issue of toxicities is especially challenging in adjuvant setting because of the lengthy treatment and its lasting impact on patients' quality of life. Moreover, benefits of adjuvant chemotherapy in patients with lower risk of recurrence remain unclear, making it questionable whether it is worthwhile for them to suffer the side effects of chemotherapy.

Angiogenesis refers to an important sequence of cellular events 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. 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.
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.

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-I) and VEGFR-2 (Flk-I/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.

In addition to being an angiogenic factor, VEGF, as a pleiotropic growth factor, exhibits multiple biological effects in other physiological processes, such as endothelial cell survival and proliferation, vessel permeability and vasodilation, monocyte chemotaxis, and calcium influx. Moreover, other 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.

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.

Since cancer remains one of the most deadly diseases additional treatments, such as adjuvant therapy, are desirable. The present invention addresses these and other needs, as will be apparent upon review of the following disclosure.

Summary of the Invention

The use of VEGF-specific antagonists in combination with chemotherapy has been shown to be beneficial in patients with cancer, e.g., metastatic colorectal cancer, non-small
cell lung cancer, breast cancer, etc., but less is known about the use of anti-VEGF antibodies in adjuvant therapy. The invention herein concerns the results obtained in clinical studies of the adjuvant use of AVASTIN® in human subjects with nonmetastatic, colorectal cancer.

Accordingly, the invention features a method of adjuvant therapy comprising

administering to a patient with cancer an effective amount of a VEGF-specific antagonist, e.g., an anti-VEGF antibody, for more than one year. In some embodiments the method of adjuvant therapy extends disease free survival (DFS) or overall survival (OS) in the patient. In some embodiments the DFS or OS is evaluated, e.g., analyzed about 2 to 5 years after initiation of treatment. Also provided is a method of adjuvant therapy comprising

administering to a patient with cancer an effective amount of a VEGF-specific antagonist, wherein progression of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment lasts for more than one year. In some embodiments the progression of cancer is prevented or delayed for about 3 months or 6 months after active treatment with the VEGF-specific antagonist has ceased. The invention further provides a method of adjuvant therapy comprising administering to a patient with cancer an effective amount of a VEGF-specific antagonist, wherein recurrence of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment with the VEGF-specific antagonist lasts for more than one year. In some embodiments the recurrence of cancer is prevented or delayed for about 3, 4, 5 or 6 months after active treatment with the VEGF-specific antagonist has ceased. In certain embodiments the patient is administered the VEGF-specific antagonist following definitive surgery. In certain embodiments the adjuvant therapy comprising administration of the anti-VEGF antibody is continued for at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more after initiation of treatment.

The invention provides a method of adjuvant therapy comprising administering to a patient who has undergone definitive surgery for cancer, an effective amount of a VEGF-specific antagonist so as to extend DFS or OS in the patient, wherein the VEGF-specific antagonist is administered for more than one year. In some embodiments the DFS or OS is evaluated about 2 to 5 years after initiation of treatment. Also provided is a method of

adjuvant therapy comprising administering to a patient who has undergone definitive surgery for cancer, e.g., a primary tumor, an effective amount of a VEGF-specific antagonist, wherein
progression of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment lasts for more than one year. In some embodiments the progression of cancer is prevented or delayed for about 3, 4, 5 or 6 months after active treatment with the VEGF-specific antagonist has ceased. The invention further provides a method of adjuvant therapy comprising administering to a patient who has undergone definitive surgery for cancer, e.g., a primary tumor, an effective amount of a VEGF-specific antagonist, wherein recurrence of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment with the VEGF-specific antagonist lasts for more than one year. In some embodiments the recurrence of cancer is prevented or delayed for about 3, 4, 5 or 6 months after active treatment with the VEGF-specific antagonist has ceased. In certain embodiments the adjuvant therapy comprising administration of the anti-VEGF antibody is continued for at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more after initiation of treatment.

The invention further provides a method of treating a patient who has undergone definitive surgery for cancer, e.g., a primary tumor, comprising administering to the patient adjuvant therapy comprising an effective amount of a VEGF-specific antagonist so as to extend DFS or OS in the patient, wherein the VEGF-specific antagonist is administered for more than one year. In some embodiments the DFS or OS is evaluated, e.g., analyzed about 2 to 5 years after initiation of treatment. Also provided is a method of treating a patient who has undergone definitive surgery for cancer, e.g., the primary tumor, comprising administering to the patient adjuvant therapy comprising an effective amount of a VEGF-specific antagonist, wherein progression of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment lasts for more than one year. In some embodiments the progression of cancer is prevented or delayed for about 3, 4, 5 or 6 months after active treatment with the VEGF-specific antagonist has ceased. The invention further provides a method of treating a patient who has undergone definitive surgery for cancer, e.g., primary tumor, comprising administering to the patient adjuvant therapy comprising an effective amount of a VEGF-specific antagonist, wherein recurrence of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment with the VEGF-specific antagonist lasts for more than one year. In some embodiments the recurrence of cancer is prevented or delayed for about 3, 4, 5
or 6 months after active treatment with the VEGF-specific antagonist has ceased. In certain embodiments the method comprises administration of the anti-VEGF antibody for at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more after initiation of treatment.

The invention also provides a method of preventing or delaying cancer recurrence in a patient comprising administering to the patient an effective amount of a VEGF-specific antagonist, e.g., an anti-VEGF antibody, for more than one year, wherein said administering of the VEGF-specific antagonist, e.g., anti-VEGF antibody, prevents cancer recurrence. The invention further provides a method of decreasing the likelihood of cancer recurrence in a patient comprising administering to the patient an effective amount of a VEGF-specific antagonist, e.g., an anti-VEGF antibody, for more than one year, wherein said administering of the VEGF-specific antagonist, e.g., anti-VEGF antibody, decreases the likelihood of cancer recurrence.

In some embodiments of any of the methods of the invention, said administering of the VEGF-specific antagonist prevents or reduces the likelihood of occurrence of a clinically detectable tumor, or metastasis thereof.

In each of the methods of the invention the administration of the VEGF-specific antagonist, e.g., anti-VEGF antibody, is continued for at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more after initiation of treatment. In some embodiments the administration of the VEGF-specific antagonist, e.g., anti-VEGF antibody, is continued until death.

In each of the methods of the 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. The anti-VEGF antibody 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®), G6-31, B20-4.1, and fragments thereof. In some embodiments the anti-VEGF antibody comprises a heavy chain variable region comprising the following amino acid sequence:

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EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQA
PGKGLEWGVINTYGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAED
TAVYYCAKYPHYSGHWWYFDVWGQGTLYVTSSEQIDNO:1
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and a light chain variable region comprising the following amino acid sequence:

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

In certain embodiments of the methods of the invention the anti-VEGF antibody is bevacizumab.

Each of the methods of the 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 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, breast cancer, 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 nonmetastatic colorectal cancer. In some embodiments of the methods of the invention the subject has metastatic colorectal cancer. In some embodiments the subject has resected stage II or stage III carcinoma of the colon.

In embodiments where the subject has undergone definitive surgery, the VEGF-specific antagonist, e.g., anti-VEGF antibody, is generally administered after a period of time in which the subject has recovered from the surgery. This period of time can include the period required for wound healing or healing of the surgical incision, the time period required to reduce the risk of wound dehiscence, or the time period required for the subject to return to a level of health essentially similar to or better than the level of health prior to the surgery. The period between the completion of the definitive surgery and the first administration of the anti-VEGF antibody can also include the period needed for a drug holiday, wherein the subject requires or requests a period of time between therapeutic regimes. Generally, the time period between completion of definitive surgery and the commencement of the anti-VEGF antibody therapy can include less than one week, 1 week, 2 weeks, 3 weeks, 4 weeks (28 days), 5 weeks, 6 weeks, 7 weeks, 8 weeks, 3 months, 4 months, 5 months, 6 months, 7
months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, 3 years, or more. In one embodiment, the period of time between definitive surgery and administering the anti-VEGF antibody is greater than 2 weeks and less than 1 year. In one embodiment, the period of time between definitive surgery and administering the anti-VEGF antibody is at least 28 days.

Each of the above aspects can further include monitoring the subject for recurrence of the cancer. Monitoring can be accomplished, for example, by evaluating disease free survival (DFS) or overall survival (OS). In one embodiment, the DFS or the OS is evaluated about 2 to 5 years after initiation of treatment. In one embodiment, the subject is disease free for at least 1 to 5 years after 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 or 10 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 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 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 or 10 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.

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 some embodiment, the treatment with an anti-VEGF antibody is prolonged until the patient has been cancer free for a time period selected from the group consisting of, 1 year, 2 years, 3 years, 4 years 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, and 12 years.

In other embodiments, treatment with the VEGF-specific antagonist is a monotherapy or a monotherapy for the duration of the VEGF-specific antagonist treatment period, as assessed by the clinician or described herein.
In other embodiments, treatment 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, and an anti-proliferative compound. Treatment with the VEGF-specific antagonist can also include any combination of the above types of therapeutic regimens. In addition, cytotoxic agents, anti-angiogenic and anti-proliferative agents can be used in combination with the VEGF-specific antagonist. In one embodiment, the anti-cancer therapy is chemotherapy. For example, the chemotherapeutic agent is selected from, e.g., alkylating agents, antimetabolites, folic acid analogs, pyrimidine analogs, purine analogs and related inhibitors, vinca alkaloids, epipodophyllotoxins, antibiotics, L-Asparaginase, topoisomerase inhibitor, interferons, platinum coordination complexes, anthracenedione substituted urea, methyl hydrazine derivatives, adrenocortical suppressant, adrenocorticosteroids, progestins, estrogens, antiestrogen, androgens, antiandrogen, gonadotropin-releasing hormone analog, etc. In some aspects, 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 anti-cancer therapy is chemotherapy which includes the administration of oxaliplatin, 5-fluorouracil, leucovorin or combinations thereof. In one embodiment, the VEGF-specific antagonist, administered either alone or with an anti-cancer therapy, can be administered as maintenance therapy.

The method of the invention are also advantageous in preventing the recurrence of a tumor or the regrowth of a tumor, for example, a dormant tumor that persists after removal of the primary tumor, or in reducing or preventing the occurrence or proliferation of micrometastases.

In additional embodiments of each of the above aspects of the invention, the VEGF-specific antagonist is administered in an amount or for a time (e.g., for a particular therapeutic regimen over time) to increase or extend (e.g., by 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more) survival of a subject who has undergone definitive surgery to treat colorectal cancer. In one example, the survival is measured as DFS or OS in the subject, wherein the DFS or the OS is evaluated about 2 to 5 years after initiation of adjuvant
treatment with a VEGF-specific antagonist. In some additional embodiments, the VEGF-specific antagonist is used to prevent or decrease likelihood of the reoccurrence of cancer or cancer progression in the subject.

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**

FIGURE 1 depicts the treatment regimen for the C-08 trial. Arm A: modified FOLFOX6 (oxaliplatin (85 mg/m²) with concurrent leucovorin (400 mg/m²) and 5-FU (400 mg/m² IV bolus) on Day 1 and 5-FU (2400 mg/m²) over 46 hours on Day 1 and Day 2) q 14 days for 12 cycles (6 months); Arm B: modified FOLFOX6 q 14 days for 12 cycles plus bevacizumab administered before oxaliplatin on Day 1 of each chemotherapy cycle (5 mg/kg IV) q 14 days for 1 year.

FIGURE 2 depicts the study design for the NSABP C-08 trial. Group 1: modified FOLFOX6 (oxaliplatin (85 mg/m²) with concurrent leucovorin (400 mg/m²) and 5-FU (400 mg/m² IV bolus) on Day 1 and 5-FU (2400 mg/m²) over 46 hours on Day 1 and Day 2) q 14 days for 12 cycles (6 months); Group 2: modified FOLFOX6 q 14 days for 12 cycles plus bevacizumab administered before oxaliplatin on Day 1 of each chemotherapy cycle (5 mg/kg IV) q 14 days for 1 year.

**Detailed Description**

1. **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-I/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 may be identified in the present application, e.g., by "VEGF (8-109)," "VEGF (1-109)" or "VEGF 165." 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-I receptors comparable to native VEGF.

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 Kd 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.

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.

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.

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.

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.

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), expressly incorporated herein by reference. The "EU index as in Kabat" refers to the residue numbering of the human IgGl EU antibody.

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(I 09) 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). 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(I 09) 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 TV-ethyl TV- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and 7V-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 M\(^{-1}\) 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.

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 vascular endothelial cell proliferation
or to induce vascular permeability. Preferred blocking antibodies or antagonist antibodies
completely inhibit the biological activity of the antigen.

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.

"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 CH\(_1\) domains; (ii) the Fab' fragment, which is a Fab
fragment having one or more cysteine residues at the C-terminus of the CH\(_1\) domain; (iii) the
Fd fragment having VH and CH\(_1\) domains; (iv) the Fd' fragment having VH and CH\(_1\)
domains and one or more cysteine residues at the C-terminus of the CH\(_1\) 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 93/1 1161; and Hollinger et al, Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)); (xi) "linear antibodies" comprising a pair of tandem Fd segments (VH-CHI-VH-CHI) 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).

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 present 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.

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.

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.

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.

"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 CDRHI includes amino acids H26-H35, the heavy chain FR1 residues are at positions 1-25 and the FR2 residues are at positions 36-49.

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.

"Single-chain Fv" or "scFv" antibody fragments comprise the V\textsubscript{H} and V\textsubscript{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\textsubscript{H} and V\textsubscript{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).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V\textsubscript{H}) connected to a light chain variable domain (V\textsubscript{L}) in the same polypeptide chain (V\textsubscript{H} and V\textsubscript{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 93/11611; and Hollinger et al, Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).

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_{H1}C_{H1}-V_{H2}C_{H2}) which, together with complementary light chain polypeptides, form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

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)).

"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).

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., Bio/Technology 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.

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.

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.

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.

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 x 10^-7 M, preferably no more than about 1 x 10^-8 M and most preferably no more than about 1 x 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.
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.

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 WO 1/45746. In one embodiment, the serum albumin peptide to be attached comprises an amino acid sequence of DICLPRWGCLW. 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.

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 as VEGF receptor protein. In certain embodiments, the chimeric VEGF receptor protein is capable of binding to and inhibiting the biological activity of VEGF.

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.

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.

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 above, e.g., antibodies to VEGF-A or to the VEGF-A receptor (e.g., KDR receptor or Flt-I receptor), 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).

A "loading dose" herein generally comprises an initial dose of a therapeutic agent administered to a patient, and is followed by one or more maintenance dose(s) thereof. Generally, a single loading dose is administered, but multiple loading doses are contemplated herein. Usually, the amount of loading dose(s) administered exceeds the amount of maintenance dose(s) administered and/or the loading dose(s) are administered more frequently than the maintenance dose(s), so as to achieve the desired steady-state concentration of the therapeutic agent earlier than can be achieved with the maintenance dose(s).

A "maintenance" dose herein refers to one or more doses of a therapeutic agent administered to the patient 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.

"Operable" cancer refers to cancer which is confined to the primary organ and suitable for surgery.

"Survival" refers to the patient remaining alive, and includes disease free survival (DFS) 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.

"Disease free survival (DFS)" refers to the patient remaining alive, without return of the cancer, 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 one aspect of the invention, DFS is analyzed according to the intent-to-treat principle, i.e., patients are evaluated on the basis of their assigned therapy. The events used in the analysis of DFS can include local, regional and distant recurrence of cancer, occurrence of secondary cancer, and death from any cause in patients without a prior event (e.g., colorectal cancer recurrence or second primary cancer).

"Overall survival" refers to the patient 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.

By "extending survival" or "increasing the likelihood of survival" is meant increasing DFS and/or OS or increasing the probability of remaining alive and/or disease-free at a given point in time in a treated patient relative to an untreated patient (i.e. relative to a patient not treated with a VEGF-specific antagonist, e.g., an anti-VEGF antibody), or relative to a control treatment protocol, such as treatment only with the chemotherapeutic agent, such as those use in the standard of care for colorectal cancer, e.g., leucovorin, 5-fluorouracil, oxaliplatin, irinotecan or combinations thereof. Survival is monitored for at least about 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.

"Hazard ratio" in survival analysis is a summary of the difference between two survival curves, representing the reduction in the risk of death on treatment compared to control, over a period of follow-up. Hazard ratio is a statistical definition for rates of events. For the purpose of the present 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.

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).
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.

"Adjuvant therapy" herein refers to therapy given after definitive surgery, after which no evidence of residual disease can be detected, so as to reduce the risk of disease recurrence, either local or metastatic. The goal of adjuvant therapy is to prevent or delay recurrence of the cancer, and therefore to reduce the chance of cancer-related death.

By "maintenance therapy" is meant a therapeutic regimen that is given to reduce the likelihood of disease recurrence or progression after a beneficial outcome of an initial therapeutic intervention. 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.

Herein, "standard of care" chemotherapy refers to the chemotherapeutic agents routinely used to treat a particular cancer.

"Definitive surgery" is used as that term is used within the medical community, and typically refers to surgery where the outcome is potentially curative. Definitive surgery includes, for example, procedures, surgical or otherwise, that result in removal or resection of the tumor, including those that result in the removal or resection of all grossly visible tumor.

Definitive surgery includes, for example, complete or curative resection or complete gross resection of the tumor. Definitive surgery includes procedures that occurs in one or more stages, and includes, for example, multi-stage surgical procedures where one or more surgical or other procedures are performed prior to resection of the tumor. Definitive surgery includes procedures to remove or resect the tumor including involved organs, parts of organs and tissues, as well as surrounding organs, such as lymph nodes, parts of organs, or tissues.
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 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, breast cancer, 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.

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 believed to be 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.
By "micrometastasis" is meant a small number of cells that have spread from the primary tumor to other parts of the body. Micrometastasis may or may not be detected in a screening or diagnostic test.

"Cancer recurrence" herein refers to a return of cancer following treatment, and includes return of cancer in the primary organ, as well as distant recurrence, where the cancer returns outside of the primary organ.

A subject at "high risk of cancer recurrence" is one who has a greater chance of experiencing recurrence of cancer. For example, relatively young subjects (e.g., less than about 50 years old), those with positive lymph nodes, particularly 4 or more involved lymph nodes (including 4-9 involved lymph nodes, and 10 or more involved lymph nodes), and those with tumors greater than 2 cm in diameter, e.g., in breast cancer patients. A subject's risk level can be determined by a skilled physician. Generally, such high risk subjects will have lymph node involvement (for example with 4 or more involved lymph nodes); however, subjects without lymph node involvement are also high risk, for example if their tumor is greater or equal to 2 cm.

"Decrease in risk of cancer recurrence" is meant reducing the likelihood of experiencing recurrence of cancer relative to an untreated patient (i.e., relative to a patient not treated with a VEGF-specific antagonist, e.g., an anti-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 colorectal cancer, e.g., leucovorin, 5-fluorouracil, oxaliplatin, irinotecan or a combination thereof. Cancer recurrence is monitored for at least about 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.

"Initiation of treatment" refers to the start of a treatment regimen following surgical removal of the tumor. In one embodiment, such may refer to administration of one or more chemotherapeutic agents following surgery. Alternatively, this can refer to an initial administration of a VEGF-specific antagonist, e.g., an anti-VEGF antibody, and one or more chemotherapeutic agent.

By "curing" cancer is herein is meant the absence of cancer recurrence at about 2, 3, 4 or about 5 years after beginning adjuvant therapy, depending on the type of cancer.
"Tumor", as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

By "tumor dormancy" is meant a prolonged quiescent state in which tumor cells are present but tumor progression is not clinically apparent. A dormant tumor may or may not be detected in a screening or diagnostic test.

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.

A "population" of subjects refers to a group of subjects with cancer, such as in a clinical trial, or as seen by oncologists following approval, e.g., FDA approval, for a particular indication, such as cancer adjuvant therapy.

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, 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 of two or more of these agents are also included in the invention.

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. At²¹, 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.
A "chemotherapeutic agent" 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, imposulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylene phosphoramide, triethylenethiophosphoramide and trimethylololomelamine; 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 CBI-TMI); eleutherobin; pancratistatin; azasarcodictyin; 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 ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall (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, carubicin, carbinomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epidurubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peptomycin, 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, thiamicrine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine,
enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, 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; nitaerine; 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; trichotheccenes (especially T-2 toxin, verracurin A, roardin A and anguidine); urethan; vindesine; dacarbazine; mannunostine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotaipa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE® Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® doxetaxel (Rhone-Poulenc Rorer, Antony, France); chloranbucil; 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; capetitabine; combretastatin; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); 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.

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, LY1 17018, onapristone, and FARESTON- toremifene; aromatase inhibitors that inhibit the enzyme
aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, 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-alpha, Ral 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.

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-alpha and -beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-alpha; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, -beta and -gamma 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-lalpha, 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-alpha or TNF-beta; 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.

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 alkylation 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

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.
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.

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.

The term "intravenous infusion" refers to introduction of a drug into the vein of an animal or human patient 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.

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.

The term "subcutaneous administration" refers to introduction of a drug under the skin of an animal or human patient, 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.

The term "subcutaneous infusion" refers to introduction of a drug under the skin of an animal or human patient, 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 patient, 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.
The term "subcutaneous bolus" refers to drug administration beneath the skin of an animal or human patient, 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.

A "disorder" is any condition that would benefit from treatment with the anti-VEGF 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, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

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. For the treatment of tumor dormancy or micrometastases, the therapeutically effective amount of the drug may reduce the number or proliferation of micrometastases; reduce or prevent the growth of a dormant tumor; or reduce or prevent the recurrence of a tumor after treatment or removal (e.g., using an anti-cancer therapy such as surgery, radiation therapy, or chemotherapy). 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, disease free survival (DFS), time to disease progression (TTP), duration of progression free survival (PFS), the response rates (RR), duration of response, time in remission, and/or quality of life. The effective amount may improve disease free survival (DFS), improve overall survival (OS), decrease likelihood of recurrence, extend time to recurrence, extend time to distant recurrence (i.e., recurrence...
outside of the primary site), cure cancer, improve symptoms of cancer (e.g., as gauged using a cancer specific survey), reduce appearance of second primary cancer, etc.

"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, including those in which the occurrence or recurrence of cancer is to be prevented.

"Active treatment" as used herein refers to the period of time during which the therapeutic drug is being administered to the patient. For example, if a therapeutic drug is being administered to the patient every 2 weeks over the course of one year followed by no treatment or other therapy, then the active treatment with the therapeutic drug is the one year period during which time that drug was being administered to the patient.

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.

All publications, patent applications, and patents mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

II. Anti-VEGF Antibodies and Antagonists

(i) VEGF Antigen

The VEGF antigen to be used for production of antibodies maybe, e.g., the VEGFi\textsubscript{65} 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.

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 VEGFi\textsubscript{65}. 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.

Although a vascular endothelial cell growth factor could be isolated and purified from natural sources for subsequent therapeutic use, the relatively low concentrations of the protein in follicular cells and the high cost, both in terms of effort and expense, of recovering VEGF proved commercially unavailing. Accordingly, 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).

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 Argno-AIa_{111}. 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.


Two VEGF receptors have been identified, Flt-1 (also called VEGFR-I) and KDR (also called VEGFR-2). Shibuya et al. (1990) Oncogene 8:5 19-527; 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). 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 present, 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.

**Anti-VEGF Antibodies**

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.

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.

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, the content of these patent applications are expressly incorporated herein by reference. 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 2005012126; 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, 191, K101, E103, and C104 or, alternatively, comprising residues F17, Y21, Q22, Y25, D63, 183 and Q89.

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

```
EVQLVESGGGLVQPSGLRLS CAAASGYTFT NYGMNWVRQAG PKGKLEWVGW
INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP
```

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

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DIQMTQSPSSLSLVGIYDRTITCSASQDIS NYLNWYQQKP GKAIPVLVYF
TSSLHSGVPS RFSGSGSGTD FTTLTISLQP EDFATYYCQQ YSTVPWTFGQ
```

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, the entire disclosure of which is expressly incorporated herein by reference. See also PCT Publication No. WO2005/044853, the entire disclosure of which is expressly incorporated herein by reference. In one embodiment, the G6 series antibody binds to a functional epitope on human VEGF comprising residues F17, Y21, Q22, Y25, D63, 183 and Q89.

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, the entire disclosure of which is expressly incorporated herein by reference. See also PCT Publication No. WO2005/044853, and US Patent Application 60/991,302, the content of these patent applications are expressly incorporated herein by reference. In one embodiment, the B20 series antibody binds to a functional epitope on human VEGF comprising residues F17, M18, D19, Y21, Y25, Q89, 191, KIO1, E103, and C104.

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 2ug/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).

(Uii) VEGF receptor molecules

The two best characterized VEGF receptors are VEGFR1 (also known as Flt-1) and VEGFR2 (also known as KDR and FLK-I 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. The full length Flt-I receptor includes an extracellular domain that has seven Ig domains, a transmembrane domain, and an intracellular domain with tyrosine kinase activity. The extracellular domain is involved in the binding of VEGF and the intracellular domain is involved in signal transduction.

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-l. 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.

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 invention 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-l/Fc, KDR/Fc, or Flt-l/KDR/Fc
(also known as VEGF Trap). (See for example PCT Application Publication No. WO97/44453)

A soluble VEGF receptor protein or chimeric VEGF receptor proteins of the invention 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

The invention provides a method of adjuvant therapy comprising administering a VEGF-specific antagonist, e.g., an anti-VEGF antibody, to a subject for more than one year. In some embodiments the subject has nonmetastatic colorectal cancer. In some embodiments of the method the VEGF-specific antagonist is administered following definitive surgery. The subject treated herein is generally at risk of cancer recurrence.

In some embodiments the method of adjuvant therapy extends disease free survival (DFS) or overall survival (OS) in the patient. In some embodiments the DFS or OS is evaluated, e.g., analyzed, about 2 to 5 years after initiation of treatment. Also provided is a method of adjuvant therapy comprising administering to a patient with cancer an effective amount of a VEGF-specific antagonist, wherein progression of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment lasts for more than one year. In some embodiments the progression of cancer is prevented or delayed for about 3, 4, 5 or 6 months after active treatment with the VEGF-specific antagonist has ceased. The invention further provides a method of adjuvant therapy comprising administering to a patient with cancer an effective amount of a VEGF-specific antagonist, wherein recurrence of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment with the VEGF-specific antagonist lasts for more than one year. In some embodiments the recurrence of cancer is prevented or delayed for about 3, 4, 5 or 6 months after active treatment with the VEGF-specific antagonist has ceased. In certain embodiments the patient is administered the VEGF-
specific antagonist following definitive surgery. In certain embodiments the adjuvant therapy comprising administration of the anti-VEGF antibody is continued for at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more after initiation of treatment.

The invention provides a method of adjuvant therapy comprising administering to a patient who has undergone definitive surgery for cancer, e.g., a primary tumor, an effective amount of a VEGF-specific antagonist so as to extend DFS or OS in the patient, wherein the VEGF-specific antagonist is administered for more than one year. In some embodiments the DFS or OS is evaluated, e.g., analyzed, about 2 to 5 years after initiation of treatment. Also provided is a method of adjuvant therapy comprising administering to a patient who has undergone definitive surgery for cancer, e.g., a primary tumor, an effective amount of a VEGF-specific antagonist, wherein progression of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment lasts for more than one year. In some embodiments the progression of cancer is prevented or delayed for about 3, 4, 5 or 6 months after active treatment with the VEGF-specific antagonist has ceased. The invention further provides a method of adjuvant therapy comprising administering to a patient who has undergone definitive surgery for cancer, e.g., a primary tumor, an effective amount of a VEGF-specific antagonist, wherein recurrence of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment with the VEGF-specific antagonist lasts for more than one year. In some embodiments the recurrence of cancer is prevented or delayed for about 3, 4, 5 or 6 months after active treatment with the VEGF-specific antagonist has ceased. In certain embodiments the adjuvant therapy comprising administration of the anti-VEGF antibody is continued for at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more after initiation of treatment.

The invention further provides a method of treating a patient who has undergone definitive surgery for cancer, e.g., a primary tumor, comprising administering to the patient adjuvant therapy comprising an effective amount of a VEGF-specific antagonist so as to extend DFS or OS in the patient, wherein the VEGF-specific antagonist is administered for more than one year. In some embodiments the DFS or OS is evaluated, e.g., analyzed, about 2 to 5 years after initiation of treatment. Also provided is a method of treating a patient who has undergone definitive surgery for cancer, e.g., a primary tumor, comprising administering
to the patient adjuvant therapy comprising an effective amount of a VEGF-specific antagonist, wherein progression of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment lasts for more than one year. In some embodiments the progression of cancer is prevented or delayed for about 3, 4, 5 or 6 months after active treatment with the VEGF-specific antagonist has ceased. The invention further provides a method of treating a patient who has undergone definitive surgery for cancer, e.g., a primary tumor, comprising administering to the patient adjuvant therapy comprising an effective amount of a VEGF-specific antagonist, wherein recurrence of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment with the VEGF-specific antagonist lasts for more than one year. In some embodiments the recurrence of cancer is prevented or delayed for about 3, 4, 5 or 6 months after active treatment with the VEGF-specific antagonist has ceased. In certain embodiments the method comprises administration of the anti-VEGF antibody for at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more after initiation of treatment.

For example, a method can include the following steps: a) a first stage comprising a plurality of cycles wherein each cycle comprises administering to the subject an effective amount of a VEGF-specific antagonist, e.g., an anti-VEGF antibody such as bevacizumab, and optionally, at least one chemotherapeutic agent at a predetermined interval; and b) a second stage comprising a plurality of cycles wherein each cycle comprises administering to the subject an effective amount of a VEGF-specific antagonist, e.g., an anti-VEGF antibody such as bevacizumab, without any chemotherapeutic agent at a predetermined interval; wherein the combined first and second stages last for at least one year after the initial postoperative treatment. In some embodiments the combined first and second stages last for more than one year after initial postoperative treatment. In some embodiments the second stage lasts for more than 1 year, at least 2 years, at least 3 years, at least 4 years, at least 5 years or at least 10 years after the initial postoperative treatment. In one embodiment, the first stage comprises a first plurality of treatment cycles wherein a VEGF-specific antagonist, e.g., bevacizumab, and a first chemotherapy regimen are administered, followed by a second plurality of treatment cycles wherein a VEGF-specific antagonist,
e.g., an anti-VEGF antibody such as bevacizumab, and a second chemotherapy regimen are administered.

In one example, the method includes administration of modified FOLFOX6 (oxaliplatin (85 mg/m²) with concurrent leucovorin (400 mg/m²) and 5-FU (400 mg/m² IV bolus) on Day 1 and 5-FU (2400 mg/m²) over 46 hours on Day 1 and Day 2) q 14 days for 12 cycles (6 months) plus bevacizumab administered before oxaliplatin on Day 1 of each chemotherapy cycle (5 mg/kg IV) q 14 days for 1 year or more.

In one administration schedule, the adjuvant therapy of the invention comprises a first stage wherein a VEGF-specific antagonist, e.g., an anti-VEGF antibody, and one or more chemotherapeutic agents are administered to a patient in a plurality of treatment cycles; and a second stage wherein a VEGF-specific antagonist, e.g., an anti-VEGF antibody, is used as a single agent in a plurality of maintenance cycles. Each treatment cycle consists of one to three weeks, depending on the particular treatment plan. For example, a treatment cycle can include bevacizumab as the VEGF-specific antagonist and can be three weeks, which means patients receive one dose of chemotherapy and one dose of bevacizumab every three weeks. A treatment cycle can also be two weeks, which means patients receive one dose of chemotherapy and one dose of bevacizumab, every other week. The entire first stage of treatment can last for about 4-12 cycles. During the second, maintenance stage, bevacizumab may be given biweekly or triweekly, depending on the length of the particular cycle, and for a total about 10-50 cycles. In certain embodiments, the adjuvant therapy lasts for at least one year from the initiation of the treatment (e.g., initial postoperative treatment), and the subject's progress will be followed after that time. In some embodiments the anti-VEGF antibody adjuvant therapy lasts for more than 1 year, at least 2 years, at least 3 years, at least 4 years, at least 5 years or at least 10 years from the initiation of treatment or until death.

Depending on the type and severity of the disease, preferred dosages for the anti-VEGF antibody are in the range from about 1 ug/kg to about 50mg/kg, most preferably from about 5mg/kg to about 15mg/kg, including but not limited to 7.5 mg/kg or 10 mg/kg. In some aspects, the chemotherapy regimen involves the traditional high-dose intermittent administration. In some other aspects, the chemotherapeutic agents are
administered using smaller and more frequent doses without scheduled breaks ("metronomic chemotherapy"). The progress of the therapy of the invention is easily monitored by conventional techniques and assays.

Administration of the antibody and chemotherapy can decrease the likelihood of disease recurrence (cancer recurrence in the primary organ and/or distant recurrence), in a cancer patient compared to subjects treated with chemotherapy (e.g., leucovorin, oxaliplatin, 5-FU, irinotecan or combinations thereof) alone.

In one aspect, the invention provides a method of adjuvant therapy comprising administering to a patient with cancer, following definitive surgery, an effective amount of an anti-VEGF antibody so as to extend disease free survival (DFS) or overall survival (OS) in the patient. The DFS or OS may be evaluated, e.g., analyzed, about 2 to 5 years after initiation of treatment. In some embodiments the DFS or OS is evaluated, e.g., analyzed, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years after initiation of treatment. The invention also provides a method of preventing cancer recurrence in a patient comprising administering to the patient an effective amount of an anti-VEGF antibody wherein said administering of the anti-VEGF antibody prevents cancer recurrence. The invention further provides a method of decreasing the likelihood of cancer recurrence in a patient comprising administering to the patient an effective amount of an anti-VEGF antibody wherein said administrating of the anti-VEGF antibody decreases the likelihood of cancer recurrence. In some embodiments of the methods of the invention, said administrating of the VEGF-specific antagonist prevents or reduces the likelihood of occurrence of a clinically detectable tumor, or metastasis thereof.

For adjuvant therapy, the VEGF-specific antagonist can be administered in an amount or for a time (e.g., for a particular therapeutic regimen over time) to reduce (e.g., by 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more) or inhibit tumor metastasis; to reduce or inhibit tumor growth or tumor cell proliferation; to reduce or prevent the growth of a dormant tumor; to reduce or prevent the growth or proliferation of a micrometastases; to reduce or prevent the re-growth of a tumor after treatment or removal; and/or to relieve to some extent one or more of the symptoms associated with the cancer.

The VEGF-specific antagonist is generally administered after a period of time in which the subject has recovered from the surgery. This period of time can include the period
required for wound healing or healing of the surgical incision, the time period required to
reduce the risk of wound dehiscence, or the time period required for the subject to return to a
level of health essentially similar to or better than the level of health prior to the surgery. The
period between the completion of the definitive surgery and the first administration of the
VEGF-specific antagonist can also include the period needed for a drug holiday, wherein the
subject requires or requests a period of time between therapeutic regimes. Generally, the time
period between completion of definitive surgery and the commencement of the VEGF-
specific antagonist therapy can include less than one week, 1 week, 2 weeks, 3 weeks, 4
weeks (28 days), 5 weeks, 6 weeks, 7 weeks, 8 weeks, 3 months, 4 months, 5 months, 6
months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, 3 years, or
more. In one embodiment, the period of time between definitive surgery and administering the
VEGF-specific antagonist is greater than 2 weeks and less than 1 year.

In one example, the VEGF-specific antagonist, e.g., an anti-VEGF antibody, is
administered in an amount effective to extend disease free survival (DFS) or overall
survival (OS). The DFS or the OS may be evaluated, e.g., analyzed, about 2 to 5 years
after an initial administration of the antibody. In certain embodiments, the subject's
DFS or OS is evaluated, e.g., analyzed, about 3-5 years, about 4-5 years, or at least
about 4, or at least about 5 years after initiation of treatment or after initial diagnosis.

The VEGF-specific antagonist may be administered as single agent. The invention
also features the use 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.

In certain aspects, the VEGF-specific antagonist is used in combination with one or
more chemotherapeutic agents for adjuvant therapy for the treatment of a colorectal cancer
following definitive surgery. A variety of chemotherapeutic agents may be used in the
combined treatment methods of the invention. An exemplary and non-limiting list of
chemotherapeutic agents contemplated is provided herein under the "Definitions" section, or described hererin.

In one example, the invention features the use of a VEGF-specific antagonist with one or more chemotherapeutic agents (e.g., a cocktail). In some embodiments where the cancer is colorectal cancer, the chemotherapeutic agent may be one that is specifically used for colorectal cancer and includes, but is not limited to, leucovorin, 5-fluorouracil, oxaliplatin, irinotecan or combinations of two or more of such chemotherapeutic agents. 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.

The combined administration includes coadministration or concurrent administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein optionally there is a time period while both (or all) active agents simultaneously exert their biological activities. Thus, the chemotherapeutic agent may be administered prior to, or following, administration of the VEGF-specific antagonist, e.g., an anti-VEGF antibody. In this embodiment, the timing between at least one administration of the chemotherapeutic agent and at least one administration of the VEGF-specific antagonist, e.g., an anti-VEGF antibody, is preferably approximately 1 month or less, and most preferably approximately 3 weeks, 2 weeks or less. Alternatively, the chemotherapeutic agent and the anti-VEGF antibody are administered concurrently to the patient, in a single formulation or separate formulations. Treatment with the combination of the chemotherapeutic agent (e.g. leucovorin, oxaliplatin, 5-FU, irinotecan or combinations
thereof) and the anti-VEGF antibody (e.g. bevacizumab) may result in a synergistic, or
greater than additive, therapeutic benefit to the patient.

The chemotherapeutic agent, if administered, is usually administered at
dosages known therefor, or optionally lowered due to combined action of the drugs or
negative side effects attributable to administration of the antimetabolite
chemotherapeutic agent. Preparation and dosing schedules for such chemotherapeutic
agents may be used according to manufacturers' instructions or as determined
empirically by the skilled practitioner.

In some other aspects, other therapeutic agents useful for combination tumor therapy
with the anti-VEGF 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
patient. In a preferred embodiment, the anti-VEGF antibody is co-administered with a growth
inhibitory or cytotoxic agent. For example, the growth inhibitory or cytotoxic agent may be
administered first, followed by the anti-VEGF antibody. However, simultaneous
administration or administration of the anti-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.

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 small molecule VEGFR antagonist. Such molecules are suitably present in
combination in amounts that are effective for the purpose intended.

In certain aspects, 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 Nature 407(6801):249-57 (2000). Preferably, 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 patient.

For the adjuvant therapy, the appropriate dosage of VEGF-specific antagonist may depend on the type of disease to be treated, as defined above, the severity and course of the disease, previous therapy, the patient's clinical history and response to the VEGF-specific antagonist, and the discretion of the attending physician. The VEGF-specific antagonist may be suitably administered to the patient 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 prevent cancer recurrence.

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.

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.

In addition to the above therapeutic regimes, the patient may be subjected to radiation therapy.
In certain embodiments, the administered anti-VEGF antibody is an intact, naked antibody. However, the anti-VEGF antibody may be conjugated with a cytotoxic agent. In certain embodiments, 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.

IV. Dosages and Duration

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 patient, 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, a benign, precancerous, or early stage cancer; or to treat or prevent the occurrence or recurrence of a tumor, a dormant tumor, or a micrometastases, for example, in the neoadjuvant or adjuvant setting. 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.

Depending on the type and severity of the disease, about 1 µg/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 patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to about 100 mg/kg or more, depending on the factors mentioned above. Particularly desirable dosages include, for example, 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 7.5 mg/kg or 10 mg/kg. The progress of the therapy of the invention is easily monitored by conventional techniques and assays.

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 some embodiments the therapy is continued for more than one year. In certain embodiments, the VEGF-specific antagonist therapy is continued for 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. In some embodiments the therapy is continued until disease progression. In some embodiments, the therapy is continued in the absence of disease recurrence.

The VEGF-specific antagonists of the invention are administered to a subject, e.g., a human patient, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Local administration is particularly desired if extensive side effects or toxicity is associated with VEGF antagonism. An \textit{ex vivo} strategy can also be used for therapeutic applications. \textit{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.

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.

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.

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.

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**

Therapeutic formulations of the antibodies used in accordance with the present invention are prepared using standard methods known in the art, e.g., by mixing the antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences (20th edition), ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, PA), 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 dextrins; 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™, PLURONIC™ or polyethylene glycol (PEG). Preferred lyophilized anti-VEGF antibody formulations are described in WO 97/04801, expressly incorporated herein be reference.

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%.

In one embodiment, 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 maybe 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 may be 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 polysorbate 20, and Water for Injection, USP.

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 small molecule VEGFR antagonist. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsule prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacrylate) microcapsule, 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, supra.

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.

The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.
VI. Efficacy of the Treatment

The present invention provides methods of adjuvant therapy in cancer patients where the treatment produces beneficial anti-cancer effects without causing significant toxicities or adverse effects. Efficacy of the treatment of the invention can be measured by various endpoints commonly used in evaluating cancer treatments, including but not limited to, duration of survival, disease free survival, progression free survival, time to disease progression, time in remission, and/or 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. The anti-VEGF antibody of the invention may cause inhibition of metastatic spread 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.

In one embodiment the present invention provides methods of preventing or decreasing the likelihood of cancer recurrence in a human patient.

In one example, the VEGF-specific antagonist, e.g., an anti-VEGF antibody, is administered in an amount effective to extend DFS or OS, wherein the DFS or the OS is evaluated, e.g., analyzed, about 2 to 5 years after an initial administration of the antibody. In certain embodiments, the subject's DFS or OS is evaluated, e.g., analyzed, about 3-5 years, about 4-5 years, or at least about 4, or at least about 5 years, or at least about 6 years, or at least about 7 years, or at least about 8 years, or at least about 9 years, or at least about 10 years after initiation of treatment or after initial diagnosis.

In one embodiment, the methods of the present invention can be used for increasing the duration of survival of a subject susceptible to or diagnosed with a cancer or cancer recurrence. Duration of survival is defined as the time from first administration of the drug to death. Duration of survival can also be measured by stratified hazard ratio (HR) of the treatment group versus control group, which represents the risk of death for a patient during the treatment.

In yet another embodiment, the treatment of the invention significantly increases response rate in a group of subjects, e.g., human patients, susceptible to or diagnosed with a
cancer who are treated with various anti-cancer therapies. Response rate is defined as the percentage of treated patients who responded to the treatment. In one aspect, the combination treatment of the invention using an anti-VEGF antibody and surgery, radiation therapy, or one or more chemotherapeutic agents significantly increases response rate in the treated patient group compared to the group treated with surgery, radiation therapy, or chemotherapy alone.

VII. Antibody Production

(i) Polyclonal antibodies

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, SOCl₂, or \( R'N=C=NR \), where \( R \) and \( R' \) are different alkyl groups.

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

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

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)).

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.

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)).

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).
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.

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.

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.

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. ScL 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.

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.

(II) Humanized and human antibodies

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.

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)).

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.

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.

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 (JH) 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 Immunol., 7:33 (1993); and Duchosal et al. Nature 355:258 (1992).

Alternatively, phage display technology (McCafferty et al., Nature 348:552-553 (1990))
can be used to produce human antibodies and antibody fragments \textit{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 M13 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.


As discussed above, human antibodies may also be generated by \textit{in vitro} activated B cells (see U.S. Patents 5,567,610 and 5,229,275).

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

\textit{(iv) Antibody fragments}

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, \textit{e.g.}, Morimoto \textit{et al.}, \textit{Journal of Biochemical and Biophysical Methods} 24:107-117 (1992) and Brennan \textit{et al.}, \textit{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 \textit{E. coli} and chemically coupled to form F(ab')\textsubscript{2} fragments (Carter \textit{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.

(vi) Other amino acid sequence modifications

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.

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.

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.

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.

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), He (I), Pro (P), Phe (F), Trp (W), Met (M)
2. uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), GIn (Q)
3. acidic: Asp (D), Glu (E)
4. basic: Lys (K), Arg (R), His(H)

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

1. hydrophobic: Norleucine, Met, Ala, Val, Leu, He;
2. neutral hydrophilic: Cys, Ser, Thr, Asn, GIn;
3. acidic: Asp, Glu;
4. basic: His, Lys, Arg;
5. residues that influence chain orientation: Gly, Pro;
6. aromatic: Trp, Tyr, Phe.
Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

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).

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.

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.

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-acetylglucosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

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).

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 Al, 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 WO03/01 1878, 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.

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-

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 Clq binding and/or CDC.

Antibodies with altered Clq binding and/or complement dependent cytotoxicity (CDC) are described in WO99/51642, US Patent No. 6,194,551Bl, US Patent No. 6,242,195Bl, US Patent No. 6,528,624Bl 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).

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.

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.

Engineered antibodies with three or more (preferably four) functional antigen binding sites are also contemplated (US Appln No. US2002/0004587 Al, Miller et al).

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.

(v) **Immunoconjugates**

The invention also pertains to 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).

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 $^{212}$Bi, $^{131}$I, $^{131}$In, $^{90}$Y and $^{186}$Re.

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 l-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/1 1026.

In another embodiment, the antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, 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).

(vi) **Immunoliposomes**

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.

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 present 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 (such as Doxorubicin) is optionally contained within the liposome. See Gabizon et al. J. National Cancer Inst.81(19)1484 (1989)
VIII. Articles of Manufacture and Kits

In another embodiment of the invention, 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 insert with instructions for use, including for example a warning that the composition is not to be used in combination with another composition, or instructing the user of the composition to administer to a patient the anti-VEGF antibody composition alone or in combination with an anti-cancer composition, e.g., leucovorin, 5-FU, oxaliplatin, irinotecan or combinations thereof. The term "instructions for use" means providing directions for applicable therapy, medication, treatment, treatment regimens, and the like, by any means, e.g., in writing, such as in the form of package inserts or other written promotional material.

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 patients, 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. The kit may be manufactured as a single use unit dose for one patient, multiple uses for a
particular patient (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 patients ("bulk packaging"). The kit components may be assembled in cartons, blister packs, bottles, tubes, and the like.

The invention provides a kit for treating a patient who has undergone definitive surgery for cancer, e.g., a primary tumor, comprising a package, wherein the package comprises an anti-VEGF antibody composition and instructions for using the anti-VEGF antibody composition in adjuvant therapy, wherein the instructions recite that the DFS at 1 year after initiation of the adjuvant therapy for patients receiving the adjuvant therapy was 94.3 with a hazard ratio of 0.60.

**Deposit of Materials**

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:

<table>
<thead>
<tr>
<th>Antibody Designation</th>
<th>ATCC No.</th>
<th>Deposit Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4.6.1</td>
<td>ATCC HB-10709</td>
<td>March 29, 1991</td>
</tr>
</tbody>
</table>

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

**EXAMPLES**

**Example 1. Bevacizumab adjuvant therapy in patients with colorectal cancer**

This example concerns analysis of results obtained from colorectal cancer subjects treated in the National Surgical Adjuvant Breast and Bowel Project (NSABP C-08) clinical trial. The primary aim of the study was to determine the clinical benefit of adding bevacizumab to standard chemotherapy for treating colorectal cancer, as measured by disease-free survival (DFS). A secondary goal was to determine if there was clinical benefit in prolonging overall survival. The standard chemotherapy used in this trial was a combination
of leucovorin, 5-fluorouracil and oxaliplatin. This trial evaluated the efficacy of bevacizumab (AVASTIN®) as adjuvant therapy for patients with resected stages II and III carcinoma of the colon.

Study Design

The design of the NSABP C-08 study is depicted in Figures 1 and 2.

In the NSABP C-08 trial, the following treatment protocol was used:

Arm A/Group 1: modified FOLFOX6 (mFOLFOXβ: oxaliplatin (85 mg/m²) with concurrent leucovorin (400 mg/m²) and 5-FU (400 mg/m² IV bolus) on Day 1 and 5-FU (2400 mg/m²) over 46 hours on Day 1 and Day 2) q 14 days for 12 cycles (6 months);

Arm B/Group 2: modified FOLFOX6 q 14 days for 12 cycles (6 months) plus bevacizumab administered before oxaliplatin on Day 1 of each chemotherapy cycle (5 mg/kg IV) q 14 days for 1 year.

Bevacizumab (AVASTIN®) was supplied as a clear to slightly opalescent, sterile liquid ready for parenteral administration in two vial sizes: each 100 mg (25 mg/ml - 4 ml fill) glass vial contained bevacizumab with phosphate, trehalose, polysorbate 20 and Sterile Water for Injection, USP and each 400 mg (25 mg/ml - 16 ml fill) glass vial contained bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection, USP. AVASTIN® was administered by withdrawing the necessary amount for a dose of 5 mg/kg and diluted in a total volume of 100 ml of 0.9% Sodium Chloride Injection, USP before intravenous administration.

To qualify for these trials, patients were required to have histologically confirmed adenocarcinoma of the colon that met one of the following stages:

(1) Stage II carcinoma (T_{3or4}.N₀, M₀) (The tumor has invaded through the muscularis propria into the subsetrosa or into non-peritonealized pericoilic or perirectal tissues (T3); or has directly invaded other organs or structures, and/or perforates visceral peritoneum (T₄)) or

(2) Stage III carcinoma (any T, N₁ or ₂, M₀) (The tumor has invaded to any depth, with involvement of regional lymph nodes).

Patients with T4 tumors that involved an adjacent structure (e.g., bladder, small intestine, ovary, etc.) by direct extension from the primary tumor were eligible if all of the following conditions were met:

(1) all or a portion of the adjacent structure was removed en bloc with the primary tumor;
(2) in the opinion of the surgeon, all grossly visible tumor was completely resected ("curative resection");
(3) histological evaluation by the pathologist confirmed that the margins of the resected specimen are not involved by malignant cells; and
(4) local radiation therapy would not be utilized.

Patients with more than one synchronous primary colon tumor were eligible with staging classification being based on the more advanced primary tumor.

Patients must have had an en bloc complete gross resection of tumor (curative resection) by open laprotomy or laparoscopically-assisted colectomy. Patients who had a two-stage surgical procedure to first provide a decompressive colostomy and then in a later procedure to have the definitive surgical resection were eligible. The distal extent of the tumor must be greater than or equal to 12 cm from the anal verge on endoscopy. If the patient was not a candidate for endoscopy then the distal extent of the tumor must be greater than or equal to 12 cm from the anal verge as determined by surgical examination.

Patients were 18 years of age or older, had an ECOG performance status of 0 or 1 and in the opinion of the investigator must have had a life expectancy of at least 5 years, excluding their diagnosis of cancer.

At the time of randomization, patients must have had a postoperative absolute granulocyte count (AGC) of greater than or equal to 1500 mm$^3$ (or less than 1500/mm$^3$ if in the opinion of the investigator this represented an ethnic or racial variation of normal) and a postoperative platelet count of greater than or equal to 100,000/mm$^3$. Patients also had normal hepatic and renal function.

Patients with prior malignancies, including colorectal cancers, were eligible if they had been disease-free for at least 5 years and were deemed by their physician to be at low risk for recurrence. Patients with squamous or basal cell carcinoma of the skin, melanoma in situ, carcinoma in situ of the cervix, carcinoma in situ of the colon or rectum that have been effectively treated even if these conditions were diagnosed within 5 years prior to randomization were also eligible.

Patients were ineligible if they had any of the following conditions: colon cancer other than adenocarcinoma, rectal tumors, isolated distant or non-contiguous intra-abdominal metastases (even if resected), systemic or radiation therapy initiated for the malignancy,
significant bleeding unrelated to the primary colon tumor within 6 months before study entry, serious or non-healing wound, skin ulcers or bone fracture, gastroduodenal ulcer determined by endoscopy to be active, major surgical procedure, open biopsy or significant traumatic injury within 28 days prior to randomization, anticipation of need for major surgical procedure during the course of the trial, core biopsy or other minor procedure, excluding placement of a vascular access device, within 7 days prior to randomization, uncontrolled blood pressure (greater than 150/90 mmHg), previous history of CNS cerebrovascular ischemia, history of peripheral arterial ischemia within 6 months, history of visceral arterial ischemia within 6 months, concomitant halogenated antiviral agents, clinically significant peripheral neuropathy at the time of randomization (grade 2 or greater neurosensory or neuromotor toxicity using the NCI Common Terminology Criteria for Adverse Events Version 3.0), non-malignant systemic disease that would have precluded use of any of the study drugs used in the trial, pregnancy or lactation at the time of randomization, psychiatric or addictive disorders or other conditions that in the opinion of the investigator would have precluded the patient from meeting the study trial requirements, PT (INR) > 1.5 unless the patient was on full-dose anticoagulants and the subject had an in-range INR on a stable dose of warfarin or stable dose of low molecular weight heparin and the subject had not had active bleeding or a pathological condition that was associated with a high-risk of bleeding.

The primary endpoint of this trial was duration of disease free survival (DFS). Events for DFS included first documented evidence of colon cancer recurrence, second primary cancer or death from any cause. Secondary endpoints were duration of overall survival (OS) and toxicity related to study therapy. Events for overall survival included death from any cause.

Diagnosis of colon cancer recurrence was made using the following criteria. For abdominal and/or pelvic sites: positive cytology or biopsy if anastomatic;

Abdominal, pelvic and retroperitoneal nodes: (1) positive cytology or biopsy, (2) progressively enlarging node(s) as evidenced by two CT or MRI scanscs separated by at least a 4 week interval, (3) ureteral obstruction in the presence of a mass as documented on CT or MRI scan or (4) a single CT or MRI scan showing a definite mass which is confirmed to be malignant by a positive PET scan at that site.
Peritoneum (including visceral and parietal peritoneum or omentum): (1) positive cytology or biopsy or (2) progressively enlarging intraperitoneal solid mass as evidenced by two CT or MRI scans separated by at least 4 week interval, or a single scan confirmed to be malignant by a positive PET scan at that site.

Ascites: positive cytology

Liver: (1) positive cytology or biopsy or (2) three of the following which are not associated with benign disease: (i) recent or progressive hepatomegaly, abnormal liver contour; (ii) positive radionucleotide liver scan or sonogram; (iii) positive PET scan which confirms abnormal CT scan or MRI scan and is associated with a rising CEA; (iv) abnormal liver function studies; or (V) elevated CEA, i.e., a persistent rise in CEA titer of 10 times the upper normal value, confirmed on two determinations separated by a 4-week interval, in patients who had a normal postoperative CEA value (the determination should be performed by the same laboratory, using the same method.)

Pelvic mass not otherwise specified (NOS): (1) positive cytology or biopsy or (2) progressively enlarging intrapelvic solid mass as evidenced by two CT or MRI scans separated by at least a 4-week interval or (3) a solid mass on a single CT scan confirmed by a positive PET scan at that site.

Abdominal wall, perineum and scar: positive cytology or biopsy

Nonabdominal and nonpelvic sites:

Skeletal: for all bone-only recurrence, a biopsy is required

Lung: (1) positive cytology, aspirate or biopsy or (2) radiologic evidence of multiple pulmonary nodules that re felt to be consistent with pulmonary metastases.

Bone marrow: positive cytology, aspirate, biopsy or MRI scan

Central nervous system: (1) positive CT or MRI scan, usually in a patient with neurologic symptoms; or (2) biopsy or cytology (for a dianosis of meningeal involvement).

The diagnosis of a second primary cancer was confirmed histoloigcally whenever possible.

Results

The results from this trial indicate that addition of AVASTIN® to chemotherapy significantly increased DFS as compared to chemotherapy alone, during the first year, which
corresponds to the active treatment phase. The data show that this significant benefit was not associated with any increased toxicities or adverse effects.

2,710 patients were accrued to the study (1,356 to the control arm and 1,354 to the experimental arm). 18 patients on the control arm and 20 patients on the experimental arm were not evaluated for efficacy due to no follow-up or positive surgical margins. In addition, 22 control and 15 experimental arm patients were found to be ineligible for other reasons, but were included in the analysis. Thus, there were 1,338 and 1,334 patients in the control and experimental arms, respectively, included in these analyses. The median follow-up was 35.6 months. Patient characteristics were well balanced by treatment arm. Slightly over half of the patients were less than 60 years of age, approximately 15% were older than 70 years and there was an equal gender distribution. Stage II patients constituted approximately 25%.

Time to an event was measured from randomization. All p-values, other than the primary endpoint, were evaluated as significant at the 0.05 level two-sided. All confidence intervals were 95%. Hazard ratios (HR) were calculated from Cox models and p-values from time to event were from the log rank test. HRs and p-values were stratified by number of positive nodes whenever possible. Proportions were compared by Fischer's exact method. The primary analysis was based on the intention to treat principal excluding only patients with no follow-up and patients not at-risk for the primary endpoint at the time of randomization (known to have metastases or positive surgical margins). Smooth estimates of the underlying hazard functions were calculated by the method of Muller and Wang (Biometrics 1994 50:61-76). Smooth estimates of the ratio of the underlying hazards were calculated by the method of Gilbert et al. (Biometrics 2002 58:773-80).

The results were as follows:

<table>
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<tr>
<th></th>
<th>No. of patients</th>
<th>No. of events</th>
<th>3 year DFS (%)</th>
<th>p value</th>
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<tr>
<td>mFOLFOX6</td>
<td>1338</td>
<td>312</td>
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<td>mFOLFOX6 + bevacizumab</td>
<td>1334</td>
<td>291</td>
<td>77.4</td>
<td>0.15</td>
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For patients with stage II disease, the 3-year DFS were 87.4% and 84.7% (HR=0.82 CI 0.54-1.25; p=0.35) and for stage III, 74.2% and 72.4% (HR=0.90 CI 0.76-1.07; p=0.23) for the experimental and control arms, respectively.

The final hazard ratio (HR) was 0.888 with a p value of 0.146. The hazard ratio (HR) and p values assessed over time were as follows:

<table>
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<tr>
<th>Year(s) after initiation of treatment</th>
<th>1</th>
<th>1.25</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
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<td>HR</td>
<td>0.6</td>
<td>0.61</td>
<td>0.74</td>
<td>0.81</td>
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<td>0.87</td>
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<tr>
<td>p value</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
<td>0.004</td>
<td>0.02</td>
<td>0.05</td>
<td>0.08</td>
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The DFS at 1 year after initiation of treatment was 94.3 for patients treated with mFOLFOX 6 + bevacizumab and 90.7 for patients treated with mFOLFOX 6 alone (HR was 0.60 with a p value of 0.0004). Bevacizumab had a strong effect during the first 1.25 years (HR=0.61 95% CI 0.48-0.78, p<0.0001). These data indicate that addition of bevacizumab to chemotherapy conferred a clinically meaningful and significant benefit during the active treatment phase (first 12 months after initiation of treatment) in which bevacizumab was being administered to the patient and shortly thereafter. These results also show for the first time that administration of bevacizumab for more than 1 year would be beneficial to the patient.

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.
What is claimed is:

1. A method of adjuvant therapy comprising administering to a patient with cancer, following definitive surgery, an effective amount of a VEGF-specific antagonist so as to extend disease free survival (DFS) or overall survival (OS) in the patient, wherein the VEGF-specific antagonist is administered for more than one year.

2. The method of claim 1, wherein the DFS or OS is evaluated about 2 to about 5 years after initiation of treatment with the VEGF-specific antagonist.

3. The method of claim 1, wherein extending DFS or OS comprises preventing or delaying cancer recurrence, or preventing or delaying occurrence of a second primary cancer.

4. A method of adjuvant therapy comprising administering to a patient with cancer, following definitive surgery, an effective amount of a VEGF-specific antagonist, wherein progression of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment lasts for more than one year.

5. The method of claim 4, wherein the progression of cancer is prevented or delayed for about 6 months after active treatment with the VEGF-specific antagonist has ceased.

6. A method of adjuvant therapy comprising administering to a patient with cancer, following definitive surgery, an effective amount of a VEGF-specific antagonist, wherein recurrence of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment lasts for more than one year.

7. The method of claim 6, wherein the recurrence of cancer is prevented or delayed for about 6 months after active treatment with the VEGF-specific antagonist has ceased.

8. A method of adjuvant therapy comprising administering to a patient who has undergone definitive surgery for cancer, an effective amount of a VEGF-specific antagonist so as to extend DFS or OS in the patient, wherein the VEGF-specific antagonist is administered for more than one year.

9. The method of claim 8, wherein the DFS or OS is evaluated about 2 to about 5 years after initiation of treatment with the VEGF-specific antagonist.
10. The method of claim 8, wherein extending DFS or OS comprises preventing or delaying cancer recurrence or preventing or delaying occurrence of a second primary cancer.

11. A method of adjuvant therapy comprising administering to a patient who has undergone definitive surgery for cancer, an effective amount of a VEGF-specific antagonist, wherein progression of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment lasts for more than one year.

12. The method of claim 11, wherein the progression of cancer is prevented or delayed for about 6 months after active treatment with the VEGF-specific antagonist has ceased.

13. A method of adjuvant therapy comprising administering to a patient who has undergone definitive surgery for cancer, an effective amount of a VEGF-specific antagonist wherein recurrence of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment lasts for more than one year.

14. The method of claim 13, wherein the recurrence of cancer is prevented or delayed for about 6 months after active treatment with the VEGF-specific antagonist has ceased.

15. A method of treating a patient who has undergone definitive surgery for cancer, comprising administering to the patient adjuvant therapy comprising an effective amount of a VEGF-specific antagonist so as to extend DFS or OS in the patient, wherein the VEGF-specific antagonist is administered for more than one year.

16. The method of claim 15, wherein the DFS or OS is evaluated about 2 to about 5 years after initiation of treatment with the VEGF-specific antagonist.

17. The method of claim 15, wherein extending DFS or OS comprises preventing or delaying cancer recurrence or preventing or delaying occurrence of a second primary cancer.

18. A method of treating a patient who has undergone definitive surgery for cancer, comprising administering to the patient adjuvant therapy comprising an effective amount of a VEGF-specific antagonist, wherein progression of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment lasts for more than one year.

19. The method of claim 18, wherein the progression of cancer is prevented or delayed for about 6 months after active treatment with the VEGF-specific antagonist has ceased.
20. A method of treating a patient who has undergone definitive surgery for cancer, comprising administering to the patient adjuvant therapy comprising an effective amount of a VEGF-specific antagonist, wherein recurrence of the cancer is prevented or delayed during active treatment with the VEGF-specific antagonist, and wherein the active treatment lasts for more than one year.

21. The method of any one of claims 1-20 wherein said administering of the VEGF-specific antagonist prevents or reduces the likelihood of occurrence or recurrence of a clinically detectable tumor, or metastasis thereof.

22. A method of preventing cancer recurrence in a patient comprising administering to the patient an effective amount of a VEGF-specific antagonist for more than one year, wherein said administering of the VEGF-specific antagonist prevents cancer recurrence.

23. A method of decreasing the likelihood of cancer recurrence in a patient comprising administering to the patient an effective amount of a VEGF-specific antagonist for more than one year, wherein said administrating of the anti-VEGF antibody decreases the likelihood of cancer recurrence.

24. The method of claim 22 or claim 23, wherein the patient has undergone definitive surgery prior to the administration of the VEGF-specific antagonist.

25. The method of any one of claims 1-24, wherein the patient is identified as having a risk of cancer recurrence or low likelihood of survival following definitive surgery.

26. The method of any one of claims 1-24, wherein the method further comprises administering a chemotherapeutic agent to the patient.

27. The method of claim 26, wherein the treatment with the VEGF-specific antagonist is concurrent with the treatment with the chemotherapeutic agent.

28. The method of any one of claims 1-24, wherein the VEGF-specific antagonist is an anti-VEGF antibody.

29. The method of claim 28, wherein the anti-VEGF antibody is administered to the patient at least 28 days after definitive surgery.

30. The method of claim 28, wherein the anti-VEGF antibody is bevacizumab.
31. The method of claim 30, wherein the anti-VEGF antibody binds the same epitope as the monoclonal anti-VEGF antibody A4.6.1 produced by hybridoma ATCC HB 10709.

32. The method of claim 30, wherein the anti-VEGF antibody has a heavy chain variable region comprising the following amino acid sequence:

```
EVQLVESGGG LVQPGGLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGWIYNTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYPHYYGSSHWYF DVWGQGTLVTAQVQ (SEQ ID NO: 1)
```

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

```
DIQMTQSPSS LSASVQDRVT ITCSASQDIS NYLNWYQQKP GAKPVLQYY FITLTISSLQP ETFATYYCQQ YSTVPWTFGQ GTKVEIKR (SEQ ID NO: 2).
```

33. The method of any one of claims 1-32, wherein the cancer is colorectal cancer, breast cancer, lung cancer, renal cancer, gastric cancer, ovarian cancer or glioblastoma.

34. A kit for treating a patient who has undergone definitive surgery for cancer, comprising a package, wherein the package comprises an anti-VEGF antibody composition and instructions for using the anti-VEGF antibody composition in adjuvant therapy, wherein the instructions recite that the DFS at 1 year after initiation of the adjuvant therapy for patients receiving the adjuvant therapy was 94.3 with a hazard ratio of 0.60.
### Arm A:

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<tr>
<th>Drug</th>
<th>Dose</th>
<th>Administration</th>
<th>Dosing Interval</th>
<th>Planned Duration</th>
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<tr>
<td>Oxaliplatin</td>
<td>85 mg/m²</td>
<td>Administered IV concurrently with separate infusion bags of 250 mL D5W and separate lines connected by Y-line tubing over 2 hours</td>
<td>Day 1 q 14 days</td>
<td></td>
</tr>
<tr>
<td>Leucovorin</td>
<td>400 mg/m²</td>
<td></td>
<td></td>
<td>12 Cycles</td>
</tr>
<tr>
<td>5-FU</td>
<td>400 mg/m²</td>
<td>IV bolus over 2-4 minutes</td>
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</tr>
<tr>
<td>5-FU</td>
<td>2400 mg/m² over 46 hours</td>
<td>IV continuous infusion over 46 hours</td>
<td>Days 1 and 2 q 14 days</td>
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### Arm B:

<table>
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<tr>
<th>Drug</th>
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<th>Administration</th>
<th>Dosing Interval</th>
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<tr>
<td>Bevacizumab</td>
<td>5 mg/kg</td>
<td>IV diluted in 100 mL of 0.9% NaCl solution given over a period of: 90 minutes – 1st dose 60 minutes – 2nd dose 30 minutes – all subsequent doses Flush infusion line</td>
<td>Day 1 q 14 days</td>
<td>12 Months</td>
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<tr>
<td>Oxaliplatin</td>
<td>85 mg/m²</td>
<td>IV given concurrently with separate infusion bags of 250 mL D5W and separate lines connected by Y-line tubing over 2 hours</td>
<td>Day 1 q 14 days</td>
<td>12 Cycles (6 months)</td>
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<td>Leucovorin</td>
<td>400 mg/m²</td>
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<td>5-FU</td>
<td>400 mg/m²</td>
<td>IV bolus over 2-4 minutes</td>
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<td>5-FU</td>
<td>2400 mg/m² over 46 hours</td>
<td>IV continuous infusion over 46 hours</td>
<td>Days 1 and 2 q 14 days</td>
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**FIG. 1**
C-08 SCHEMA

PATIENTS WITH RESECTED STAGE II OR III COLON CARCINOMA

STRATIFICATION
Number of Positive Nodes (0, 1-3, >3)

RANDOMIZATION

GROUP 1
mFOLFOX6*
q 14 days x 12 cycles

GROUP 2
Bevacizumab 5 mg/kg IV
q 2 weeks x 1 year
+
mFOLFOX6*
q 14 days x 12 cycles

*modified FOLFOX6 regimen:
Oxaliplatin 85 mg/m² IV Day 1
Leucovorin 400 mg/m² IV Day 1
5-FU 400 mg/m² IV bolus Day 1
5-FU 2400 mg/m² by continuous IV infusion over 46 hours (Day 1 and Day 2)

FIG. 2
A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C

See patent family annex

Date of the actual completion of the international search

15 June 2010

Date of mailing of the international search report

25/06/2010

Name and mailing address of the ISA
European Patent Office, P B 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Authorized officer

Ferreira, Roger
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